EXPLANATORY STATEMENT

APPLICATION A566

L-5-METHYLTETRAHYDROFOLATE, CALCIUM AS A PERMITTED VITAMIN FORM OF FOLATE

For Information on matters relating to this Assessment Report or the assessment process generally, please refer to http://www.foodstandards.gov.au/standardsdevelopment/

Executive Summary

An Application was received from Axiome Pty Ltd, on behalf of Merck Eprova AG, seeking approval for L-5-methyltetrahydrofolate, calcium salt (L-MTHF) as a permitted form of the B-group vitamin, folate, in foods where voluntary folate fortification is currently permitted in the *Australia New Zealand Food Standards Code* (the Code). Folic acid, the conventional source of supplementary folate, is currently the only permitted form listed in the Code.

Prior to the commencement of mandatory folic acid fortification in September 2009, approval of this Application would allow the addition of L-MTHF to cereals and cereal products, breads, pasta, extracts of meat, vegetables or yeast, vegetable juices and beverages, analogues of yoghurt and dairy desserts and formulated beverages. Once mandatory fortification comes into force, L-MTHF will not be permitted as a fortificant in breads made from wheat flour. This Application does not seek permission to use L-MTHF in infant formula or foods for infants where addition of folic acid is specified.

L-MTHF is synthetically produced from folic acid and is suitable for use in dietary supplements or food fortification in dry crystalline or microencapsulated form. The Applicant claims nutritional benefits in using L-MTHF in place of folic acid – it is the major form of folate occurring in the body and found naturally in foods, and is less likely than folic acid to correct the anaemia associated with Vitamin B_{12} deficiency.

Risk assessment

FSANZ has undertaken a comprehensive risk assessment of L-MTHF and concluded that its use in a range of foods for voluntary fortification purposes does not raise public health and safety concerns. The available evidence indicates that L-MTHF is safe for human consumption and may be considered equivalent to folic acid as a nutritional source of folate when added to foods. The upper tolerable limits on daily intake specified for folic acid are appropriate for L-MTHF. As no extension of use is sought, dietary intakes of folate would therefore not be expected to change with approval for L-MTHF.

The evidence supporting the stability of L-MTHF in food matrices is based on studies of a liquid model food system, bread baking and the manufacture of breakfast cereal. L-MTHF appears to be stable under a range of food processing conditions. However, given the variable nature of processing and storage conditions, individual manufacturers would need to consider the appropriate use of L-MTHF based on their products and associated production processes to ensure adequate presence of the active nutrient in the final food.

Risk management

Approval to use L-MTHF as a permitted form of folate will not result in any changes to the current labelling requirements for fortified foods. If a nutrition (content) claim was made, *folate* would be required to be listed in the Nutrition Information Panel (NIP). Similarly, approval to use L-MTHF will not result in changes to the conditions applying to a nutrition claim, as L-MTHF and folic acid are considered nutritionally equivalent for voluntary fortification purposes.

However, foods fortified with L-MTHF will not be permitted to make a health claim. This is considered appropriate because the evidence supporting the connection between maternal folate consumption and reducing the risk of neural tube defects applies specifically to foods fortified with folic acid. This evidence consists of clinical studies examining the efficacy of folic acid in women of child bearing age and should not be extrapolated to an alternative form of folate. On these grounds therefore, the currently permitted health claim should continue to apply only to foods fortified with folic acid.

FSANZ has recently notified to the Australia and New Zealand Food Regulation Ministerial Council (Ministerial Council) the approval of Standard 1.2.7 – Nutrition, Health and Related Claims, which is the culmination of Proposal P293. This new Standard specifies a high level claim in relation to folic acid and the prevention of NTDs. In the interim, Standard 1.1A.2 – Transitional Standard – Health Claims requires amendment to prohibit a health claim (relating to NTD reduction and folate status in pregnancy) in relation to foods fortified with L-MTHF.

Impact of regulatory options

Permission for L-MTHF requires amendment to the Schedule in Standard 1.1.1 – Preliminary Provisions – Application, Interpretation and General Prohibitions, where permitted forms of vitamins and minerals are listed, and Standard 1.3.4 – Identity and Purity, where published food additive specifications are referenced in the Code. The only regulatory options identified were to approve or not approve L-MTHF as a permitted form of the vitamin, folate. Following analysis of the potential costs and benefits of each option on affected parties (consumers, the food industry and government), approval of this Application is the preferred option as the potential benefits to all sectors outweigh the costs associated with the approval.

Purpose

The Applicant seeks approval in the Code for L-5-methyltetrahydrofolate, calcium as a permitted vitamin form of folate, which then could be used as an alternative to folic acid for the voluntary fortification of specified foods.

Decision

Vary Standards 1.1.1, 1.1A.2 and 1.3.4 of the Code to approve L-5-methyltetrahydrofolate, calcium as a permitted form of folate for the voluntary fortification of certain foods where permitted.

Reasons for Decision

A variation to the Code approving L-5-methyltetrahydrofolate, calcium salt is approved on the basis of the available scientific evidence for the following reasons:

- the proposed draft variations to the Code are consistent with the section 18 objectives of the *Food Standards Australia New Zealand Act 1991*; and

- a regulation impact assessment process concluded that approval of L-MTHF as a permitted form of folate potentially provides a net benefit to consumers and the food industry.

Consultation

The Initial and Draft Assessments were open for public comment for a period of six weeks respectively. Seven submissions were received during the first consultation period and nine submissions were received during the second round. A summary of the issues from the second round of submissions is attached to this Report (Attachment 6).

FSANZ has taken the submitters' comments into account in preparing the final assessment of this Application. No opposition to the Application was expressed in submissions and specific issues relating to folate fortification are addressed in this Report.

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INTRODUCTION

Axiome Pty Ltd, on behalf of the Applicant, Merck Eprova AG, made an Application to FSANZ seeking approval for L-5-methyltetrahydrofolate, calcium salt (L-MTHF)[1] as a permitted form of the vitamin for use in specified foods where voluntary folate fortification is currently permitted in the Code. The Application was received on 6 July 2005 and was placed in Work Plan Group 2 (non-paid). FSANZ commenced assessment of the Application in the last quarter of 2006.

A Final Assessment has been completed, including a full scientific evaluation of L-MTHF to assess its safety for human consumption and suitability for the fortification of certain foods, and consideration of issues raised in two rounds of public consultation.

1. Background

Folate is a B-group vitamin and the general name for a group of structurally-related compounds, both naturally-occurring and synthetic. Folates are widely distributed in nature and are essential for the maintenance of cellular functions and health. As humans (and other mammals) cannot synthesise folates, they must be obtained via the diet. However, folates that occur naturally in foods are susceptible to oxidation and losses can occur during food processing, manufacturing and storage. Whilst procedures can be implemented during food processing operations to minimise these losses, fortification of foods with folate can compensate for the losses and assist in maintaining adequate dietary intakes.

Folic acid is the most common synthetic form of folate used in the fortification of foods and in the majority of dietary supplements. Although folic acid rarely occurs naturally in foods, it is currently the only permitted form of folate listed in the Code. Folic acid does not function directly as a coenzyme, but undergoes reduction to L-5-methyltetrahydrofolate (L-5-MTHF) following absorption from the small intestine. In fact, irrespective of whether ingested food contains natural or synthetically-derived folates, all are metabolised to L-5-MTHF on uptake.

L-MTHF is the calcium salt of a synthetically produced derivative of folic acid and readily dissociates to Ca^{2+} and L-5-MTHF in aqueous media. Unlike folic acid, L-5-MTHF is the predominant form of folate found naturally in foods and is the essential endogenous form utilised and stored in the human body. Synthetically produced L-MTHF is absorbed directly and is then metabolically indistinguishable from any other absorbed folates.

1.1 Historical Background

Standards are in place to regulate the addition of vitamins to food and the claims that manufacturers may make concerning the presence of a vitamin in a food. Under Standard 1.3.2 – Vitamins and Minerals, a vitamin or mineral must not be added to a food unless there is a specific permission listed in the Table to clause 3 of the Standard, or elsewhere in the Code.

[1] Synonyms for L-5-methyltetrahydrofolate, calcium salt include: L-5-methyltetrahydrofolic acid, calcium salt; (6S)-5-methyltetrahydrofolic acid, calcium; L-methyltetrahydrofolate, calcium salt; L-methylfolate, calcium; and L-MTHF-Ca. It also has the marketing name of Metafolin®. For the purposes of this report, L-MTHF is used as the abbreviated name for L-5-methyltetrahydrofolate, calcium salt.

The vitamin or mineral must also be in a permitted form specified in the Schedule to Standard 1.1.1 – Preliminary Provisions – Application, Interpretation and General Prohibitions. The purpose of these restrictions and the associated labelling requirements are to ensure that consumers are not misled by statements made by manufacturers in relation to the vitamin content of foods.

1.2 Current Standard

Since 1995 in Australia, and 1996 in New Zealand, folic acid has been permitted to be voluntarily added to the following foods: flour; savoury biscuits; breads; breakfast cereals; vegetable, yeast and meat extracts; pasta; fruit and vegetable juices and drinks; and beverages derived from legumes. Folic acid may also be added to legume analogues of dairy foods and meat but in smaller amounts. More recently, voluntary folic acid fortification permissions have been extended to cereal based beverages e.g. rice and oat 'milks' and formulated beverages. These permissions are provided in Standard 1.3.2 – Vitamins and Minerals. Folate may also be added to formulated meal replacements and formulated supplementary foods (under Standard 2.9.3) and to formulated supplementary sports foods (under Standard 2.9.4).

From 13 September 2009 in Australia and 27 September 2009 in New Zealand, permissions for the *voluntary* addition of folate to bread will apply only to breads that are not made from wheat flour. From these dates, it will be mandatory for breads made from wheat flour to specifically contain folic acid as the permitted form of the vitamin.

1.2.1 Permitted claims

Under the existing food regulations, permitted claims made on the presence of a vitamin and mineral in a food refer to the total of both naturally-occurring and added forms of the nutrient. In the case of dietary folate in food, the amount declared on a label is the sum of naturally-occurring folate and added folic acid and is listed as 'folate' in the Nutrition Information Panel.

In addition, under Standard 1.1A.2 – Transitional Standard – Health Claims, a health claim referring to a link between increased maternal dietary folate intake and a reduction in NTD risk is permitted for some fortified and non-fortified foods that contain at least 40 µg folate per serving. The claim should state that increased maternal folate consumption in at least the month before and three months following conception may reduce the risk of NTDs. It must also include the recommendation that women consume a minimum of 400 µg of folate per day during this time.

1.3 Change to the scope of the Application

Under Standard 2.9.2 – Foods for Infants, folate may also be added to cereal-based and non-cereal based foods for infants however the permitted form is folic acid as specified in the Schedule to Standard 2.9.1 – Infant Formula Products. At Initial Assessment, the scope of this Application included seeking an amendment to Standard 2.9.1 to include L-MTHF as a permitted form of folate. Such an amendment would have permitted the voluntary addition of L-MTHF in foods for infants and also given permission for the mandatory fortification of infant formula products with L-MTHF.

During the assessment of this Application, FSANZ was advised that this Application does not seek permission to use L-MTHF in infant formula products or foods for infants and therefore an amendment to Standard 2.9.1 is no longer required.

1.4 Regulatory status overseas

In the USA, L-MTHF is 'generally recognised as safe' (GRAS) for use as a source of folate in conventional and medical foods and dietary supplements.

In the European Union, the European Food Safety Authority (EFSA) evaluated the data submitted with this Application and concluded that L-MTHF in dry crystalline or microencapsulated form is not a safety concern as a source of folate in dietary supplements, foods for specific nutritional uses (except infant formula) and other foods, with a tolerable upper intake level of 1 mg/adult/day (EFSA, 2004).

The EU Commission recently determined that L-MTHF would require authorisation as a novel food before it could be incorporated in the lists of vitamins authorised for the addition to food (food fortification) and the addition to infant formula. A novel food application for L-MTHF (commercially known as Metafolin®) was subsequently submitted to Ireland as the first assessing Member State. In the meantime, a favourable opinion of the Irish competent authority (FSAI) was issued and forwarded to all other Member States for comment. No 'reasoned objection' was received within the comment period. The Applicant received authorisation for Metafolin® as a novel food on 4 January 2008.

Following this approval, the Applicant intends to request inclusion of Metafolin® in the EU positive lists of vitamins that may be added to foods and to infant formula. The first request will trigger a merely administrative procedure that would be expected to lead to an amendment of the food fortification regulation in 2008. The request for use in infant formula will be forwarded to EFSA for a scientific opinion. Pending a favourable opinion, the positive list of vitamins for use in infant formula would be amended accordingly.

In Japan, a dossier for authorisation of Metafolin® as a 'designated food additive' for use in foods with a health claim has been accepted by the Ministry of Health and Welfare for evaluation. The safety assessment is in progress.

2. The Issue / Problem

Under Standard 1.1.1, folic acid is currently the only form of folate permitted in the Code for food use. The Applicant has developed L-MTHF as a synthetically produced form of folate suitable for use in food and pharmaceutical products. The Applicant claims that L-MTHF is nutritionally preferable over folic acid because it is the form of folate that is (i) normally present in the body, (ii) naturally present in foods, and (iii) unlikely to correct the clinical symptoms of a vitamin B_{12} deficiency. The use levels of L-MTHF would be equivalent on a molar basis to the levels currently permitted in Standard 1.3.2 for folic acid.

Approval for L-MTHF would also require a consequential amendment to clause 2 of Standard 1.3.4 – Identity and Purity to include reference to new specifications for L-MTHF prepared at the 65th meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2005). These specifications have been published in the FAO Compendium of Food Additive Specifications Addendum 13 (FNP, 2005).

3. Objectives

The purpose of this assessment is to determine whether it would be appropriate to vary Standards 1.1.1 and 1.3.4 of the Code to approve the use of L-MTHF as a permitted form of folate for the voluntary fortification of foods. Section 18(1) of the *Food Standards Australia New Zealand Act 1991* (FSANZ Act) provides that the objectives of FSANZ in developing or reviewing food regulatory measures or variations of food regulatory measures are:

- the protection of public health and safety;
- the provision of adequate information relating to food to enable consumers to make informed choices; and
- the prevention of misleading or deceptive conduct.

Section 18(2) requires FSANZ to also have regard to:

- the need for standards to be based on risk analysis using the best available scientific evidence;
- the promotion of consistency between domestic and international food standards;
- the desirability of an efficient and internationally competitive food industry;
- the promotion of fair trading in food; and
- any written policy guidelines formulated by the Ministerial Council.

3.1 Ministerial policy guideline

The Ministerial Council Policy Guideline on the *Fortification of Food with Vitamins and Minerals* was adopted by the Ministerial Council in May 2004. The Policy Guideline provides guidance on development of new permissions for the addition of vitamins and minerals to food (Ministerial Council 2004).

This Application seeks approval for L-MTHF as a permitted form of folate for use in specified foods where voluntary folate fortification is permitted in the Code. If approved, manufacturers of those foods, with an existing permission for folate fortification, would be able to choose to use L-MTHF instead of folic acid according to their specific requirements. This Application does not seek to extend the range of foods in which voluntary fortification is currently permitted. The risk assessment has therefore focussed on whether L-MTHF can be regarded as equivalent to folic acid in terms of safety and nutritional efficacy, which is consistent with the Policy Guideline endorsed by the Ministerial Council.

RISK ASSESSMENT

4. Key Assessment Questions

FSANZ has considered the following key questions at Draft Assessment:

- Is L-MTHF safe for human consumption?
- Is L-MTHF nutritionally equivalent to folic acid?
- Are the upper tolerable limits on daily intake specified for folic acid appropriate for L-MTHF?
- Are there benefits in using L-MTHF instead of folic acid in fortified foods?
- Is L-MTHF stable when added to processed food products?

In addressing these questions, FSANZ has considered information provided by the Applicant and other resource material including published scientific literature and general technical information available in the public domain. The summary and conclusions from separate nutrition, safety, dietary intake and food technology assessments are at **Attachments 2, 3, 4 and 5** respectively, and are presented below.

5. Risk Assessment Summary

5.1 Nutrition Assessment

FSANZ has completed a comprehensive nutritional assessment of L-MTHF based on data and information provided by the Applicant and obtained from the scientific literature. In supporting the use of L-MTHF as an alternative form of folate, the Applicant claims that L-MTHF demonstrates similar bioavailability and bioefficiency to folic acid, and has the potential advantage that it is unlikely to mask a vitamin B_{12} deficiency. The Applicant also suggests that L-MTHF may be a better alternative to folic acid for people with a genetically-determined reduced activity of the enzyme methylenetetrahydrofolate reductase.

Dietary L-MTHF can be directly absorbed and utilised by the body (also see Section 5.2). The available literature clearly shows that in adults L-MTHF is as effective in improving folate status and reducing homocysteine concentrations as an equivalent dose of folic acid. While there is a lack of direct data in children, the biochemistry is well known and FSANZ considers it reasonable to extrapolate these results to children.

Vitamin B_{12} deficiency causes a breakdown in an essential metabolic pathway resulting in both adverse haematological (megaloblastic anaemia) and neurological consequences. High doses of folic acid have the potential to correct the anaemia, but not the neuropathy associated with vitamin B_{12} deficiency, thereby potentially masking the deficiency. Current understanding of folate metabolism and *in vitro* evidence support the claim that L-MTHF would not correct megaloblastic anaemia caused by vitamin B_{12} deficiency.

However, the diagnosis of vitamin B_{12} deficiency need not rely solely on haematological symptoms, and there is no clear indication that folic acid in the food supply is delaying the diagnoses of vitamin B_{12} deficiency.

Evidence that L-MTHF is a better alternative to folic acid for those with reduced methylenetetrahydrofolate reductase activity is currently very limited. Further studies on this issue would be required to reach a firm conclusion.

On the basis of all available evidence including direct comparisons of L-MTHF and folic acid in human studies, L-MTHF can be considered as effective as folic acid as a nutritional source of folate.

5.2 Safety Assessment

The FSANZ assessment was based on chemistry, metabolism and toxicity data on L-MTHF provided by the Applicant and obtained from the scientific literature. Once ingested, L-MTHF would readily dissociate to Ca^{2+} and L-5-MTHF in the aqueous environment of the digestive tract. L-5-MTHF, like all folates, is then absorbed across the small intestine by carrier-mediated transport and is indistinguishable from all other folates in the diet, including folic acid. Recent human studies on the bioavailability of L-MTHF have been reviewed in the Nutrition Assessment and indicate that L-MTHF and folic acid are essentially bioequivalent.

The toxicological database for L-MTHF is adequate to define the hazard. It includes a number of published and unpublished studies in laboratory animals, in addition to a number of human studies examining the metabolism of L-5-MTHF relative to folic acid or the effects on certain health endpoints.

Laboratory animal studies indicated that L-MTHF has low acute and repeat-dose toxicity. There was no evidence of developmental toxicity in rats and no evidence that L-MTHF is genotoxic. Studies conducted on a number of manufacturing impurities and a racemic mixture of L-MTHF confirm the low toxicity potential of this folate. Based on these considerations, it is concluded that there are no safety concerns with regard to the use of L-MTHF as an alternative form of folate for the voluntary fortification of foods.

5.3 Dietary intake assessment

A dietary intake assessment was undertaken to assess the potential impact of fortifying certain foods with L-MTHF if used as an alternative form of folate. Food consumption data from the 1995 Australian and 1997 New Zealand National Nutrition Surveys were used for the intake assessments.

A dietary intake assessment for L-MTHF was derived from the estimated folic acid intakes from food alone (not including dietary supplements), based on current uptake of voluntary folic acid permissions outlined in Standard 1.3.2 of the Code. The intake assessment assumed that there would be 100% replacement of folic acid with L-MTHF for foods currently fortified, and the resulting intakes of L-MTHF would be equivalent to that of folic acid. The dietary intake assessment did not take into account naturally occurring folates in food or L-MTHF from supplements or multivitamins.

The dietary intake assessment was conducted for the age groups specified in the National Health and Medical Research Council (NHMRC) Nutrient Reference Values for Australia and New Zealand (NHMRC, 2006). This allowed a comparison of estimated L-MTHF intakes with the relevant Nutrient Reference Value (NRV) for folic acid which is the Upper Level (UL).

The results of the dietary intake assessment for L-MTHF indicated that:

- as shown in previous modelling for folic acid, breakfast cereals would be the major contributors to L-MTHF intakes for both the Australian (2 years and above) and New Zealand (15 years and above) populations;

- yeast extracts and breads would be major contributors to intake of L-MTHF in Australia. For New Zealand, yeast extracts would be a major contributor while breads would not;

- the New Zealand population has an overall lower L-MTHF intake from food in all age groups assessed relative to Australia, due to a lower level of uptake of voluntary fortification by the food industry; and

- children aged 2-3 years are the age group most likely to exceed the UL for folic acid, based on estimated intakes of folic acid.

The intake of additional calcium from the use of L-MTHF was also considered. Based on estimated intake of food containing L-MTHF, the additional calcium intake would be less than 1 mg per day. This additional intake of calcium is considered insignificant in relation to the total diet.

5.4 Food technology assessment

The FSANZ food technology report was based on technical information provided by the Applicant, published studies in scientific journals and other technical references. L-MTHF is a white to light yellowish, almost odourless, crystalline powder which is sparingly soluble in water. It is manufactured synthetically from folic acid under conditions of Good Manufacturing Practice (GMP) and is intended for use as an alternative form of the vitamin for use in dietary supplements and for food fortification purposes.

L-MTHF is reported to be stable in crystalline form during long-term storage (48 months at 40^{o}C and up to 75% relative humidity), after micronising or milling, and when compounded into vitamin and mineral tablets. On the basis of the available data, L-MTHF in the dry crystalline or microencapsulated form appears to demonstrate comparable stability to folic acid when tested during bread baking, in the manufacture of breakfast cereal and in liquid foods under certain conditions. However, limited information is available (either from the Application or from the literature) on the stability of added L-MTHF during a broader range of food production and food storage conditions. Based on the limited data on the stability of L-MTHF in different food matrices, food manufacturers would be responsible for ensuring that L-MTHF used as a fortificant, either in crystalline or microencapsulated form, was appropriate for their products to ensure sufficient quantities of the active nutrient were available in the final food.

6. Risk characterisation

There is no upper limit of folate intake for the Australian and New Zealand populations if the folate is intrinsic to the food; upper levels only apply to added folic acid. The estimated UL of 1 mg folic acid/day for adults is based on the neurological effects seen with a vitamin B_{12} deficiency (NHMRC, 2006), and have been conservatively applied to L-MTHF in this assessment.

Although no direct evidence is available, based on the biochemical processes involving L-MTHF in the body, correcting the anaemia associated with a vitamin B_{12} deficiency in theory would not be expected.

The dietary modelling indicates that intakes of L-MTHF used for the voluntary fortification of certain foods would generally be below the UL determined for folic acid. The modelling found the age group most likely to exceed the UL would be children 2-3 years of age, who are the group least likely to have a vitamin B_{12} deficiency. Therefore L-MTHF intakes slightly above the UL for folic acid do not represent a safety concern.

A reduction in the incidence of neural tube defects is associated specifically with maternal consumption of folic acid at specified levels and at critical times pre- and post- conception. Based on a raft of clinical studies using folic acid, the mandatory fortification of breads produced from wheat flour will come into force in Australia and New Zealand from September 2009. Although permissions for the voluntary addition of folate to both cereal flours and bread (other than wheat bread) will be retained, the commencement of mandatory fortification requirements will effectively reduce the permitted uses of L-MTHF.

The modelling in this assessment indicates that, given the current pattern of uptake of voluntary fortification by the food industry, breakfast cereals would be the major contributors to L-MTHF intakes in both Australia and New Zealand. However, breads would be a major contributor to intake of L-MTHF in the Australian and, to a lesser extent, New Zealand populations. Assuming that L-MTHF had completely replaced folic acid in fortified foods, the introduction of mandatory fortification of wheat breads with folic acid would therefore be expected to significantly impact on dietary intakes of L-MTHF via voluntary fortification. The dietary modelling conducted for Proposal P295[2] confirms that once mandatory fortification commences, breads made from wheat flour will be the major source of added folate in the diet.

The nutrition and safety assessments demonstrate that L-MTHF is safe for human consumption and has a similar bioavailability to folic acid. For foods where there is a mandatory requirement to add folate for a specific nutritional purpose, data on stability in the food matrix and during storage is considered essential. In this case, where folate fortification is the choice of the manufacturer, FSANZ considers the data provided are adequate to support the addition of L-MTHF in certain foods. Manufacturers would need to consider stability in particular applications and adopt practices that accounted for losses of L-MTHF during production, processing and storage of their product.

RISK MANAGEMENT

7. Technical and industry considerations

The Applicant highlights certain nutritional properties of L-MTHF in support of its use as an alternative form of folate for the fortification of food products. The risk assessment was primarily concerned with any potential risks to public health and safety through a permission to use L-MTHF as a folate fortificant.

[2] Proposal P295 – Consideration of mandatory fortification with folic acid, www.foodstandards.gov.au/standardsdevelopment

The nutrition and safety assessments concluded that L-MTHF is bioequivalent to folic acid however its stability in a range of foods and under different processing conditions is based on limited information.

FSANZ notes that the stability of folic acid when used in supplements and for the fortification of foods is well demonstrated. As L-MTHF is produced synthetically from folic acid, it is also likely that there would be no major cost advantage in using L-MTHF. The stated benefits in using L-MTHF instead of folic acid therefore may not be perceived by some manufacturers as reasons to alter their established practices.

Nevertheless, the Application is supported by the food industry in submissions. The Australian Food and Grocery Council and George Weston Foods both claim that food manufacturers would benefit from the choice of using either L-MTHF or folic acid according to the specific requirements of their products. These submissions also claim that any increased costs in using L-MTHF may be off-set by the potential nutritional benefits as stated by the Applicant.

8. Labelling including nutrition, health and related claims

8.1 Labelling

The purpose of food labelling is to provide consumers with information about food to enable them to make informed food choices. Labelling provides an important source of information for consumers regarding fortification, and enables consumers to make informed decisions regarding their consumption of fortified foods.

The generic labelling requirements of the Code applicable to foods that are fortified with folate include:

- listing of ingredients (Standard 1.2.4); and
- the conditions applying to nutrition claims about vitamins and minerals (Standard 1.3.2).

FSANZ considers the generic requirements of the Code to be appropriate for providing consumers with information regarding folate fortification, including if L-MTHF was used as the fortificant.

If a nutrition claim is made in relation to *folate* on a food fortified with L-MTHF, *folate* would be required to be listed in the Nutrition Information Panel (NIP) under current labelling requirements. Approval to use L-MTHF as a permitted form of folate will therefore not result in any changes to the current labelling requirements.

8.2 Nutrition and health claims

Approval for L-MTHF as a permitted form of folate will not result in changes to the conditions applying to a nutrition claim, as L-MTHF and folic acid are considered nutritionally equivalent for voluntary fortification purposes. In order to make a nutrition claim, it would be the responsibility of manufacturers to ensure the presence of the active nutrient in the final food to avoid potential consumer deception issues.

In relation to health claims, the currently permitted claim relating to maternal folate consumption and reducing the risk of neural tube defects specifically applies to food fortified with folic acid (currently the only permitted form of folate). These claims are supported by a raft of clinical studies testing the efficacy of folic acid in women of child bearing age. In contrast, there is no direct evidence to support the view that foods fortified with L-MTHF would have similar efficacy in reducing the incidence of neural tube defects on a population basis. On these grounds, the permitted health claim will be restricted to foods fortified with folic acid.

FSANZ recently notified to the Australia and New Zealand Food Regulation Ministerial Council the approval of draft Standard 1.2.7 – Nutrition, Health & Related Claims, which is the culmination of Proposal P293. This new draft Standard specifies a high level claim in relation to folic acid and the prevention of NTDs. In the interim, Standard 1.1A.2 – Transitional Standard – Health Claims requires amendment to prohibit a health claim (relating to NTD reduction and folate status in pregnancy) in relation to foods fortified with L-MTHF.

9. Options

The two regulatory options available for this Application are:

9.1 Option 1 – Prohibit the use of L-MTHF as a permitted form of folate

Maintain the *status quo* by rejecting the Application.

9.2 Option 2 – Approve the use of L-MTHF as a permitted form of folate

Vary the Schedule to Standard 1.1.1, and Standards 1.1A.2 and 1.3.4, to include L-MTHF as a permitted form of folate for the voluntary fortification of certain foods specified in the Code.

10. Impact Analysis

FSANZ is required to consider the impact of various regulatory (and non-regulatory) options on all sectors of the community, which includes consumers, food industries and governments in Australia and New Zealand.

10.1 Affected Parties

Parties possibly affected by the regulatory options outlined above include:

1. consumers, including the elderly, a group at risk of vitamin B_{12} deficiency;

2. manufacturing and retail sectors of the food industry; and

3. Governments of Australia and New Zealand, generally.

10.2 Benefit Cost Analysis

In the course of developing food regulatory measures suitable for adoption in Australia and New Zealand, FSANZ is required to consider the impact of all options on all sectors of the community, including consumers, the food industry and governments in both countries.

The regulatory impact assessment identifies and evaluates, though is not limited to, the costs and benefits of the regulation, and its health, economic and social impacts. During public consultation on the Initial Assessment Report, FSANZ sought comment on the costs and benefits of the regulatory options proposed. Comments are summarised at **Attachment 6** and, where relevant, have been considered in the benefit cost analysis.

10.2.1 Option 1 – Prohibit the use of L-MTHF as a permitted form of folate

Under Option 1, the affected parties and potential impacts are as follows:

- Maintaining restrictions on the permitted form of folate would disadvantage manufacturers of L-MTHF and would have a negative impact on manufacturers wishing to produce foods containing L-MTHF and importers of foods containing L-MTHF as they would be unable to innovate and take advantage of market opportunities for the development and sale of products containing L-MTHF.

- Consumers would be unable to take advantage of any potential benefits provided by food fortified with a form of folate found naturally in the body and which is unlikely to correct the anaemia associated with Vitamin B_{12} deficiency.

- There is no perceived impact on Government, generally.

10.2.2 Option 2 – Approve the use of L-MTHF as a permitted form of folate

Under Option 2, the affected parties and potential impacts are as follows:

- Food manufacturers would have choice in the form of folate that could be used in food products thereby allowing innovation in the processed food industry. Importers of foods containing L-MTHF would be able to take advantage of market opportunities for the development and sale of products containing L-MTHF.

- As L-MTHF is reportedly four times more expensive than folic acid it is reasonable to assume that increased manufacturing costs would be passed on to consumers. However, such costs could be minimised by the choice that manufacturers will have to use *either* L-MTHF or folic acid depending on the technological need of a product. Expanded market opportunities may also offset such costs.

- There may be costs to industry associated with any changes to product labels but these may be absorbed through normal business cycles.

- As L-MTHF is bioequivalent to folic acid and raises no safety concerns, consumers would not be disadvantaged if L-MTHF were permitted as an alternative vitamin form of folate. In fact, consumers may consider there are potential benefits with fortifying food with a form of folate found naturally in the body, and which is unlikely to mask a Vitamin B_{12} deficiency.

- There is no perceived impact on Government, generally.

10.3 Comparison of Options

Option 1 disadvantages industry in terms of its potential to take advantage of any market opportunities for products containing a new form of a nutritive substance which has been assessed as safe both in Australia and overseas. Further, consumers who select products on the basis of perceived benefits provided by folate fortification would have reduced choice in the marketplace and would have restricted access to foods containing a form of folate that is considered unlikely to correct the anaemia associated with Vitamin B_{12} deficiency.

Option 2 may result in increased costs of products fortified with L-MTHF *versus* those fortified with folic acid. However, these costs are likely to be minimal on the basis of the following: new market opportunities may arise through the use of L-MTHF; industry will have the choice to use either L-MTHF or folic acid as appropriate for their products; labelling costs may be absorbed through normal business cycles. There may also be a direct benefit to some consumers through consuming a form of folate that is considered unlikely to mask symptoms of a Vitamin B_{12} deficiency.

An assessment of the costs and benefits of the two options indicates that there would be a net benefit in permitting the use of L-MTHF as an alternative vitamin form of folate. The draft variations to the Code giving effect to the decision are at **Attachment 1**.

COMMUNICATION

11. Communication and consultation strategy

FSANZ has applied a communication strategy to this Application that involves advertising the availability of assessment reports for public comment in the national press and placing the reports on the FSANZ website for free public access. In addition, FSANZ issued media releases drawing journalists' attention to this Application. Two rounds of public comment were conducted as part of the normal application process in use prior to 1 October 2007.

The Draft and Final Assessments are distributed directly to major stakeholders. The Applicant and individuals and organisations who make submissions on this Application were notified at each stage of the Application. After draft amendment to the Code is approved, the Board's decision will be notified to the Ministerial Council. If the approval is not subject to review, the Applicant and stakeholders, including the public, will be notified of the gazettal of changes to the Code in the national press and on the website.

12. Consultation

12.1 Consultation on the Initial Assessment

The Initial Assessment Report was advertised for comment between 13 December 2006 and 7 February 2007; seven submissions were received during this period. Three submissions supported the approval of L-MTHF as a permitted form of folate wherever voluntary fortification is currently permitted in the Code. The remaining four submissions supported progression of the Application to Draft Assessment. Comments from two submitters regarding the revision of the folate advisory statement were considered to be outside the scope of the current Application.

12.2 Consultation on the Draft Assessment

Following release of the Draft Assessment, the Application was open for public comment between 12 December 2007 and 6 February 2008; nine submissions were received during this period. A summary of these submissions in included at **Attachment 6** to this report. All submissions, including several from the food industry, supported approval of this Application. Submitters' comments in relation to the mandatory fortification of foods with folic acid have been noted and any relevant issues have been addressed in the following sections of this report.

12.2.1 Proposed drafting for new health claims standard

In their submission, several jurisdictions pointed to an apparent loophole in the previously proposed drafting for the new health claims standard (Standard 1.2.7), that would enable a folate-NTD claim for foods fortified with L-MTHF. This would present an inconsistency with the recommendation at Draft Assessment, which proposed disallowing a claim when L-MTHF was used as the form of folate, based on the conclusion that relevant studies associated NTD reduction specifically with maternal consumption of folic acid.

12.2.1.1 Response

The conditions for a high level health claim about folic acid and neural tube defects were amended from those proposed in the Preliminary Final Assessment Report for Proposal P293 – Nutrition, Health & Related Claims. Standard 1.2.7 – Nutrition, Health and Related Claims, which was recently approved and notified to the Ministerial Council, now prescribes that the food carrying such a claim must contain at least 40 µg of folic acid, and the property of the food mentioned in the claim must be folic acid (not folate). The reasons for referring to folic acid rather than folate are:

- The NHMRC and NZ Ministry of Health (NHMRC and NZ Ministry of Health, 2006) recommendation in relation to prevention of neural tube defects refers only to folic acid, not to natural folates or total folates expressed as DFEs.

- The FSANZ high level health claim review of this relationship only indicated a 'convincing' relationship with folic acid. In regard to natural folates, the evidence was not 'convincing' largely due to issues associated with quantifying the amount of natural folate consumed, bioavailability and stability, and lack of consistency in the results of the few studies that had examined the relationship.

12.2.2 Stock-in-trade provisions after mandatory fortification

The NSW Food Authority sought information on any stock-in-trade provisions that may apply after September 2009 to L-MTHF-fortified wheat flour for making bread, following the commencement of mandatory fortification with folic acid.

12.2.2.1 Response

The mandatory requirement to add folic acid to bread making flour in Australia and bread in New Zealand only applies to folic acid and not L-MTHF.

If L-MTHF, once approved, is voluntarily added to bread making flour, this practice should be discontinued prior to the implementation date for the mandatory folic acid Standards (13 September 2009 in Australia and 27 September 2009 in New Zealand).

The purpose of the two year transitional period for the implementation of the mandatory folic acid fortification Standard is to give manufacturers sufficient time to adjust their manufacturing practices accordingly. As the compliance point for mandatory folic acid fortification is at the milling stage (in Australia), it is expected that any voluntary use of L-MTHF following approval of this Application, would cease in time to meet mandatory requirements. In effect therefore, there are no provisions for stock-in-trade that would apply to wheaten bread-making flour fortified with L-MTHF.

12.2.3 Nutrient Reference Values

The New Zealand Food Safety Authority considers that this Application presents an opportune time to align the recommended dietary intakes of folate listed in the Code with the recently published Nutrient Reference Values, and to standardise the use of the terms folate and folic acid within the Code.

12.2.3.1 Response

FSANZ is currently scoping a project to assess the adoption into the Code of a set of regulatory Nutrient Reference Values based on the revised official Nutrient Reference Values for Australia and New Zealand. It is intended that one or more Proposals will be prepared to address this issue holistically rather than on a case-by-case, or nutrient specific, basis. This approach should ensure consistency throughout the Code with respect to terms used for nutrients.

12.2.4 Naming of L-MTHF

Following Draft Assessment, the European manufacturer of L-MTHF, Merck Eprova AG, noted that the divalent cation (calcium) was not specified in the draft variation to the Code giving approval to L-MTHF as a permitted form of folate.

12.2.4.1 Response

FSANZ was already aware that the published specifications for L-MTHF relate to its calcium salt. To avoid any potential ambiguity in the Code, FSANZ has reviewed and amended the proposed drafting to reflect approval for L-methyltetrahydrofolate, calcium. This is a minor drafting change which ensures that the term used in the Code precisely reflects synthetically produced L-MTHF.

12.2.5 Chemical description of L-MTHF

The applicant suggested that in various parts of the Draft Assessment Report, the term ‘diastereoisomeric mixture’ should be used because L-5-MTHF-Ca has two asymmetric (chiral) centres.

12.2.5.1 Response

While in general two chiral centres would preclude the use of the term racemic mixture, there are conventions surrounding L-5-MTHF-Ca which are intended to simplify identifying all four possible stereoisomers. By convention, both chiral centres in L-MTHF-Ca have the natural L-configuration (6S, αS). In the D-isomer (D-5-MTHF-Ca), the configuration of the chiral carbons is (6R, αS) i.e. the α-C being the same as in the L- form. So the difference between the D and L forms occurs as a function of a difference at only one chiral centre. Hence, it is considered appropriate to refer to a mixture of D and L forms of 5-MTHF-Ca as a racemic mixture. This was explained in the safety assessment under 'Chemistry' (Attachment 3) and therefore a terminology change is considered unnecessary.

12.3 World Trade Organization (WTO)

As members of the World Trade Organization (WTO), Australia and New Zealand are obligated to notify WTO member nations where proposed mandatory regulatory measures are inconsistent with any existing or imminent international standards and the proposed measure may have a significant effect on trade.

Currently, there are no relevant international standards relating to the use of L-MTHF as a permitted form of folate for food fortification purposes. The proposed measure (i.e. amendment to the Code) is likely to have a liberalising effect on international trade on the grounds that it does not change the permitted level of folate fortification specified in the Code and addition of folate to the specified foods is voluntary in New Zealand and Australia. In addition, the proposed amendment is consistent with the regulatory status of L-MTHF in the USA and recent approval for specified uses in Europe. On this basis, it was not considered necessary to notify the WTO regarding the proposed measure.

CONCLUSION

13. Conclusion and Decision

Based on the completion of a comprehensive risk assessment of L-MTHF as an alternative form of folate, FSANZ has concluded that use of L-MTHF for voluntary fortification purposes would raise no public health and safety concerns.

Decision

Vary Standards 1.1.1, 1.1A.2 and 1.3.4 to approve L-5-methyltetrahydrofolate, calcium as a permitted form of folate for the voluntary fortification of specified foods where permitted.

13.1 Reasons for Decision

A variation to the Code approving L-5-methyltetrahydrofolate, calcium salt is approved on the basis of the available scientific evidence for the following reasons:

- the proposed draft variations to the Code are consistent with the section 18 objectives of the FSANZ Act; and

- a regulation impact assessment process concluded that approval of L-MTHF as a permitted form of folate potentially provides a net benefit to consumers and the food industry.

13.2 Additional recommended change to the Code

The recommended approval of a new permitted form of added folate requiring amendments to the Schedule to Standard 1.1.1, presents the opportunity to delete the reference to folate in column 3 of that Schedule. Column 3 qualifies the 200 µg RDI.

Deleting the folate reference is recommended to provide clarity now that the recently published Nutrient Reference Value (NRV) report differentiates the bioavailability of synthetic folic acid from natural folates, and introduces a new term Dietary Folate Equivalents (DFE). Although the new NRVs are yet to be incorporated into the Code, this minor amendment addresses the possibility of the Code being interpreted in the light of the revised approach to nutrients. The draft variations to Standard 1.1.1 are at **Attachment 1** to this report.

14. Implementation and Review

Following notification, the proposed draft variation to the Code is expected to come into effect on gazettal, subject to any request from the Ministerial Council for a review of FSANZ's decision.

ATTACHMENTS

1. Draft variations to the *Australia New Zealand Food Standards Code*
2. Nutrition Assessment Report
3. Safety Assessment Report
4. Dietary Intake Assessment
5. Food Technology Report
6. Summary of public submissions received after Draft Assessment

Attachment 1

Draft variations to the *Australia New Zealand Food Standards Code*

Standards or variations to standards are considered to be legislative instruments for the purposes of the Legislative Instruments Act (2003) and are not subject to disallowance or sunsetting.

To commence: on gazettal

[1] ***Standard 1.1.1*** *of the Australia New Zealand Food Standards Code is varied by omitting from* Columns 2, 3 and 4 *of the* Schedule*, the entries in relation to* Folate, *substituting –*

Folic acid L-methyltetrahydrofolate, calcium	200 µg	100 µg

[2] ***Standard 1.1A.2*** *of the Australia New Zealand Food Standards Code is varied by inserting after paragraph (3)(e) –*

(ea) The reference to folate in the Table to subclause 3(e) excludes folate in the form of L-methyltetrahydrofolate, calcium.

[3] ***Standard 1.3.4*** *of the Australia New Zealand Food Standards Code is varied by omitting paragraph 2(a), substituting –*

(a) Combined Compendium of Food Additive Specifications, FAO JECFA Monograph 1 (2005) as superseded by specifications published in FAO JECFA Monographs 3 (2006) and FAO JECFA Monographs 4 (2007), Food and Agriculture Organisation of the United Nations, Rome; or

Attachment 2

Nutrition Assessment – L-5-Methyltetrahydrofolate, Calcium

Summary and conclusions

A comprehensive nutrition assessment was undertaken to determine the nutritional similarities of L-MTHF and folic acid using data and information provided by the Applicant and from the published literature. In supporting the use of L-MTHF as an alternative form of folate, the Applicant claims that L-MTHF demonstrates similar bioavailability and bioefficiency to folic acid, and has the potential advantage that it is unlikely to mask a vitamin B_{12} deficiency. The Applicant also suggests that L-MTHF may be a better alternative to folic acid for people with a genetically-determined reduced activity of the enzyme methylenetetrahydrofolate reductase.

Dietary L-MTHF can be directly absorbed and utilised by the body (also see Safety Assessment at **Attachment 3**). The available literature clearly shows that in adults L-MTHF is as effective in improving folate status and reducing homocysteine concentrations as an equivalent dose of folic acid. While there is a lack of direct data in children, the biochemistry is well known and FSANZ considers it reasonable to extrapolate these results to children.

Vitamin B_{12} deficiency causes a breakdown in an essential metabolic pathway resulting in both adverse haematological (megaloblastic anaemia) and neurological consequences. High doses of folic acid have the potential to correct the anaemia, but not the neuropathy associated with vitamin B_{12} deficiency, thereby potentially masking the deficiency. Current understanding of folate metabolism and *in vitro* evidence support the claim that L-MTHF would not correct megaloblastic anaemia caused by vitamin B_{12} deficiency. However, the diagnosis of vitamin B_{12} deficiency need not rely solely on haematological symptoms, and there is no clear indication that folic acid in the food supply is delaying the diagnoses of vitamin B_{12} deficiency.

Evidence that L-MTHF is a better alternative to folic acid for those with reduced methylenetetrahydrofolate reductase activity is currently very limited. Further studies on this issue would be required to reach a firm conclusion.

On the basis of all available evidence including direct comparisons of L-MTHF and folic acid in human studies, L-MTHF can be considered as effective as folic acid as a nutritional source of folate.

Introduction

The aim of this nutritional assessment is to review the information on the nutritional role of folates and to assess the biological similarity or bioequivalence of L-5-methyltetrahydrofolate and folic acid. The applicant claims that L-MTHF *demonstrates similar bioavailability and bioefficiency to folic acid, and has the potential advantage that it is unlikely to mask the clinical symptoms of vitamin B_{12} deficiency.* The applicant also suggests that L-MTHF may be a better alternative to folic acid for people with a genetically-determined reduced activity of the enzyme methylenetetrahydrofolate reductase.

Background

Folate is a water soluble B-group vitamin essential to cellular functions. Humans cannot synthesise folate; it must therefore be derived from the diet.

Forms of folate

The term folate is used generically to refer to the various forms of the vitamin, both naturally-occurring and synthetic, which share similar structure and biological activity. Folates consist of a pteroyl group joined to as many as 11 glutamic acid residues. In addition to varying numbers of glutamic acid residues, folates also vary in the extent of the reduction state of the pteroyl group. Figure 1 shows the generic form of folate along with the one-carbon substituents that can occur at the N-5 and N-10 positions.

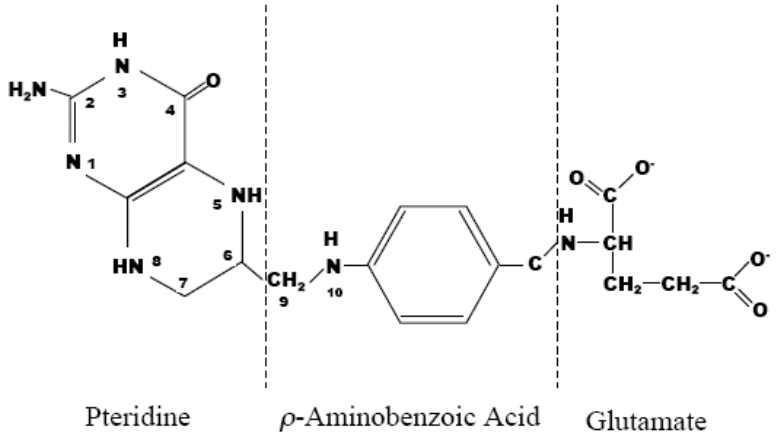
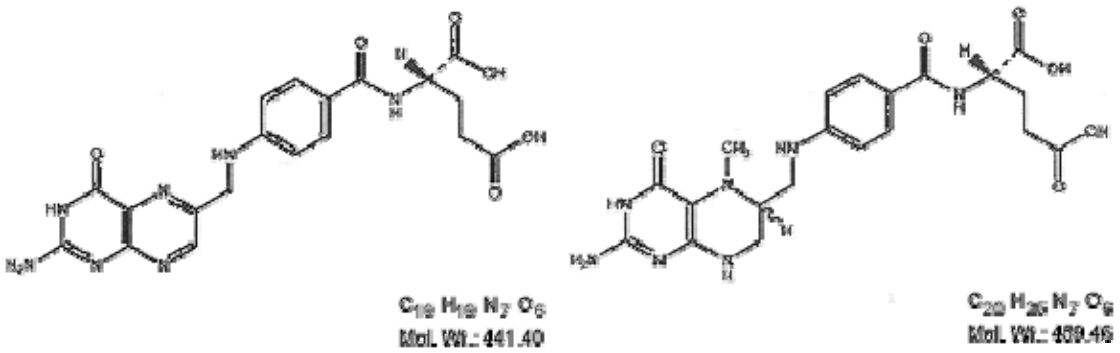


Figure 1: Generic Structure of Folate

One Carbon Substitute		Position	Oxidation State
Methyl	$-CH_3$	N-5	Methanol
Methylene	$-CH_2-$	N-5, N-10	Formaldehyde
Methenyl	-CH=	N-5, N-10	Formate
Formyl	-CHO-	N-5, or N10	Formate
Formimino	HN=CH-	N-5	Formate

The structures of L-MTHF and folic acid are shown in Figure 2. L-MTHF is the predominant form circulating in plasma. Folic acid, also called pteroylglutamic acid, is the most common synthetic form of folate currently used in fortification and in the majority of dietary supplements.

The key structural differences between the two compounds are that L-MTHF is methylated at the N-5 position, and folic acid occurs in the oxidized state, whereas L-MTHF occurs in the reduced state, as do other naturally occurring folates.



Folic Acid **L-5-Methyltetrahydrofolate**

Figure 2: The key structural difference between the two molecules is that L-MTHF has a methyl group attached to the N at position 5. In addition, folic acid occurs in the oxidized state whereas L-MTHF occurs in the reduced state.

Figure 2: Structure of Folic Acid and L-5-Methyltetrahydrofolate

Absorption and bioavailability of folates

Dietary folates, including folic acid and L-MTHF, are predominantly absorbed in the proximal small intestine (Shane, 2000). In the case of polyglutaminated folates, brush border membrane γ-glutamylhydrolases remove all but one of the glutamate residues prior to absorption. Folates naturally found in food are converted to L-MTHF before being released into the portal circulation. This is also true of physiological doses, ~ 200 μg, of folic acid. However, excess folic acid can enter the circulation unmetabolised (Kelly *et al*, 1997). A portion of the absorbed folate is used by enteric cells; the remainder enters the portal circulation with much of this portion being taken up by the liver for storage and later release (Shane, 2000; Wright *et al*, 2005). Figure 3 outlines the absorption and metabolism of folate.

Bioavailability refers to *the proportion of the ingested nutrient absorbed and utilized through normal metabolic pathways* (Hurrel, 2002). *It is influenced by dietary factors and host related factors* (Gibson, 2007). Much remains to be determined about the bioavailability of different forms of folate in foods. However, several factors that can or may influence folate bioavailability have been identified (Brouwer *et al*, 2001) including:

- the chemical form;
- the amount ingested;
- the number of glutamic acid residues attached to the pteroyl group;
- the food matrix in which the folate occurs;
- the folate status of the host;
- host genetic factors
- physiological state of the host; and
- drugs or other substances that may interfere with folate absorption or metabolism.

To date, studies comparing the bioavailability of folic acid and L-MTHF have used supplements. Of most relevance to this assessment is that folate taken in the form of a supplement on an empty stomach has a higher bioavailability than folates consumed as part of a food matrix (Brouwer *et al*, 2001).

Metabolism and physiological functions of folate

Folate's main physiological function is as an acceptor and donator of one-carbon units in amino acid and nucleic acid metabolism. Specifically, folate is required for the synthesis of the purines required for the formation of DNA and RNA, and the conversion of the amino acid homocysteine to methionine (Shane, 2000). Additionally, folate plays a role in the conversion of vitamin B_{12} to one of its coenzymes and is required for the methylation of DNA and RNA. During times of high DNA and RNA synthesis, such as during the rapid cell division that occurs during foetal development, folate requirements are increased (Pitkin, 2007). Figure 3 provides a basic summary of folate metabolism.

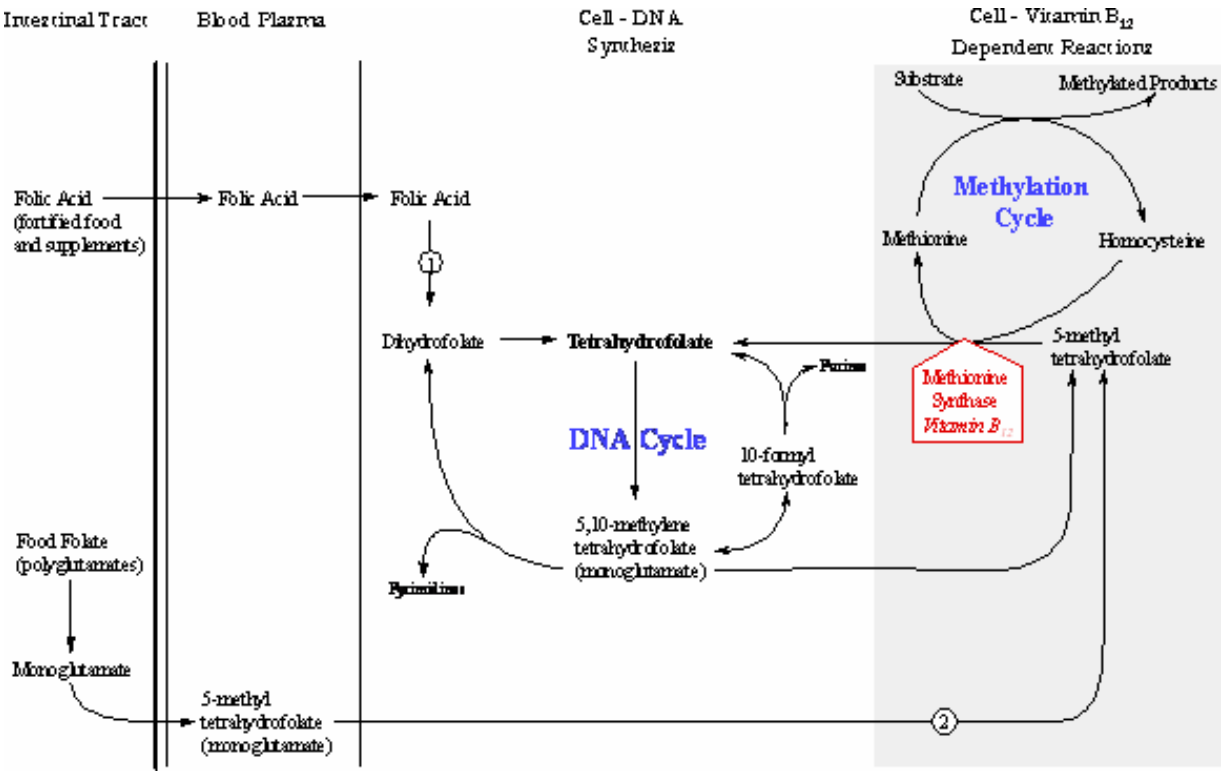


Figure 3: Food folate, including folic acid, is absorbed from the intestines into the circulation. The liver converts most folate, including folic acid, to L-MTHF, some of which then re-enters the circulation, becoming available for absorption by the cells of the body. Inside cells, L-MTHF can enter the methylation or DNA cycle. Although folic acid is normally converted to L-MTHF by the liver as part of first pass metabolism, any unmetabolised folic acid can enter the DNA cycle at a different point to L-MTHF.

Figure 3: Diagram of Folate Metabolism

Sources of folate

Foods naturally high in folate include green leafy vegetables (such as broccoli and spinach), liver, nuts, orange juice, some fruits and dried beans and peas. Cereals are moderate sources of folate.

Based on the national nutrition surveys conducted in Australia and New Zealand in 1995 and 1997 respectively, cereals and cereal-based dishes, vegetables and legumes contributed nearly 60% of naturally-occurring folate in the adult diet (NZMoH, 1999; ABS, 1999). These surveys were conducted prior to or about the time of the introduction of voluntary fortification.

Nutrient Reference Values for folate

The reference values for folate intakes are set out in the *Nutrient Reference Values for Australia and New Zealand*[3]. Several types of nutrient reference values (NRVs) exist for folate including the estimated average requirement (EAR[4]), the recommended dietary intake (RDI[5]) and the upper level of intake (UL[6]). In the absence of sufficient data to determine an EAR and RDI, an adequate intake (AI[7]) was established for infants. The new EAR and RDI for folate are expressed as 'dietary folate equivalents' or DFEs[8], which reflect the difference in bioavailability between different forms of folate. The revised NRVs established higher levels of folate intake than those previously published in 1991. NRVs for folate are given in Table 1 arranged by age, gender and physiological state.

Table 1: Australian and New Zealand Nutrient Reference Values for Folate

	Age	AI	EAR	RDI	UL
		(µg DFE per day)			
Infants	0-6 months	65 (as folate)			
	7-12 months	80			
Children & Adolescents	1-3 years		120	150	300
	4-8 years		160	200	400
	9-13 years		250	300	600
	14-18 years		330	400	800
Adults	19+ years		320	400	1000
Pregnancy	14-18 years		520	600	800
	19-50 years		520	600	1000
Lactation	14-18 years		450	500	800
	19-50 years		450	500	1000

[3] This document is available online at http://www.nhmrc.gov.au/publications/synopses/n35syn.htm.
[4] A daily nutrient level estimated to meet the requirements of half the healthy individuals in a particular life stage and gender group.
[5] The average daily dietary intake level that is sufficient to meet the nutrient requirements of nearly all (97-98%) healthy individuals in a particular life stage and gender group.
[6] The highest average daily nutrient intake level likely to pose no adverse health effects to almost all individuals in the general population. As intake increases above the UL, the potential risk of adverse effects increases. A UL has only been established for folic acid, not for other forms of folate.
[7] The average daily nutrient intake level 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.
[8] DFEs is a term used to accommodate the various bioavailabilities of folate. One µg DFE = 1 µg food folate = 0.5 µg of folic acid on an empty stomach = 0.6 µg of folic acid with meals.

The revised NRVs also advises women capable of, or planning, pregnancies to consume additional folic acid as a supplement or in the form of fortified foods at a level of 400 µg/day of folic acid for at least one month before and three months after conception (NHMRC *et al*, 2006).

The Code contains a composite of RDIs for folate. The revised NRVs have not yet been adopted into the Code nor has the principle of DFEs.

Standard 1.1.1 – Preliminary Provisions – Application, Interpretation and General Prohibitions currently specifies an RDI of 200 µg and 100 µg of *folic acid* for the general population and 1-3 year-olds respectively.

Consequences of folate deficiency

Due to the importance of folate in cell division, subadequate folate intake is first detected in rapidly dividing tissue such as blood cells. Hypersegmented neutrophils are one of the earliest signs of deficiency (Gibson, 2005). If it progresses, folate deficiency can lead to megaloblastic anaemia; this is characterised by the appearance of red blood cells that are enlarged and contain approximately twice the amount of DNA of normal red blood cells (Shane, 2000). It should be noted that megaloblastic anaemia is not restricted to folate deficiency but may also be caused by vitamin B_{12} deficiency (Gibson, 2005). Blood morphology is therefore not a specific marker for folate status.

Folate-deficiency anaemia is rare in developed countries. As little as 100 µg food folate is thought sufficient to prevent deficiency (Gibson, 2005). Since folate requirements are increased during pregnancy and lactation, the main concern in developed countries is not with nutritional folate deficiency, but the added benefit of high folate intakes for the prevention of neural tube defects (NTDs) (Pitkin, 2000). These are a group of birth defects, which arise during the early development of the brain and spinal cord *in utero* (Shane, 2000). It has consistently been shown that folic acid supplementation during the periconceptional period reduces the risk of NTD affected pregnancies (Pitkin, 2000). This is recognised in the NRVs described above.

Biological equivalence of L-MTHF and folic acid

Biological equivalence (bioequivalence) implies that the physiological effects of ingesting two compounds in the same molar dose are essentially the same with respect to efficacy and safety. This section explores the measures relevant to the assessment of bioequivalence in the context of folate, and summarises the relevant research pertaining to the bioequivalence of folic acid and L-MTHF.

Indices of folate status

The liver is the main storage organ for folate (Gibson, 2005). It is not generally feasible to measure liver folate directly. However, a number of easily measured biomarkers for folate status are available; the most commonly used are briefly described below. Each of these indices can be influenced by various factors other than folate status; it is therefore advisable to measure multiple indices.

Serum and plasma folate

Methyltetrahydrofolate is the predominant form of folate found in serum, with much of the folate bound to proteins, including albumin (Gibson, 2005). Serum or plasma folate concentrations are an indicator of short term changes in folate intake. They increase rapidly over a period of several days following high folate intake. Similarly they decrease rapidly when folate intakes drop markedly. Therefore, serum and plasma folate concentrations are not necessarily indicative of body folate stores or long-term folate status.

In addition to changes in folate intake, serum or plasma folate concentrations may also be influenced by pregnancy, some oral contraceptive agents, smoking, some drugs, and some diseases including liver and kidney disease (Gibson, 2005).

Red Blood Cell folate

Red blood cells incorporate folate as they develop in the bone marrow (Shane, 2000). Once red blood cells are fully formed, folate levels remain stable throughout the approximately four month life span of the cell (Gibson, 2005). Red blood cell folate therefore reflects folate stores and long-term folate status.

In addition to changes in folate intake, red blood cell folate concentrations may also be influenced by factors such as smoking, oral contraceptive use, vitamin B_{12} deficiency, pregnancy, parity, some disease states, and iron deficiency (Gibson, 2005).

Serum or plasma homocysteine

Folate is required for the conversion of the amino acid homocysteine to methionine; without sufficient folate, serum and plasma homocysteine concentrations begin to rise (Gibson, 2005). Elevated homocysteine concentrations can be detected even in the early stages of tissue folate depletion making it a useful surrogate measure for folate stores when these are not replete.

The conversion of homocysteine to methionine is also dependent on the actions of vitamin B_{12} and indirectly vitamin B_6 (Gibson, 2005). Several other factors have also been shown to influence homocysteine concentrations including age, gender, ethnicity, pregnancy, alcohol intake, recent intake of food, caffeine intake, smoking, and some medications. Hence elevated homocysteine concentrations alone are not necessarily indicative of low folate stores.

Acute dose comparison of folic acid and L-5-methyltetrahydrofolate

The bioavailability of different folates has been studied by measuring the plasma response to a single oral dose of the test form of folate relative to the response to folic acid in terms of the rate of increase, the maximum increase, or the rise in concentration above fasting baseline levels, i.e. area under the curve (AUC) (Brouwer *et* al, 2001; Wright *et al*, 2003).

A number of studies examining the bioavailability of L-MTHF relative to folic acid have been summarised previously (Brouwer *et al*, 2001). These studies all involve a period of loading with folic acid prior to the administration of oral doses of folic acid, L-MTHF, or other forms of folate.

The studies assessed bioavailability by measuring changes in serum and/or urinary folate concentrations over periods ranging from 2-48 hours. Results of these studies are conflicting and inconclusive. The short duration of the period of measurement with respect to serum folate has been suggested as a serious weakness in the study designs, as these periods may not have allowed for complete absorption of the test dose.

Some recent studies have used isotope labelled forms of folate to allow for better differentiation between the test folate and existing folate stores (Wolfe *et al*, 2001). The most recent findings using stable isotope labelled folates, including folic acid, show that the appearance of total folate in the plasma following an oral dose (Wright *et al*, 2003):

1. is greater than the appearance of labelled folate;
2. the average plasma labelled AUC the test folate was 221% ($p<0.001$) of the response to folic acid;
3. the average unlabelled AUC response was the same for folic acid and the test folate; and
4. there was no within-subject association between labelled and unlabelled plasma AUC responses.

The appearance of substantial quantities of unlabelled folate in the plasma indicates that folic acid and other forms of folate can induce the acute release of folate from endogenous stores, and that these account for the majority of the rise in the serum in the short-term rather than the test dose. Thus, there is considerable doubt about the ability of acute dose studies to provide a reliable index of relative bioavailability until the apparent differences in the metabolism of folic acid vs. other forms of folate are better understood (Wright *et al*, 2003, Wright *et al*, 2005).

Chronic dose comparison of folic acid and L-5-methyltetrahydrofolate

Comparisons between L-MTHF and folic acid have been carried out over a number of weeks using supplements manufactured by Merck Eprova AG, the originator of this Application. These studies do not suffer from the aforementioned uncertainties currently surrounding acute dose studies, and provide good information on the outcome of chronic intake by measuring a combination of biomarkers of folate status. Results of these studies have been summarised in Table 2.

The studies in Table 2 all administered equimolar amounts of folic acid or L-MTHF in the form of supplements. The studies were conducted in a range of healthy adult male and female subjects aged 19 years to over 50 years. One study consisted exclusively of postpartum lactating women (Houghton *et al*., 2006). The studies reported either no difference between subjects given folic acid and those given L-MTHF in terms of changes to indices of folate status or they indicated a greater positive change with L-MTHF.

One of the key limitations of these studies is that they were done exclusively in adult subjects. However, there is no indication in the literature that children absorb or metabolise folate differently to adults. Therefore the overall conclusion that L-MTHF is bioequivalent to folic acid in adults is likely to also hold true for children.

Table 2: Comparison of Folic Acid with L-MTHF in Chronic Supplementation Trials

Citation	Sample Size & Descriptors	Protocol	Measured Endpoints & Outcomes
(Venn *et al*., 2002)	97 healthy non-pregnant, non-lactating women aged 18-49 years	Parallel placebo RCT. Women received 100 µg, 227 nmol/d folic acid (n = 31) or 113 µg, 227 nmol/d L-MTHF (n = 38) for 24 weeks	Plasma folate concentration: • Baseline adjusted mean weekly increase of 6.9 nmol/L and 9.2 nmol/L for L-MTHF and folic acid respectively with no statistically significance (p>0.05) difference. Red cell folate: • Baseline adjusted mean weekly increases of 7.4 nmol/L and 8.3 nmol/L for L-MTHF and folic acid respectively with no statistically significance (p>0.05) difference. There were no differences in the slope between folic acid and L-MTHF for either plasma or red cell folate (P= 0.7)
(Venn *et al*., 2003)	155 healthy adults (104 females, and 51 males), mean age 45 years	Parallel placebo RCT. Participants received equimolar amounts of folic acid at 100 µg/d (n=52), L-MTHF at 113 µg/d (n=53), or placebo (n=50) for 24 weeks	After adjustment for baseline values there was no significant difference (P>0.05) in red blood cell or plasma folate, after 24 weeks, between folic acid and L-MTHF groups. Statistically significant (P=0.045) greater reduction in plasma homocystine concentration in those receiving L-MTHF (14.6%) vs. those given folic acid (9.3%).
(Lamers *et al*, 2003; Lamers *et a*., 2004, Lamers *et al*, 2006)	135 healthy non-pregnant, non-lactating women aged 19-33 years	Parallel placebo RCT. Women received 400 µg, 906 nmol/d folic acid (n = 34), or 416 µg, 906 nmol/d L-MTHF (n = 35), 208 µg, 453 nmol/d L-MTHF (n = 33), or placebo (n = 34) for 24 weeks	Greater rate of plasma and red blood cell folate accumulation with L-MTHF than with folic acid (P < 0.05) No difference in plasma homocysteine reduction between L-MTHF and folic acid (P > 0.05)
(de Meer *et al*, 2005)	14 healthy females (7 under 30 years, 7 over 50 years old 10 healthy males (5 under 30 years, 5 over 50 years old)	Parallel randomised intervention. Subjects received 400 µg folic acid (n=12), or an equimolar dose of L-MTHF (n=12), for 5 weeks.	Intervention / Baseline / 5 weeks Plasma folate (nmol/L) L-MTHF (<30 years) 9.9 21.6 Folic acid (<30 years) 13.3 23.7 L-MTHF (>50 years) 21.0 26.6 Folic acid (>50 years) 16.7 22.1 No significant difference between interventions

Citation	Sample Size & Descriptors	Protocol	Measured Endpoints & Outcomes			
(Houghton *et al*., 2006)	64 post partum women aged 20-38 years	Parallel placebo RCT. All women supplemented with 1000 µg folic acid during pregnancy. Post partum, lactating women received 400 µg, 906 nmol/d folic acid (n = 24), or 416 µg, 906 nmol/d L-MTHF (n = 22), or placebo (n =23) for 16 wk	Treatment	Baseline	16 Weeks	Change
			RBC folate (nmol/L)			
			L-MTHF	2998	2178	-820
			Folic Acid	3635	1967	-1668
			Plasma folate (nmol/L)			
			L-MTHF	85	91	+6
			Folic Acid	102	94	-8
			Plasma tHcy (µmol/L)			
			L-MTHF	7.9	8.1	+0.2
			Folic Acid	8.7	8.7	0

L-MTHF – L-5-methyl-tetrahydrofolate, RBC – red blood cell, RCT – randomised controlled trial

Correcting the anaemia associated with the diagnosis of vitamin B12 deficiency

It has been suggested that high folic acid intakes (>1,000 µg per day) could delay the diagnosis and eventual treatment of severe vitamin B_{12} deficiency in older people (Capra *et al*., 2005). This could occur by correcting the anaemia that may accompany vitamin B_{12} deficiency which is one of the clinical signs traditionally relied on for diagnosis. A UL of 1,000 µg folic acid per day in adults has been set based on the neurological effects seen with vitamin B_{12} deficiency (NHMRC, 2006).

Vitamin B_{12} deficiency is recognised through presentation of clinical signs of abnormal haematology or neuropathy and a definitive diagnosis is usually obtained from serum vitamin B_{12} levels which are lower in deficiency states. Doctors are advised to consider vitamin B_{12} deficiency as a possible cause when presented with individuals who have clinical signs of anaemia or neuropathy. Among countries that have introduced mandatory fortification with folic acid, there have been no reports of adverse effects on neurological function, especially in people aged 65 years and over with low vitamin B_{12} status (SACN, 2005).

The Applicant suggests that L-MTHF is unlikely to mask the haematological symptoms of vitamin B_{12} deficiency. This assumption is based on the nature of folate metabolism illustrated in Figure 3. Folic acid that is not converted to L-MTHF before entering the circulation has the potential to be converted to dihydrofolate in the absence of vitamin B_{12}. Dihydrofolate thus formed can then take part in the DNA cycle (Scott *et al*, 1981). This would allow cell division to continue, thereby delaying megaloblastic anaemia even in the presence of vitamin B_{12} deficiency. However, the methylation cycle would still be affected by the deficiency leading to the progression of non-haematological symptoms. In contrast, L-MTHF requires the action of vitamin B_{12} before it can enter the DNA cycle and should therefore not be able to delay manifestation of megaloblastic anaemia.

Three investigations showing that bone marrow cells from vitamin B_{12} deficient patients treated with folic acid were able to continue normal DNA biosynthesis unlike those treated with L-MTHF, provide more direct evidence that L-MTHF is unlikely to address the anaemia and therefore mask vitamin B_{12} deficiency (Metz *et al*, 1967; Ganeshugura *et al*, 1978; Zittoun *et al*, 1978). Further evidence comes from a case study showing the administration of 100 µg L-MTHF orally or intravenously did not improve the haematological status of a vitamin B_{12} deficient patient (Gutstein *et al*, 1973).

Genetic interactions

The applicant suggests that L-MTHF may be a better alternative to folic acid for people with a genetically-determined reduced activity of the enzyme methylenetetrahydrofolate reductase (MTHFR). MTHFR catalyses the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, an intermediary step in the metabolism of folic acid. A reduced activity of this enzyme could, in theory, slow the conversion of folic acid to the metabolically active L-MTHF.

A significant interaction between genotype and treatment using folic acid or racemic (D, L) L-MTHF has been reported (Fohr *et al*., 2002). However, the numbers of participants homozygous for the polymorphism was small (n = 5 and n = 7 in the folic acid and 5-methyltetrahydrofolate groups, respectively) and no statistical comparison was made between them. The hypothesis that L-MTHF may be a better alternative to folic acid for people with a reduced activity of MTHFR requires further testing.

Conclusion

Equimolar amounts of supplemental folic acid or L-MTHF over several weeks show that L-MTHF results in elevation of plasma/serum folate concentrations and decreases in serum homocysteine of equivalent or greater magnitude than folic acid. L-MTHF can therefore be considered bioequivalent to folic acid over the long-term.

References

Adams, T. and Jeffreson, S. (1996) Australia implements voluntary folate fortification. *Am.J.Public Health* 86(4):593-594.

Bonaa, K.H., Njolstad, I., Ueland, P.M., Schirmer, H., Tverdal, A., Steigen, T., Wang, H., Nordrehaug, J.E., Arnesen, E. and Rasmussen, K. (2006) Homocysteine lowering and cardiovascular events after acute myocardial infarction. *N.Engl.J.Med.* 354(15):1578-1588.

Brouwer, I.A., van Dusseldorp, M., West, C.E., Steegers-Theunissen, R.P.M. (2001) Bioavailability and bioefficacy of folate and folic acid in man. *Nutr.Res.Rev.* 14:267-93.

Brown, J.P., Scott, J.M., Foster, F.G. and Weir, D.G. (1973) Ingestion and absorption of naturally occurring pteroylmonoglutamates (folates) in man. *Gastroenterology* 64(2):223-232.

De Meer, K., Smulders, Y.M., Dainty, J.R., Smith, D.E., Kok, R.M., Stehouwer, C.D., Finglas, P.M. and Jakobs, C. (2005) [6S]5-methyltetrahydrofolate or folic acid supplementation and absorption and initial elimination of folate in young and middle-aged adults. *Eur.J.Clin.Nutr.* 59(12):1409-1416.

Ganeshaguru, K. and Hoffbrand, A.V. (1978) The effect of deoxyuridine, vitamin B12, folate and alcohol on the uptake of thymidine and on the deoxynucleoside triphosphate concentrations in normal and megaloblastic cells. *Br.J.Haematol.* 40(1):29-41.

Gibson, R.S. (2005) *Principles of Nutritional Assessment*. 2nd Ed. Oxford University Press, New York, NY.

Gibson, R.S. (2007) The role of diet- and host-related factors in nutrient bioavailability and thus in nutrient based dietary requirement estimates. *Food Nutr. Bull.* 28(suppl):S77–100

Green, T.J., Venn, B.J., Skeaff, C.M. and Williams, S.M. (2005) Serum vitamin B12 concentrations and atrophic gastritis in older New Zealanders. *Eur J Clin Nutr* 59(2):205-210.

Gregory, J.F., III, Bhandari, S.D., Bailey, L.B., Toth, J.P., Baumgartner, T.G. and Cerda, J.J. (1992) Relative bioavailability of deuterium-labeled monoglutamyl tetrahydrofolates and folic acid in human subjects. *Am.J.Clin.Nutr.* 55(6):1147-1153.

Hurrell, R. (2002) Bioavailability—a time for reflection. *Int. J. Vitam. Nutr. Res.*72:5–6.

Flood, V.M., Webb, K.L., Smith, W., Rochtchina, E. and Mitchell, P. (2004) Prevalence of low serum folate, red cell folate, serum vitamin B12 and elevated homocysteine. *Asia Pac.J Clin Nutr* 13(Suppl):S85.

Fohr, I.P., Prinz-Langenohl, R., Bronstrup, A., Bohlmann, A.M., Nau, H., Berthold, H.K. and Pietrzik, K. (2002) 5,10-Methylenetetrahydrofolate reductase genotype determines the plasma homocysteine-lowering effect of supplementation with 5-methyltetrahydrofolate or folic acid in healthy young women. *Am.J.Clin.Nutr.* 75(2):275-282.

Hokin, B.D. and Butler, T. (1999) Cyanocobalamin (vitamin B-12) status in Seventh-day Adventist ministers in Australia. *Am.J.Clin.Nutr.* 70(3 Suppl):576S-578S.

Houghton, L.A., Sherwood, K.L., Pawlosky, R., Ito, S. and O'Connor, D.L. (2006) [6S]-5-Methyltetrahydrofolate is at least as effective as folic acid in preventing a decline in blood folate concentrations during lactation. *Am.J.Clin.Nutr.* 83(4):842-850.

Institute of Medicine. (1998) Folate. In: *Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin, and Choline*. Chapter 8. National Academy Press, Washington, D.C., pp 198-305.

Lamers, Y., Prinz-Langenohl, R., Moser, R. and Pietrzik, K. (2003) [6S]-5-methyltetrahydrofolate is as effective as folic acid in lowering plasma total homocysteine. *J.Inherit.Metab.Dis.* 26 (supplement 1):10.

Lamers, Y., Prinz-Langenohl, R., Moser, R. and Pietrzik, K. (2004) Supplementation with [6S]-5-methyltetrahydrofolate or folic acid equally reduces plasma total homocysteine concentrations in healthy women. *Am.J.Clin.Nutr.* 79(3):473-478.

National Health and Research Council, Department of Health and Ageing, Ministry of Health (2006) *Nutrient Reference Values for Australia and New Zealand.*

McMahon, J.A., Green, T.J., Skeaff, C.M., Knight, R.G., Mann, J.I. and Williams, S.M. (2006) A controlled trial of homocysteine lowering and cognitive performance. *N.Engl.J.Med.* 354(26):2764-2772.

Metz, J., Kelly, A., Swett, V.C., Waxman, S. and Herbert, V. (1968) Deranged DNA synthesis by bone marrow from vitamin B-12-deficient humans. *Br.J.Haematol.* 14(6):575-592.

Perry, J. and Chanarin, I. (1970) Intestinal absorption of reduced folate compounds in man. *Br.J.Haematol.* 18(3):329-339.

Pietrzik, K. and Remer, T. (1989) Zur bioverfugbarkeitsprufung von mikronahrstroffen (bioavailability study of micronutrients). *Zeitschrift fur Ernahrungswissenschaft* 28:130-141.

Pikin, R.M. (2007) Folate and neural tube defects. *Am.J.Clin.Nutr.* 85(suppl):285S-8S.

SACN (2005) *Folate and disease prevention. Draft report*. http://www.sacn.gov.uk/reports/#

Scott, J.M. and Weir, D.G. (1981) The methyl folate trap. A physiological response in man to prevent methyl group deficiency in kwashiorkor (methionine deficiency) and an explanation for folic-acid induced exacerbation of subacute combined degeneration in pernicious anaemia. *Lancet* 2(8242):337-340.

Shane, B. (2000) Folic acid, vitamin B12, and vitamin 6. In: Stipanuk, M.H., ed. *Biochemical and Physiological Aspects of Human Nutrition*. W.B. Saunders Company, Philadelphia, Penn, pp 483-500.

Smulders, Y.M., Smith, D.E., Kok, R.M., Teerlink, T., Swinkels, D.W., Stehouwer, C.D. and Jakobs, C. (2006) Cellular folate vitamer distribution during and after correction of vitamin B12 deficiency: a case for the methylfolate trap. *Br.J.Haematol.* 132(5):623-629.

Tamura, T. and Stokstad, E.L. (1973) The availability of food folate in man. *Br.J.Haematol.* 25(4):513-532.

Venn, B.J., Green, T.J., Moser, R. and Mann, J.I. (2003) Comparison of the effect of low-dose supplementation with L-5-methyltetrahydrofolate or folic acid on plasma homocysteine: a randomized placebo-controlled study. *Am.J.Clin.Nutr.* 77(3):658-662.

Venn, B.J., Green, T.J., Moser, R., McKenzie, J.E., Skeaff, C.M. and 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(11):3353-3355.

Wright, A.J., Finglas, P.M., Dainty, J.R., Hart, D.J., Wolfe, C.A., Southon, S. and Gregory, J.F. (2003) Single oral doses of 13C forms of pteroylmonoglutamic acid and 5-formyltetrahydrofolic acid elicit differences in short-term kinetics of labelled and unlabelled folates in plasma: potential problems in interpretation of folate bioavailability studies. *Br.J.Nutr.* 90(2):363-371.

Attachment 3

Safety Assessment Report L-5-methyltetrahydrofolate, calcium salt

Summary and conclusions

The FSANZ assessment was based on chemistry, metabolism and toxicity data on L-MTHF provided by the Applicant and obtained from the scientific literature. Once ingested, L-MTHF would readily dissociate to Ca^{2+} and L-5-MTHF in the aqueous environment of the digestive tract. L-5-MTHF, like all folates, is then absorbed across the small intestine by carrier-mediated transport and then becomes indistinguishable from all other absorbed folates (including folic acid), which are actually metabolised to L-5-MTHF. Therefore, L-5-MTHF can be considered the point of convergence for all absorbed dietary folates, whether synthetic or naturally-derived.

The toxicological database for L-MTHF is adequate and includes a number of published and unpublished studies in laboratory animals, in addition to a number of human studies examining the metabolism of L-5-MTHF relative to folic acid or the effects on certain health endpoints. Recent human studies on the bioavailability of L-MTHF *versus* folic acid have been reviewed in the Nutrition Assessment and indicate that L-MTHF is bioequivalent to folic acid in humans.

Laboratory animal studies indicated that L-MTHF has low acute and repeat-dose toxicity. There was no evidence of developmental toxicity in rats and no evidence that L-MTHF is genotoxic. Studies conducted on a number of manufacturing impurities and a racemic mixture of L-MTHF confirm the low toxicity potential of this folate. The Australian upper level of intake (UL) for folate in fortified foods or supplements is between 300-1000 µg/day. This UL is based on the neurological effects in the presence of vitamin B_{12} deficiency in humans and has been used in the dietary intake assessment of L-MTHF (see **Attachment 4**). The absence of any toxicological effects in laboratory animals at doses at least four orders of magnitude higher than this intake indicates that the ingestion of L-MTHF at the levels proposed is unlikely to be a safety concern.

Based on these considerations, it is concluded that there are no safety concerns with regard to the use of L-MTHF as an alternative form of folate for the voluntary fortification of foods.

Introduction

This safety assessment was conducted to identify potential public health and safety risks associated with the addition of L-5-methyltetrahydrofolic acid, calcium salt (L-MTHF) for the nutrient fortification of certain foods. The assessment was based on data on the chemistry, metabolism and toxicity of L-MTHF provided by the applicant and obtained from the scientific literature. In addition, safety assessments conducted by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and European Food Safety Authority (EFSA) were considered.

Chemistry

Details of the physicochemical properties of L-MTHF are summarised in the Food Technology Report (Attachment 5).

L-MTHF has two chiral carbon atoms: one in position 6 of the pteroyl moiety and the α-C in the L-glutamic acid moiety. As a result, four diastereoisomers are possible.
Due to complexity in isomeric forms of naturally occurring tetrahydrofolate, it has been agreed that all natural diastereoisomers of reduced folates be defined as the L- isomer. By convention, both chiral centres in L-MTHF have the natural L-configuration (6S, αS). In the unnatural isomer, D-5-MTHF-Ca, the configuration of the chiral carbons is (6R, αS), as the α-C is the same as in the L- form. The applicant stated that the D-isomer is not biologically active.

Manufacturing process

The applicant indicated that L-MTHF is produced synthetically from folic acid. Full details of the manufacturing process have been declared 'Commercial-in-Confidence'.

Proposed specifications

The Applicant supplied an analysis of five representative batches of L-MTHF, which showed the purity to be generally above 97%. Draft product specification for food-grade L-MTHF as provided in the Application are as follows

Constituent	Concentration
L-MTHF	95.0 – 102.0 %
D-5-MTHF-Ca (unnatural isomer)	≤ 1.0 %
Individual related compounds	≤ 1.0 %
Other folates and related compounds[1]	≤ 2.5 %
Calcium	7.0 – 8.5 %
Boron	≤ 20 ppm
Water	≤ 17.0 %
Ethanol	≤ 0.5 %
Heavy metals (as lead)	≤ 20 ppm
Total viable aerobic counts	≤ 1000 CFU/g

New specifications of identity and purity for L-MTHF were prepared at the 65th meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2005) and subsequently published in the FAO Compendium of Food Additive Specifications Addendum 13 (FNP 52 Add 13, 2005).

Impurities

The applicant indicated that three types of impurities have been identified in preparations of L-MTHF:

(a) Reaction by-products – minor amounts of other folates including folic acid, tetrahydrofolic acid and 5,10 methylenetetrahydrofolic acid. The latter two folate compounds occur naturally in the body;

(b) Breakdown and oxidation products (without folate activity) including 4-aminobenzoylglutamic acid, 5-methyltetrahydropteroic acid, 4α-hydroxy-5-methyltetrahydrofolatic acid and a pyrazino-s-triazine derivative. The first three compounds have been identified in food as degradation products of endogenous folate. 4α-Hydroxy-5-methyltetrahydrofolic acid has been detected in the urine of rats as a metabolite of orally administered folic acid; and

(c) Other related compounds including D-5-MTHF-Ca [the (6R)-diastereoisomer] and the dimethylated form of tetrahydrofolic acid.

Maximum limits of these impurities in commercial L-MTHF-Ca have been established. Impurities listed above in (a) and (b) are not more than 2.5% of the product. The D- form is not more than 1%. The content of the L- and D- forms and other folates are determined by two different HPLC methods using pure L-MTHF-Ca (Merck Eprova AG) as a reference standard. The first analysis separates 5-MTHF (L- and D- forms) from other folates; the second analysis separates L-MTHF from the D- form.

Toxicological Assessment

Absorption, distribution, metabolism and excretion

Folates are coenzymes that function in the transfer, oxidation and reduction of one carbon units involved in the metabolism of certain amino acids (serine, glycine, methionine and histidine), methionine regeneration and synthesis of nucleic acids (Baily & Gregory 1999). As humans (and other mammals) cannot synthesise folates, they must be obtained via the diet. In comparison to folic acid, the bioavailability of natural folates is ≤50% (Sauberlich et al 1987).

While folic acid (pteroylmonoglutamic acid) contains only one glutamate residue, natural dietary folates typically have 2-7 glutamate moieties per molecule (Brouwer et al 2001). The absorption of folates from the digestive tract is dependent on them being in the monoglutamated form and therefore polyglutamyl folates must first be enzymatically deconjugated by γ-glutamyl hydrolase [Enzyme Classification (EC) No. 3.4.19.9; also known as folate conjugase], present on the intestinal brush border and in certain fresh vegetables, to a monoglutamyl form (Brouwer et al 2001; Gregory et al 1992; Konings et al 2002; Leichter et al 1979). However, before monoglutamated forms of folic acid can enter the portal circulation they are reduced to tetrahydrofolate (THF) and then either methylated or formylated during passage through mucosal cells in the jejunum (Brouwer et al 2001). The length of the glutamyl side-chain is therefore a key factor in the absorption efficiency of folates, with the polyglutamate chain shown to reduce bioavailability relative to monoglutamyl folic acid (Melse-Boonstra et al 2004). For example, the bioavailability of folic acid is approximately >90% (Lin et al 2004), while the bioavailability of polyglutamyl folic acid is approximately 60-70% (Melse-Boonstra et al 2004).

As folates are hydrophilic molecules they show minimum potential to cross cell membranes by diffusion (Hou et al 2005). Therefore, specific uptake from the digestive tract occurs by saturable carrier-mediated transport via the highly-specific reduced folate carrier, which is expressed on the brush border membranes of intestinal cells (predominantly in the jejunum) (Selhub & Rosenberg 1981).

Following saturation of the reduced folate carrier, folates can also be absorbed via a non-saturable mechanism involving passive diffusion (Selhub & Rosenberg 1981). In adults, ingestion of greater than 200 µg folic acid as a single bolus dose saturated the conversion process as shown by the presence of unmodified free folic acid in plasma (Kelly et al 1997).

Irrespective of whether ingested food contains natural or synthetically-produced folates, dietary folates absorbed into the intestinal mucosal cells are metabolised into L-5-MTHF on uptake. Synthetically produced L-5-MTHF is absorbed directly and is then metabolically indistinguishable from any other absorbed folates.

Once absorbed into the portal circulation, folates are transported to the liver, excreted into the bile, reabsorbed and then distributed back to the liver and other tissues (Steinberg et al 1979). In fact the slower appearance of monoglutamyl folates in the plasma following administration of folic acid relative to natural folates has been attributed to the high proportion of folates (~70%) entering the enterohepatic circulation and being stored in the liver (Kok et al 2004; Wright et al 2003).

The uptake of circulating L-5-MTHF (monoglutamate) into various tissues occurs via the reduced folate carrier and two high affinity receptors that mediate cellular uptake by endocytosis (Qiu et al 2006). Once inside the cell, the metabolically inactive L-5-MTHF is demethylated to tetrahydrofolate (THF) by the enzyme methionine synthase (EC 2.1.1.13), producing methionine from homocysteine in the process; THF is then available to participate in DNA biosynthesis (Kelly et al 1997).

Excretion of oxidised, free and conjugated folates occurs via the bile or urine. The major urinary catabolite is p-aminobenzoylglutamic acid in both the free and acetylated form (Kownacki-Brown et al 1993; Lin et al 2004). Other urinary metabolites in humans following administration of folic acid include N10-formyl folate, N-5-MTHF and pteroylglutamic acid (McLean & Chanarin 1966).

Toxicity

Animal data

The applicant submitted a number of unpublished *in vivo* and *in vitro* toxicity studies on L-MTHF and some of the impurities generated during the manufacturing process, which are summarised in the Tables below. The full evaluation of these studies is at attachment A. In addition, the applicant summarised the results of unpublished toxicity studies conducted on a racemic mixture of 50% L-MTHF and 50% and D-5-MTHF-Ca, which were submitted to the FDA as part of the approval process for its use as a dietary supplement; the results of these studies are also provided in a Table below.

Human data

Human data pertinent to the bioavailability and bioequivalence of L-MTHF and folic acid have been evaluated in the Nutrition Assessment (see Attachment 4). These data indicated that L-MTHF is bioequivalent to folic acid, with some suggestion that it may be marginally more bioavailable.

A number of studies have investigated the absorption/metabolism of L-MTHF or L-5-MTHF and/or the effects of supplementation on certain health endpoints (for examples see Bostom et al 2000; Venn et al 2002; Venn et al 2003; de Meer et al 2005; Lamers et al 2006). While these studies were not specifically designed to assess toxicity *per se*, the absence of adverse effects provides additional assurances of safety. In particular, Bostom et al (2000) administered repeated high oral dose of L-5-MTHF (17 mg/day) to a group of 25 haemodialysis patients for 12 weeks without any evidence of adverse health effects.

While there are reports in the scientific literature regarding hypersensitivity to folic acid (it is contraindicated in persons receiving folic acid therapy) there is no known hypersensitivity to the natural folates, including L-5-MTHF.

Results of toxicity studies on L-MTHF

Study Type	Species/Strain	Dose levels	Results	Reference
Acute (oral)	Rat (Wistar) HsdCpb:WU	2000 mg/kg bw	No deaths or clinical signs LD_{50} > 2000 mg/kg bw	Heusener & von Eberstein (1998a)
Subchronic (oral)	Rat Hanlbm:WIST	0, 25, 100 & 400 mg/kg bw/d for 13 weeks	No treatment-related effects at any dose. NOEL = 400 mg/kg bw/d	Hamann et al (2001)
Developmental (oral)	Rat (Wistar) HsdCpb:WU	0, 100, 300 & 1000 mg/kg bw/d on days 5-19 of gestation	No maternotoxicity, foetotoxicity or developmental toxicity at any dose NOEL = 1000 mg/kg bw/d	Schubert et al (2003)
Reverse mutation (Ames test)	Bacteria (*Salmonella typhimurium*) TA 98, 100, 102, 1535 &1537 Bacteria (*Escherichia coli*) WP2 uvrA pkM101	5-5000 µg/plate ± metabolic activation with S9 mix	Negative	Utesch (1999a-e)
Forward mutation	Mouse lymphoma cells L5178Y $TK^{(+/-)}$	5-5000 µg/plate ± metabolic activation with S9 mix	Negative	Utesch (2000a)
Unscheduled DNA synthesis	Rats (Han Wistar) [Crl:WI (GIx/BRL/Han) Br]	0, 800 & 2000 µg/kg bw (oral)	Negative	Howe (2002)
Micronucleus test	Rats (Wistar) HsdCpd:WU	2000 mg/kg bw (oral)	Negative	Utesch (2000b)

NOEL = No-Observed-Effect-Level

Results of toxicity studies on a racemic mixture of L- and D-5-MTHF-Ca

Study Type	Species/Strain	Dose levels	Results
Acute (oral)	Rat	5000 mg/kg bw	No deaths. Transient reaction to treatment. LD_{50} > 5000 mg/kg bw
4-week (oral)	Rat	0, 40, 120 & 360 mg/kg bw/d	NOEL = 360 mg/kg bw/d
	Dog		NOEL = 120 mg/kg bw/d, based on decreased food consumption at 360 mg/kg bw/d
Subchronic (oral)	Rat	0, 40, 120 & 360 mg/kg bw/d	Reduced bodyweight gain at 120 & 360 mg/kg bw/d. Elevated serum glucose at 360 mg/kg bw/d.
	Dog	0, 20, 60 & 180 mg/kg bw/d	NOEL = 180 mg/kg bw/d
2-generation reproduction (oral)	Rat	0, 40, 120 & 360 mg/kg bw/d	NOEL = 360 mg/kg bw/d
Developmental (oral)	Rat	0, 50, 150 & 450 mg/kg bw/d	NOEL = 450 mg/kg bw/d
	Rabbit		Lower bodyweight gain at 450 mg/kg bw/d during treatment. NOEL = 450 mg/kg bw/d
Developmental toxicity (oral peri/postnatal)	Rat	0, 40, 120 & 360 mg/kg bw	Slightly lower bodyweight gain during lactation at 360 mg/kg bw/d
Reverse mutation (Ames test)	Bacteria (*Salmonella typhimurium*)	1, 10, 100 & 1000 µg/plate ± metabolic activation with S9 mix	Negative
Forward mutation	Mouse lymphoma cells	10, 100, 500 & 1000 µg/plate ± metabolic activation with S9 mix	Negative
Chromosomal aberration	Human lymphocytes	1, 10, 100 & 800 µg/plate	Negative
Unscheduled DNA synthesis	HeLa cells	0, 10, 100 & 2000 µg/mL plate ± metabolic activation with S9 mix	Negative
Micronucleus test	Rats	700 mg/kg bw (ip)	Negative

NOEL = No-Observed-Effect-Level; ip = intraperitoneal

Results of toxicity studies on manufacturing impurities[1]

Study Type	Species/Strain	Dose levels	Results	Reference
Acute (oral)	Rat (Wistar) HsdCpb:WU	2000 mg/kg bw	No deaths or clinical signs LD50 > 2000 mg/kg bw	Heusener & von Eberstein (1998c-e)
Reverse mutation (Ames test)	Bacteria (*Salmonella typhimurium*) TA 98, 100, 102, 1535 &1537 Bacteria (*Escherichia coli*) WP2 uvrA pkM101	5-5000 µg/plate ± metabolic activation with S9 mix	Negative	Utesch (1999a-e)

1 = D-5-MTHF-Ca, L-Mefox-Ca (the s-triazine oxidation product of L-5-MTHF) & L-MTHPA-Ca (the hydrolysis product of L-5-MTHF)

Assessments by other agencies

Joint FAO/WHO Expert Committee on Food Additives (JECFA)

The safety of L-MTHF as an alternative vitamin form of folate was evaluated by JECFA at its 65th Meeting. The Committee concluded that there were no safety concerns for its use in dry crystalline or microencapsulated form as an alternative to folic acid used in dietary supplements, foods for special dietary uses and other foods on the basis that: (1) L-MTHF was considered to follow the same absorption and metabolic pathway as the natural folates; and (2) L-MTHF was considered to be bioequivalent to folic acid (WHO 2006).

The European Food Safety Authority (EFSA)

EFSA (via the Scientific panel on Food Additives, Flavourings, processing Aids and Materials in Contact with Food) evaluated the safety of L-MTHF as an alternative to folic acid in solid, semi-liquid or liquid dietary supplements, foods for a particular use or regular foods. EFSA concluded that the use of L-MTHF, with a tolerable upper level of 1 mg/adult person/day is not a safety concern.

US Food and Drug Administration (FDA)

The FDA (through the Centre for Food Safety and Applied Nutrition) conducted a scientific evaluation of L-5-MTHF as part of a 75-day premarket notification and concluded that there were no safety concerns with regard to its use as a dietary supplement (FDA 1998).

Discussion

Once ingested, L-MTHF would readily dissociate to Ca^{2+} and L-5-MTHF in the aqueous environment of the digestive tract. L-5-MTHF, like all folates, is then absorbed across the small intestine by carrier-mediated transport and is then indistinguishable from all other absorbed folates (including folic acid).

In fact, all absorbed folates are metabolised to L-5-MTHF by mucosal cells in the intestinal brush border. Therefore, L-5-MTHF can be considered the point of convergence for all absorbed dietary folates, whether synthetic or naturally-derived.

The toxicological database for L-MTHF is adequate and includes a number of published and unpublished studies in laboratory animals. There are also a number of human studies, which have examined the metabolism of L-5-MTHF relative to folic acid or the effects on certain health endpoints, such as homocysteine. Recent human studies on the bioavailability of L-MTHF *versus* folic acid have been reviewed in the Nutrition Assessment and indicate that L-MTHF is bioequivalent to folic acid in humans.

Laboratory animal studies indicated that L-MTHF has very low acute oral toxicity (LD_{50} >2000 mg/kg bw/d) and low repeat-dose toxicity (NOEL = 400 mg/kg bw/d). There was no evidence of developmental toxicity in rats up to a dose of 1000 mg/kg bw/d. There was no evidence that L-MTHF is genotoxic. Studies conducted on a number of manufacturing impurities and a racemic mixture of D/L-MTHF confirm the low toxicity potential of the test material. While the folate status of the test animals was not reported in any of the studies, it is likely that at such high doses, saturation of the active uptake mechanism would have occurred.

References

Baily LB & Gregory JF (1999) Folate metabolism and requirements. J Nutr. **129**: 779-782

Bostom AG, Shemin D, Bagley P, Massy ZA, Zanabli A, Chritopher K, Speigel P, Jacques PF, Dworkin L & Selhub J (1999) Controlled comparison of L-methyltetrahydrofolate versus folic acid for treatment of hyperhomocysteinemia in hemodialysis patients. Circulation **101**: 2829-2832.

Brouwer IA, van Dusseldorp M, West CE & Steegers-Theunissen RPM (2001) Bioavailability and bioefficacy of folate and folic acid in man. Nutrition Research Reviews **14**: 267-293.

De Meer K, Smulders YM, Dainty JR, Smith DEC, Kok RM, Stehouwer CDA, Finglas PM & Jakobs C (2005) [6S]-5-methyltettrahydrofolate or folic acid supplementation and absorption and initial elimination of folate in young and middle-aged adults. European Journal of Clinical Nutrition **59**: 1409-1416

FDA (1998) 5-Methytetrahydrofolate: 75-Day premarket notification for new dietary ingredients http://www.fda.gov/OHRMS/DOCKETS/DOCKETS/95s0316/rpt0023_01.pdf

Gregory JF, Bhandari S, Baily LB, Toth JP, Baumgartner TG & Cerda JJ (1992) Relative bioavailability of deuterium-labeled monoglutamyl tetrahydrofolates and folic acid in human subjects. Am J Clin Nutr **55**: 1147-53

Hamman H-J, Luetkemeir H, Knuppe C & Millar PM (2001) Calcium-L-mefolinate (L-MTHF): 13-week oral toxicity (gavage) study in Wistar rats. RCC Project No. 758316. Lab: RCC Ltd, Toxicology Division, Itingen, Switzerland) Sponsor: Merck KgaA, Darmstadt, Germany. Unpublished study report.

Heusener A & Eberstain M (1998a) Calcium-L-mefolinat – Acute toxicity study in rats after oral administration. Lab: Institute of Toxicology, Merck KGaA, Darmstadt, Germany. Sponsor: Eprova AG, Schaffhausen, Switzerland. Unpublished study report.

Heusener A & Eberstain M (1998b) Calcium-D/L-mefolinat – Acute toxicity study in rats after oral administration. Lab: Institute of Toxicology, Merck KGaA, Darmstadt, Germany. Sponsor: Eprova AG, Schaffhausen, Switzerland. Unpublished study report.

Heusener A & Eberstain M (1998c) Calcium-D-mefolinat – Acute toxicity study in rats after oral administration. Lab: Institute of Toxicology, Merck KGaA, Darmstadt, Germany. Sponsor: Eprova AG, Schaffhausen, Switzerland. Unpublished study report.

Heusener A & Eberstain M (1998d) Calcium-L-mefox – Acute toxicity study in rats after oral administration Lab: Institute of Toxicology, Merck KGaA, Darmstadt, Germany. Sponsor: Eprova AG, Schaffhausen, Switzerland. Unpublished study report.

Heusener A & Eberstain M (1998e) Calcium-L-MTHPA – Acute toxicity study in rats after oral administration Lab: Institute of Toxicology, Merck KGaA, Darmstadt, Germany. Sponsor: Eprova AG, Schaffhausen, Switzerland. Unpublished study report.

Howe J (2002) Art 100461 (Calcium-L-mefolinate): Measurement of unscheduled DNA synthesis in rat liver using an *in vivo*/*in vitro* procedure. Lab: Covance Laboratories Ltd, Harrogate, North Yorkshire, England. Sponsor: Merck KgaA, Darmstadt, Germany. Unpublished study report.

Hou Z, Stapels SE, Haska CL & Matherly (2005) Localisation of substrate binding domain of the human reduced folate carrier to transmembrane domain 11 by radioaffinity labelling and cysteine-substituted accessibility methods. Journal of Biological Chemistry **280**: 36206-36213

Kelly P, McPartlin J, Goggins M, Weir DG & Scott JM (1997) Unmetabolized folic acid in serum: acute studies in subjects consuming fortified food and supplements. Am J Clin Nutr **65**:1790-5

Kok RM, Smith DEC, Dainty JR, van den Akker JT, Finglas PM, Smulders YM, Jakobs C & de Meer K (2004) 5-Methyl hydrofolic acid and folic acid measured in plasma with liquid chromatography tandem mass spectrometry: applications to folate absorption and metabolism. Analytical Biochemistry **326**: 129-138

Kownacki-Brown PA, Wang C, Bailey LB, Toth JP & Gregory III JF (1993) Urinary excretion of deuterium-labeled folate and the metabolite p-aminobenzoylglutamate in humans. J Nutr. **123**: 1101-1108

Leichter J, Landymore AF & Krumdieck (1979) Folate conjugase activity in fresh vegetables and its effect on the determination of free folate content. The American Journal of Nutrition 32: 92-95

Lin Y, Dueker SR, Follet JR, Fadel JG, Arjomand A, Schneider PD, Miller JW, Green R, Buchholz BA, Vogel JS, Phair RD & Clifford AJ (2004) Quantitation of in vivo human folate metabolism. Am J Clin Nutr **80**: 680-91

Lamers Y, Prinz-Langenohl R, Brämswig S & Pietrzik K (2006) red blood cell folate concentrations increase more after supplementation with [6]-5-methyltetrahydrofolate than with folic acid in women of child bearing age. American Journal of Clinical Nutrition **84**: 156-161

McLean A & Chanarin L (1966) urinary excretion of 5-methyl-tetrahydrofolate in man. Blood 27(3) 386-388.

Melse-Boonstra A, West CE, Katan MB, Kok FJ & Verhoef (2004) Biovailability of heptaglutamyl relative to monoglutamyl folic acid in health adults. Am J Clin Nutr **79**: 424-9.

NHMRC (2006) Nutrient reference values for Australia and New Zealand. http://www.nhmrc.gov.au/publications/synopses/_files/n35.pdf

Sauberlich HE, Kretsch MJ, Skala JH, Johnson HL & Taylor PC (1987) Folate requirements and metabolism in nonpregnant women. Am J Clin Nutr **46**: 1016-28.

Schlede E, Mischke U, Diener W & Kayser D (1995) The international validation study of the acute toxic class method (oral). Archives of Toxicology **69**: 659-670.

Schubert Ch, Broschard T & Jacobs M (2003) Art 100461 (Metafolin) – Prenatal developmental toxicity study after oral administration to rats. Lab: Merck KGaA, Institute of Toxicology, Darmstadt, Germany. Sponsor: Merck Eprova AG, Schaffhausen, Switzerland. Unpublished study report.

Selhub J & Rosenberg IH (1981) Folate transport in isolated brush border membrane vesicles from rat intestine. Journal of Biological Chemistry **256**(9): 4489-4493

Steinberg SE, Campbell CL & Hillman RS (1979) Kinetics of the normal folate enterohepatic cycle J. Clin Invest. **64**: 83-88

Utesch D (1999a) Calcium-L-Mefolinat – Bacterial mutagenicity assay, Salmonella typhimurium and Escherichia coli. Lab: Institute of Toxicology, Merck KGaG, Darmstadt, Germany. Sponsor: Eprova AG, Schaffausen, Switzerland. Unpublished study report.

Utesch D (1999b) Calcium-D/L-Mefolinat – Bacterial mutagenicity assay, Salmonella typhimurium and Escherichia coli. Lab: Institute of Toxicology, Merck KGaG, Darmstadt, Germany. Sponsor: Eprova AG, Schaffausen, Switzerland. Unpublished study report.

Utesch D (1999c) Calcium-D-Mefolinat – Bacterial mutagenicity assay, Salmonella typhimurium and Escherichia coli. Lab: Institute of Toxicology, Merck KGaG, Darmstadt, Germany. Sponsor: Eprova AG, Schaffausen, Switzerland. Unpublished study report.

Utesch D (1999d) Calcium-L-Mefox – Bacterial mutagenicity assay, Salmonella typhimurium and Escherichia coli. Lab: Institute of Toxicology, Merck KGaG, Darmstadt, Germany. Sponsor: Eprova AG, Schaffausen, Switzerland. Unpublished study report.

Utesch D (1999e) Calcium-L-MTHPA – Bacterial mutagenicity assay, Salmonella typhimurium and Escherichia coli. Lab: Institute of Toxicology, Merck KGaG, Darmstadt, Germany. Sponsor: Eprova AG, Schaffausen, Switzerland. Unpublished study report.

Utesch D (2000a) Calcium-L-mefolinat – in vitro mammalian cell gene mutation test (L5178Y/TK$^{+/-}$). Lab: Institute of Toxicology, Merck KGaG, Darmstadt, Germany. Sponsor: Eprova AG, Schaffausen, Switzerland. Unpublished study report.

Utesch D (2000b) Calcium-L-mefolinat – micronucleus test in male rats after oral administration. Lab: Institute of Toxicology, Merck KGaG, Darmstadt, Germany. Sponsor: Eprova AG, Schaffausen, Switzerland. Unpublished study report.

Venn BJ, Green TJ, Moser R, Mckenzie JE, Skeaff M & Mann JI (2002) Increases in blood folate indices are similar in women of childbearing age supplemented with [6S]-5-methyltetrahydrofolate and folic acid. Journal of Nutrition **132**: 3353-3355

Venn BJ, Green TJ, Moser R & Mann JI (2003) comparison of the effect of low-dose supplementation with L-5-methyltetrahydrofolate or folic acid on plasma homocysteine: a randomized placebo-controlled study. American Journal of Clinical Nutrition **77**: 658-62

WHO (2006) Evaluation of certain veterinary drug residues in food (Sixty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series No. 934.

Wright AJA, Finglas PM, Dainty JR, Hart DJ, Wolfe CA, Southon S & Gregory JF (2003) Single oral doses of ^{13}C forms of pteroylmonoglutamic acid and 5-formyltetrahydrofolic acid elicit differences in short-term kinetics of labelled and unlabelled folates in plasma: potential problems in interpretation of folate bioavailability studies. British Journal of Nutrition. **90**: 363-371

Appendix A

Evaluation of submitted toxicity studies

Acute toxicity studies

Heusener and von Emerstein (1998a-e) conducted a series of acute oral toxicity studies in HsdCpd rats (7-8 weeks old; 156-181 g bodyweight; sourced from Winkelmann, Borchen, Germany), which were dosed with L-MTHF (97% purity; Batch No. ESF-118) and a number of manufacturing impurities and oxidation products [D/L-MTHF (96.2% purity; Batch No. ESC-008); D-5-MTHF-Ca (97.3% purity; Batch No. ESL-101); L-Mefox-Ca (97.5% purity; Batch No. ESJ-102-B) and MTHPA-Ca (98.6% purity; Batch No. ESK-103)]. All test substances were sourced from Eprova AG, Switzerland and were administered in an aqueous vehicle of hydroxypropyl methylcellulose (Methocel®). These studies were reportedly performed according to OECD Test Guideline 423 and the Acute Toxic Class Method (Schlede et al 1995), with statements of Good Laboratory Practice (GLP) and Quality Assurance (QA) provided. Three fasted rats/sex received a single oral gavage dose of 2000 mg/kg bw test substance and then observed for 14 days. There were no deaths or clinical signs and all rats gained weight over the observation period. No gross abnormalities were observed at necropsy. The LD_{50} for all test substances was > 2000 mg/kg bw.

Subchronic toxicity study

Experimental: Haman et al (2001) administered L-MTHF (97.1% purity; Batch No. LMCA-7077; sourced from Merck KgaA, Germany) in distilled water by oral gavage to groups of 10 Hanlbm:WIST rats/sex (6-weeks old; bodyweight range of 118-162 g for males and 112-142 g for females; sourced from RCC Ltd, Switzerland) at doses of 0, 25, 100 or 400 mg/kg bw/day for 13 weeks. Satellite groups of 5 rats/sex were dosed with the test substance at 0 or 400 mg/kg bw/d for 13 weeks followed by a 4-week recovery period. The study was reportedly performed according to OECD Test Guideline 408 and Directive 96/54/EC B 26. A statement of compliance with OECD and Swiss principles of GLP were provided in addition to a QA statement.

Observations for mortalities and clinical signs were recorded twice daily. Bodyweight and food consumption were recorded prior to treatment and on a weekly basis thereafter. At week 13, a Functional Observational Battery (FOB) was performed in addition to an assessment of locomotor activity and grip strength. Ophthalmoscopic examinations were performed pre-test and at weeks 7, 13 and 17. Fasted blood and urine samples were collected pre-treatment and at weeks 13 and 17 for analysis of the standard range of haematology, clinical chemistry and urinary parameters. Following sacrifice (at weeks 13 or 17), all rats were necropsied and their organs weighed: The standard range of tissues were histopathologically examination. Data were statistically analysed by a Dunnett-test, Steel-test, Student's t-test or a Fisher's exact test.

Findings: There were no treatment-related deaths or clinical signs. The occurrence of beige to yellow-coloured faeces at 100 and 400 mg/kg bw/d was attributable to the colour of the test material and not considered toxicologically-significant. There was no treatment-related effect on bodyweight gain or food consumption. There were no ophthalmoscopic abnormalities that were attributable to treatment. The FOB was unremarkable.

At week 13, significantly reduced ($p<0.01$ or 0.05) hindlimb grip strength occurred in females at 100 and 400 mg/kg bw/d but was not considered treatment-related due to the absence of a dose-response effect, an effect on forelimb grip strength, a similar effect in males or other signs of weakness.
There was no difference in grip strength between high-dose females and the control following the 4-week recovery period. Significant increases *and* decreases in locomotor activity relative to the control groups occurred in males and females at 100 and 400 mg/kg bw/d but due to the fluctuating nature of the findings they were considered incidental.

There was no treatment-related effect on any haematology or urinary parameters. There was a treatment-related reduction in creatinine kinase at week 13 (7.73, 4.20, 4.36 and 3.55 kat/L at 0, 25, 100 and 400 mg/kg bw/d, respectively), which was statistically significant at 25 and 400 mg/kg bw ($p<0.05$ and 0.01, respectively). No such effect was seen in high-dose males following the 4-week recovery period. CK is an enzyme primarily found in heart, skeletal muscle and brain and therefore an increase in blood levels generally indicates damage to these tissues. Therefore a decrease in CK actually has no toxicological significance. In addition, all treated groups fell within the historical control range for age-matched rats (1.42-5.26 kat/L) and therefore the result reflects an anomalous control group reading. There were no other treatment-related effects on any clinical chemistry parameters. There were no treatment-related macroscopic or microscopic abnormalities and no effect on organ weights.

The NOEL was 400 mg/kg bw/d based on the absence of any treatment-related toxicological effect at this dose.

Developmental toxicity study

Schubert et al (2003) administered L-MTHF (99.9% purity; Batch No. LMCA-7290; sourced from Merck Eprova AG, Switzerland), in aqueous 0.25% hydroxypropyl methylcellulose, by oral gavage to groups of 25 pregnant wistar rats (strain Hsd:Cpd; 12-13 weeks old; bodyweight range of 181-231 g; sourced from Harlan Winkelmann, Borchen, Germany) at doses of 0, 100, 300 or 1000 mg/kg bw from days 5-19 of gestation. A statement of compliance with OECD, German and EU principles of GLP were provided in addition to a QA statement. Clinical signs, body weight, and food and water consumption were monitored at regular intervals to day 20 of gestation when dams were sacrificed. Gravid uterine weights and the number of copora lutea, live and dead foetuses and complete, early and late resorptions were recorded. Foetuses were sexed, weighed and examined for visceral and skeletal abnormalities. Data were statistically analysed using the Dunnett test or Fisher-Pitman permutation test.

All dams survived to the end of the study. There were no treatment-related clinical signs and no differences in bodyweight gain and food consumption between control and treated dams. There was a significant ($p<0.05$), albeit slight increase in water consumption in high-dose dams relative to the control from day 15 of gestation. However, given that this group was already consuming more water than the control prior to treatment commencing, this finding was not considered treatment-related. There were no gross abnormalities detected in dams at necropsy. There was no evidence of foetal or developmental toxicity. The NOEL for maternal, foetal and developmental toxicity was 1000 mg/kg bw/d, the highest dose tested, based on the absence of any toxicological effects at this dose.

Genotoxicity studies

Reverse mutation in bacteria (Ames test)

Utesch (1999a-e) examined the mutagenicity of L-MTHF (97% purity; Batch No. ESF-118) and a number of manufacturing impurities and oxidation products [D/L-MTHF (96.2% purity; Batch No. ESC-008); D-5-MTHF-Ca (97.3% purity; Batch No. ESL-101); L-Mefox-Ca (97.5% purity; Batch No. ESJ-102-B) and MTHPA-Ca (98.6% purity; Batch No. ESK-103)] via the Ames test with and without an exogenous source of metabolic activation (S9 mix from rat liver homogenates) using *S. typhimurium* strains TA98, TA100, TA102, TA1535 and TA1537 and *E. coli* WP2 uvr A pKM101. A statement of compliance with principles of GLP were provided in addition to a QA statement. All test substances were sourced from Eprova AG, Switzerland and were administered in water. Tested concentrations were 5, 15.8, 50, 158, 500 and 1580 µg/plate, and 50, 158, 500, 1580 and 5000 µg/plate in the repeat experiment. At concentrations > 500 µg/plate, the test material was applied as a suspension. None of the test compounds caused cytotoxicity or an increase in the number of revertants relative the solvent control. Positive control compounds gave expected results (2-aminoanthracene and benzo[a]pyrene, with daunomycin, N-ethyl-N'-nitro-N-nitroguanidine, 9-aminoacridine and cumene hydroperoxide used as strain specific controls). Under the experimental conditions, neither L-MTHF nor its main impurities and oxidation products were mutagenic.

Forward mutation in eukaryotic cells

Utesch (2000a) examined the mutagenicity of L-MTHF (Batch No. LMCA-7077; sourced from Eprova AG, Switzerland) in the Mouse Lymphoma Thymidine Kinase Gene Mutation Assay (TK locus test). A statement of compliance with principles of GLP were provided in addition to a QA statement. Three independent experiments were conducted with and without an exogenous source of metabolic activation (S9 mix from rat liver homogenates) at concentrations of 50, 158, 500, 1580, 2810 and 5000 µg/mL: Cell culture media was used as the solvent. Precipitation of the test material occurred above 158 or 500 µg/mL. In the absence of S9 mix, cytoxicity occurred at and above 158 µg/mL, with a concomitant 1.8-3.4-fold increase in the frequency of mutants at the two highest concentrations. In the presence of S9 mix, no cytotoxicity occurred and there was no increase in the number of mutants relative to the negative control. Positive control compounds gave expected results (benzo[a]pyrene and 4-nitroquinoline N-oxide). Under the experimental conditions, L-MTHF was not mutagenic in the absence of frank cytotoxicity.

In vivo DNA synthesis and repair test

Howe (2002) tested the ability of L-MTHF (99.9% purity; Batch No. LMCA-7290; to induce unscheduled DNA synthesis (UDS) in rat liver. A statement of compliance with UK and OECD principles of GLP were provided in addition to a QA statement. Groups of four male Han Wistar rats [Crl:WI (GLx/BRL/Han) BR strain; sourced from Charles River Laboratories, UK] were given a single oral gavage dose of the test material at 800 or 2000 mg/kg bw in aqueous 0.25% hydroxypropyl methylcellulose. Additional groups of four rats were dosed with either 2-acetamido-fluorene (75 mg/kg bw) or dimethylnitrosamine (10 mg/kg) bw as positive controls. Two independent experiments were conducted. Rats were killed after 12-14 (Experiment 1) or 2-4 hours (Experiment 2), their livers removed and primary cultures of hepatocytes established for the analysis of UDS.

There were no signs of toxicity in any rat. There was no difference in the net grain count between treated and control rats, with positive controls giving the expected results. This study demonstrated that L-MTHF at up to 2000 mg/kg bw did not induce UDS under these experimental conditions.

In vivo micronucleus test

Utesch (2000b) administered a single gavage dose of L-MTHF (Batch No. LMCA-7077; sourced from Eprova AG, Switzerland) in aqueous 0.25% hydroxypropyl methylcellulose to 10 male wistar rats (HsdCpb:WU strain; 8-10 weeks old; sourced from Harlan Winkelmann GmbH) at a single dose of 2000 mg/kg bw in a standard micronucleus test (reportedly conducted according to OECD Test Guideline 474, Commission Directive 92/69/EEC and the ICH guidelines). At 24 or 48 hours after dosing, rats were killed and bone marrow smears prepared and erythrocytes examined for the presence of micronuclei. Cyclophosphamide served as the positive control and gave the expected results. There was no observed increase in micronucleated polychromatic erythrocytes and therefore L-MTHF does not cause cytogenetic damage.

Attachment 4

Dietary Intake Assessment report

A566 – L-5-methyltetrahydrofolate, calcium salt as a permitted vitamin form of folate

1. Introduction

Folates that occur naturally in foods are susceptible to oxidation and losses can occur during food processing, manufacturing and storage. Whilst procedures can be implemented during food processing operations to minimise these losses, fortification of foods with folate can compensate for the losses and assist in maintaining adequate daily intakes. Currently, folic acid is the only synthetic form of folate permitted in the Code for the fortification of foods.

In considering L-MTHF as an alternative synthetic form of folate for fortification purposes, a dietary intake assessment was undertaken as part of the risk assessment. Dietary intake estimates are usually compared to reference health standards for risk characterisation purposes. In the case of nutrients, the reference health standards are the National Health and Medical Research Council (NHMRC) Nutrient Reference Values (NRVs). NRVs are sets of recommendations for nutritional intake based on current available scientific knowledge for each nutrient as required. These reference values include the Estimated Average Requirement (EAR), Recommended Dietary Intake (RDI), Adequate Intake (AI) (used when an RDI cannot be determined), Estimated Energy Requirement (EER) and the Upper Level of Intake (UL).

The NRVs established for folates were the EAR and RDI or AI. ULs were not established for folates as no adverse effects were associated with the consumption of folates normally found in foods or fortified foods. However, ULs for folic acid were established as high supplemental intakes of folic acid have been shown to be related to adverse neurological effects in people with vitamin B12 deficiency (National Health and Medical Research Council, 2006).

2. Dietary modelling to estimate intakes of L-MTHF from food only

2.1 What is dietary modelling?

Dietary modelling is a tool used to estimate intakes of food chemicals from the diet as part of the FSANZ risk assessment process. To estimate dietary intake of food chemicals, records are needed of the foods people have eaten and also reports of how much of the food chemical of interest is in each food. The accuracy of these intake estimates depends on the quality of the data used in the dietary models. Sometimes all of the data needed are not available or the accuracy is uncertain so assumptions have to be made, either about the foods eaten or about chemical levels, based on previous knowledge and experience. The models are generally set up according to international conventions for food chemical intake estimates, however, each modelling process requires decisions to be made about how to set the model up and what assumptions to make; a different decision may result in a different answer. Therefore, FSANZ documents clearly all such decisions, model assumptions and data limitations to enable the results to be interpreted in the context of the data available and so that FSANZ risk managers can make informed decisions.

2.2 Dietary modelling approach

The dietary intake assessments discussed in this report were conducted using FSANZ's dietary modelling computer program, DIAMOND.

Dietary intake = food chemical concentration x food consumption amount

The intake of L-MTHF from the diet was estimated by combining usual patterns of food consumption, as derived from National Nutrition Survey (NNS) data, with current levels of fortification based on the uptake of voluntary fortification permissions by the food industry. An overview of the dietary modelling approach used to assess the intakes of L-MTHF is presented in Figure 1.

The dietary intake assessment for L-MTHF was based on the estimated current folic acid intakes from food alone, based on current uptake of voluntary folic acid permissions outlined in Standard 1.3.2 of the Code. The intake assessment assumed that there would be 100% replacement of folic acid with L-MTHF for those foods currently fortified and the resulting intakes of L-MTHF would be equivalent to those of folic acid. These dietary intake assessments did not take into account naturally occurring folates in food or L-MTHF from supplements or multivitamins.

For the purposes of the risk characterisation, the estimated intakes were compared with the UL for folic acid, as there is no specific UL for L-MTHF. There have been concerns regarding high supplemental intakes of folic acid, and as folic acid is being considered as a proxy for L-MTHF, the dietary intake assessment results were compared with the UL for folic acid.

Estimated intakes of L-MTHF were not compared to EARs for this assessment. This was because the application is considering voluntary fortification based on a substitution of one fortificant (folic acid) for another (L-MTHF), and therefore there is no need to assess whether there is a beneficial upward shift in population intakes as there is not expected to be one.

L-MTHF is in the form of a calcium salt. Therefore, a rough estimate of the impact on calcium intakes from fortifying foods with L-MTHF was developed.

2.3 Dietary survey data used

DIAMOND contains dietary survey data for both Australia and New Zealand; the 1995 NNS from Australia surveyed 13,858 people aged 2 years and above, and the 1997 New Zealand NNS surveyed 4,636 people aged 15 years and above.

Both of these surveys used a 24-hour food recall methodology. A second 24-hour recall was also collected on a subset of respondents in both surveys. Standard methodologies were used to estimate intake from a single 24 hour record (day one) and to adjust these records to estimate 'usual intake' by including information from a second 24 hour record (day two) (see Appendix 1*: How were the estimated dietary intakes estimated*).

It is recognised that these survey data have several limitations. For a complete list of limitations see Section 5*: Limitations*.

2.4 Population groups assessed

The dietary intake assessment was conducted separately for Australia and New Zealand population sub-groups.

As the Australian 1995 NNS was conducted on people aged 2 years and above, the following age groups were assessed: the population 2 years and above, 2-3 years, 4-8 years, 9-13 years, 14-18 years, 19-29 years, 30-49 years, 50-69 years and 70 years and above, and by gender. The New Zealand 1997 NNS was conducted on people aged 15 years and above so the following age groups were assessed: the population 15 years and above, 15-18 years, 19-29 years, 30-49 years, 50-69 years and 70 years and above, and by gender.

The NRVs for Australia and New Zealand (NHMRC, 2006) were used as a guide in selecting the age groups to assess. As different NRVs are determined for different age and gender groups, conducting the dietary intake assessments based on the NRV age groups allowed for a comparison of the estimated intakes with the relevant NRV for risk characterisation purposes.

2.5 Dietary modelling scenarios for assessing L-MTHF intakes

To estimate potential L-MTHF intakes from food alone, based on the current uptake of voluntary folic acid permissions by industry, two different model types were assessed:

(a) Market share model; and
(b) Consumer behaviour models.

The market share and consumer behaviour model types are discussed in detail below.

The intakes of L-MTHF were predicted for each population group assessed for Australia and New Zealand. This scenario only considers those voluntary fortification permissions (for folic acid) outlined in Standard 1.3.2 that have been taken up by industry, as evidenced by products available on the supermarket shelves. It does not include foods or food groups where voluntary fortification is permitted in the Code but has not been taken up by industry. It does not take into account naturally occurring L-MTHF in food or L-MTHF from the use of supplements or multivitamin supplements containing L-MTHF.

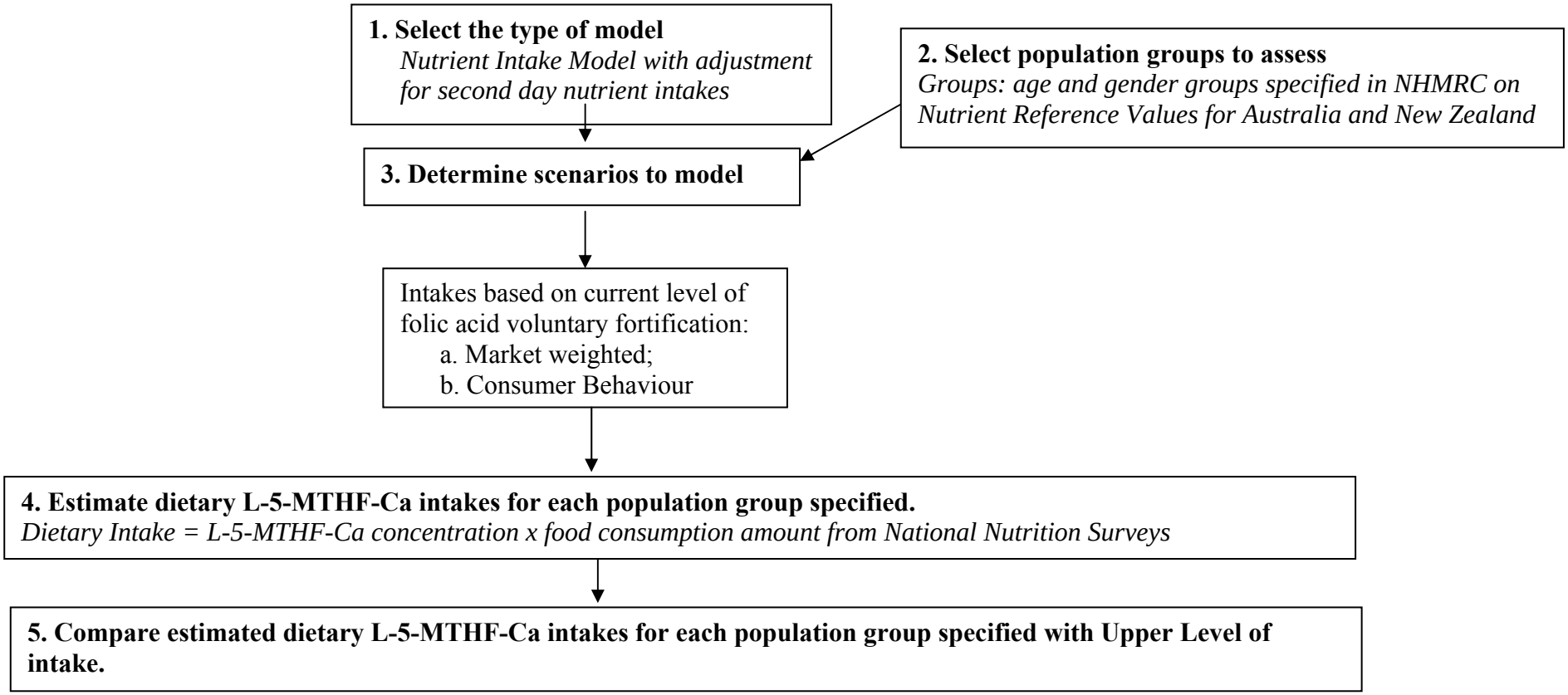


Figure 1: Dietary modelling approach used for the intake assessment for L-MTHF for the Australian and New Zealand population groups

Concentrations for foods voluntarily fortified with L-MTHF (using folic acid as a proxy) were derived from four major sources:

- unpublished FSANZ analytical data for samples purchased in Australia in 1997, 2005 and 2006; samples included in these analyses included a number of different types of common breakfast cereals, fortified breakfast juice and white bread;
- analytical data for samples purchased in New Zealand in 2003 and 2004 (Thomson, 2005); samples included in these analyses included breakfast cereals, juice, bread and food drinks;
- current label data for foods where no analytical values were available, without adjustment for potential under- or overages; and
- recipe calculation for foods that contain a fortified food as one of their ingredients (e.g. chocolate crackles that contain fortified puffed rice breakfast cereal).

Concentration data used for various foods for dietary modelling purposes were mainly based on the analytical data. The effect of cooking foods was also taken into account when constructing the concentration database. For example, when cooking bread to make toast, both losses from heat were taken into account along with concentration factors due to moisture losses when making bread into toast.

Information from the above mentioned four sources was matched against the 1995 Australian and 1997 New Zealand NNS food codes for all those foods identified as being fortified (149/4550 foods in Australia and 101/4950 foods in New Zealand). All other foods recorded as being consumed were assumed not to contain added L-MTHF. The lists of foods assumed to contain added L-MTHF are detailed in Appendix 2 (Table A2.1 for Australia and Table A2.2 for New Zealand).

2.5.1 Market share model (or population estimate)

This model aims to represent L-MTHF intakes for the average consumer i.e. reflects the typical patterns of dietary intakes over time for a whole population or population sub-group. A limitation of the market share model is that it only gives an estimate of population intakes over time. It can not predict individual behaviour or estimate L-MTHF intakes for individuals due to the use of weighted mean L-MTHF concentration values.

Weighted mean L-MTHF concentration levels were assigned to each food to reflect the current or predicted market share for fortified and unfortified products within each food category. If a fortified version of a food was not specifically identified within the NNS, but it was known that a significant proportion of the food category in the market place is now fortified, a concentration of L-MTHF was assigned to the food, and weighted to reflect the proportion of the market for that food that is now believed to be fortified. It is important to note that some foods in the NNSs were described as being folate fortified (e.g. certain breakfast cereals) therefore market weighted L-MTHF concentrations were not applied to these foods.

For example, the Australian NNS does not distinguish between the consumption of folic acid fortified white bread and unfortified white bread. The market share for folic acid fortified bread in Australia was estimated at 16% of all breads, based on sales information for a major bakery retail chain (Bakers Delight, 2006). A value representing 16% of the analysed or labelled concentration of folic acid in fortified breads was assigned to all white breads. Based on available information, fortification of breads with folic acid does not appear to be as common in New Zealand as in Australia, so different market weights were assigned.

2.5.2 Consumer behaviour model (or individual choices model)

A permission to voluntarily fortify some foods with L-MTHF presents the consumer with a choice, to avoid or positively select these foods according to their needs or those of their household. To reflect the potential differences in **individual** consumer behaviour, two options were investigated for these foods:

(a) where it was assumed that individuals would always avoid products that contain L-MTHF; and
(b) where it was assumed that individuals would always select products that contain L-MTHF.

This choice was applied to the foods reported as consumed in the NNS that did not have a sufficiently detailed description to determine whether the food was fortified or not with folic acid, and therefore L-MTHF. The model was limited as a consumer behaviour model as it was assumed that respondents ate as reported in the 1995 Australian National Nutrition Survey (NNS) and 1997 New Zealand NNS and did not change or substitute one kind of food for another. For example, it is important to note that some foods in the NNSs were described as being fortified (e.g. breakfast cereals), therefore the above options for consumer choice were not applied to these foods. The consumer behaviour models assess L-MTHF intake **for individuals only**, based on L-MTHF concentrations in certain foods. Where mean dietary L-MTHF intakes have been presented as a range, the lower bound represents option (a) and the upper bound represents option (b), as outlined above.

A limitation of this model type is that it is not a population estimate but rather gives the upper and lower ends of a range of possible intakes for an individual because it is not known how respondents in the NNS would actually have behaved had they been presented with a choice of products.

2.6 How were the estimated dietary L-MTHF intakes calculated?

A detailed explanation of how the estimated dietary intakes are calculated is at Appendix 1.

3. Assumptions used in dietary modelling

The aim of the dietary intake assessment is to make as realistic an estimate of dietary L-MTHF intake as possible. However, where significant uncertainties in the data existed, conservative assumptions were generally used to ensure that the dietary intake assessment did not underestimate intake.

The assumptions made in the dietary modelling are listed below, broken down by category.

3.1 Consumer behaviour

- People eat the same mass of bread today as they did in 1995/1997, when the NNS data were collected (see section 5 for further information).

3.2 Concentration Data

- Naturally-occurring sources of folate have not been included in the dietary intake assessment;
- if there were no Australian levels of fortification for specific foods, it was assumed that New Zealand data were representative of these food groups, and vice versa for New Zealand foods;
- if a food was not included in the intake assessment, it was assumed to contain a zero concentration of L-MTHF;
- a market share weighted L-MTHF value was assigned to food categories with voluntary permissions to fortify to reflect the proportion of products that have been fortified or, where possible, an analysis or label folic acid concentration was assigned to individual foods using up to date food composition data; and
- there was no contribution to L-MTHF intake through the use of complementary medicines (Australia) or dietary supplements (New Zealand).

3.3 General

- There are no reductions in L-MTHF concentrations due to cooking and storage; and
- for the purpose of this assessment, it is assumed that 1 millilitre is equal to 1 gram for all liquid and semi-liquid foods (e.g. orange juice).

4. Estimated dietary intakes of L-MTHF from fortification of certain foods

L-MTHF intakes were estimated for a broad range of population sub-groups as different NRVs were given to different age and gender groups. Conducting the dietary intake assessments based on the NRV age groups allowed for easy comparison of the estimated intakes with the relevant NRV for risk assessment purposes.

4.1 Estimated dietary L-MTHF intakes for population sub-groups

Comparisons between estimated mean L-MTHF intakes for population sub-groups are presented in Figure 2; the lower and upper ends of the range of mean L-MTHF intakes represent the results from the 'consumer behaviour' model – the lower bound indicates L-MTHF intakes for individuals who always avoid the products that contain L-MTHF; the upper bound indicates L-MTHF intakes for individuals who always select the products that contain L-MTHF. The results from the 'market share' model are indicated by the black line within the range of estimated L-MTHF intakes, and are representative of mean **population** intakes over a period of time. Generally, the results presented in this section refer to the 'market share' model results.

In the 'consumer behaviour' model, the estimated mean L-MTHF intakes for New Zealand did not show as large a range as for Australia. This could be due to differences in the uptake of voluntary fortification (for folic acid) between the two countries, being lower in New Zealand. The 'consumer behaviour' model results indicate that, for an individual who eats large amounts of the fortified foods and goes out of their way to select the fortified version wherever there is a choice, higher L-MTHF intakes can be achieved. However, it is considered that the number of consumers who would actually behave in this way on a regular basis is likely to be small.

The estimated mean dietary L-MTHF intakes for Australian and New Zealand population groups are shown in Table 2 and Figure 3. Full results can be found in Appendix 3 (Table A3.1 for Australia and Table A3.2 for New Zealand).

These results indicate that New Zealand population sub groups would have lower L-MTHF intakes compared to the same population group for Australia. This is due to fewer voluntary fortification permissions (for folic acid) being taken up by industry in New Zealand. One of the major areas of difference is that Bakers Delight bread, which represents approximately 15% of the bread market in Australia, is not fortified with folic acid in New Zealand, but is fortified in Australia.

4.2 Major contributors to L-MTHF intakes

The major contributors to L-MTHF intake (≥5%) were calculated for the general population for both Australia and New Zealand. Percent contributors are calculated from data from a single 24-hour recall. The results are shown in Table 2.

Breakfast cereals were the major contributors to L-MTHF intakes for both the Australian (2 years and above) and New Zealand (15 years and above) populations. Yeast extracts and breads were also major contributors to the Australian population's intakes of L-MTHF. For New Zealand, yeast extracts were a major contributor, but breads were not.

Table 1: Major contributors (>5%) to L-MTHF intakes

		Major contributors (%)		
Country	**Population group**	**Breakfast cereals**	**Yeast extracts**	**Breads**
Australia	2 years and above	51	24	19
New Zealand	15 years and above	61	35	<1

4.3 Upper level of intake

In order to determine whether intakes of L-MTHF from voluntary fortification could be a safety concern, the estimated dietary intakes were compared with the NRV for folic acid – referred to as the Upper Level (UL, see discussion in Section 1). 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' (NHMRC, 2006). The ULs specified for folic acid for each population sub-group are shown in Table 3.

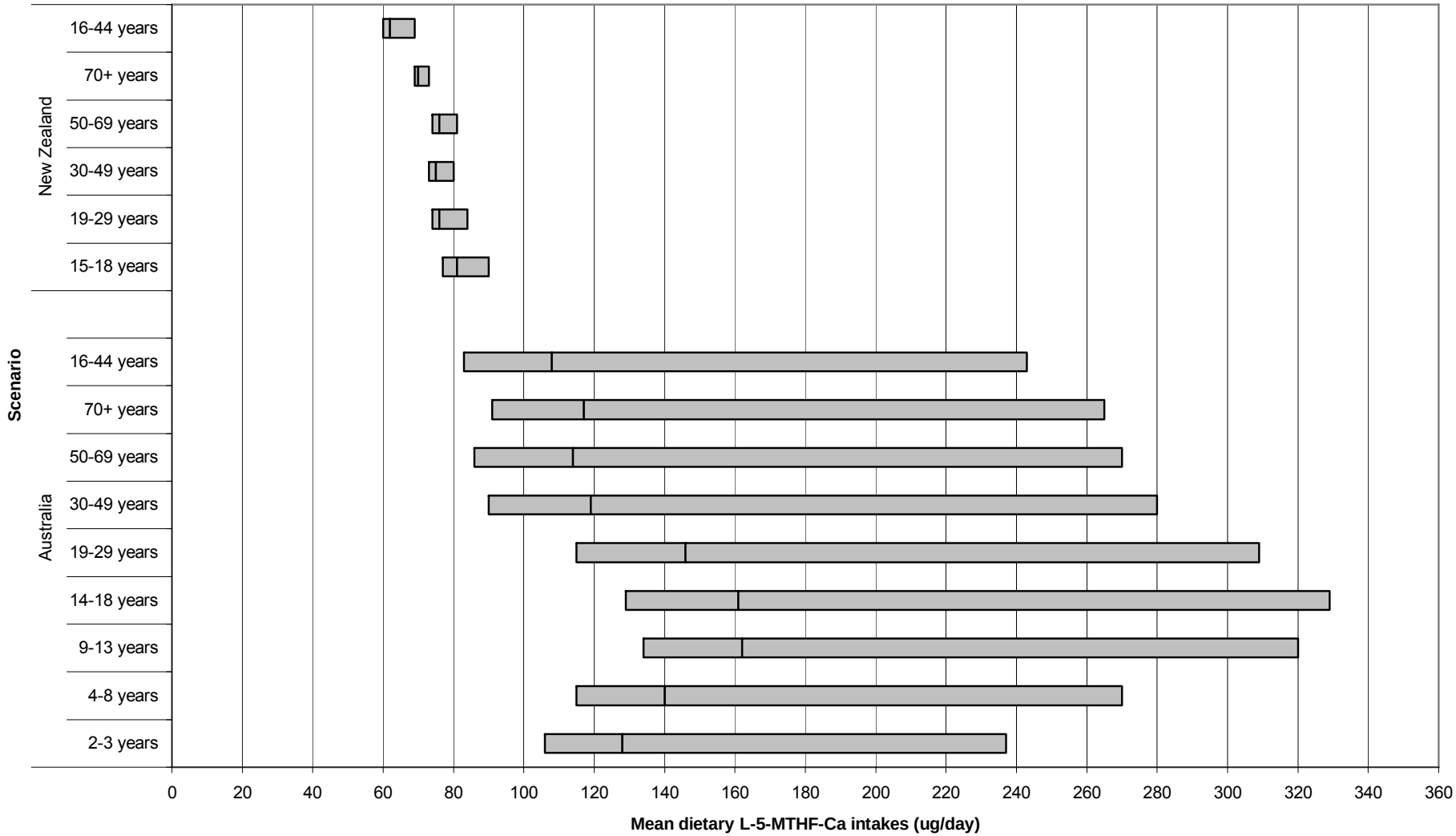


Figure 2: Comparison between estimated mean dietary L-MTHF intakes for Australian and New Zealand population groups

Table 2: Estimated mean L-MTHF intakes from food

Country	Population Group	Gender	No of respondents	Mean dietary L-MTHF intake (µg/day)		95th percentile dietary L-MTHF intake (µg/day)	
				Market weighted	Consumer behaviour	Market weighted	Consumer behaviour
Australia	2 years and above	All	13,858	129	100 - 285	346	306 - 590
New Zealand	15 years and above	All	4,636	75	73 - 81	214	209 – 229

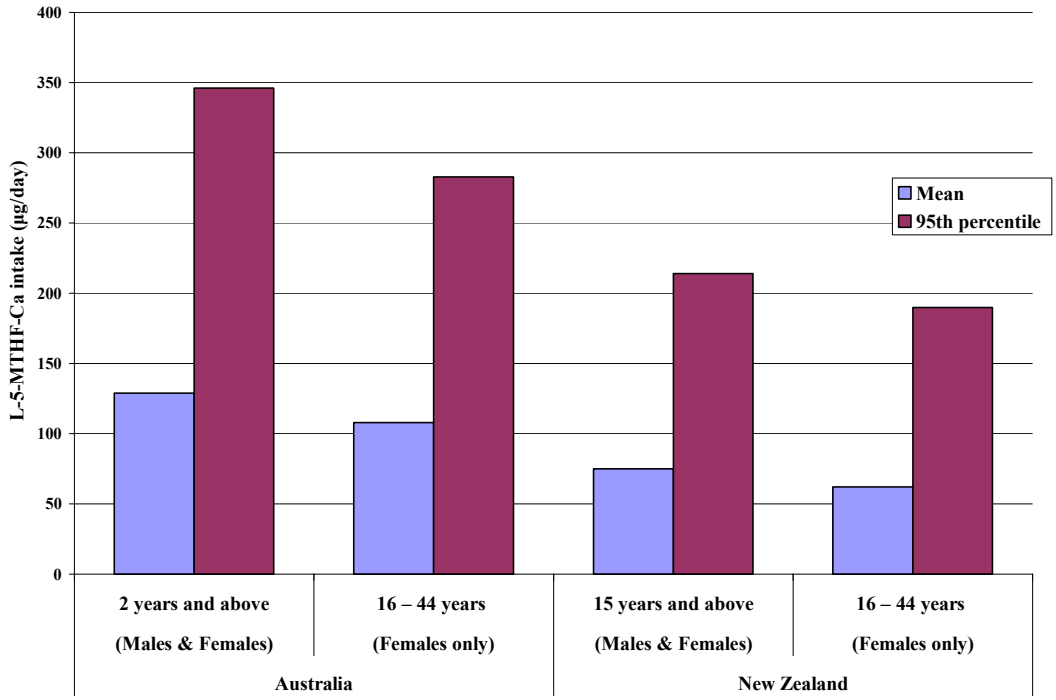


Figure 3: Estimated market weighted dietary L-MTHF intakes for Australia and New Zealand

Based on the relevant UL for folic acid for each population age group, the estimated dietary intakes for L-MTHF were determined for the Australia and New Zealand populations. Children aged 2-3 years are the population group most likely to exceed the UL for folic acid, although the overall proportion of the age groups exceeding the UL is very low. Full results can be found in Appendix 4 (Table A4.1 for Australia and Table A4.2 for New Zealand).

Table 3: Percentage of respondents with L-MTHF intakes above the Upper Level for folic acid

Country	Population Group	Upper Level (μg/day)*	No. of respondents	% respondents with L-MTHF intakes >UL folic acid
Australia	2-3 years	300	383	2
	4-8 years	400	977	1
	9-13 years	600	913	1
	14-18 years	800	734	<1
	19-29 years	1000	2,203	<1
	30-49 years	1000	4,397	<1
	50-69 years	1000	3,019	<1
	70+ years	1000	1,232	0
New Zealand	15-18 years	800	246	0
	19-29 years	1000	804	0
	30-49 years	1000	1,883	<1
	50-69 years	1000	1,147	0
	70+ years	1000	556	0

* Upper levels for folic acid used.

4.4 Calcium intakes

L-MTHF is in the form of a calcium salt and therefore contains calcium. An assessment was conducted to determine whether this form of folate would result in a significant increase in calcium intakes from the diet. Calcium forms 8% of the molecular mass of L-MTHF.

Based on estimated intakes of L-MTHF (using folic acid as a proxy), estimated additional intakes of calcium would be less than 1 mg per day, even based on the 'consumer behaviour' *always* chooses model. Intakes of calcium range between 700-1000 mg per day from the diet for Australians (McLennan and Podger, 1998) and New Zealanders (Ministry of Health (MOH), 1999; Ministry of Health (MOH), 2003). Therefore intakes of calcium from L-MTHF would be insignificant in relation to the total diet and the percentage of the UL[9] for the age groups assessed. Due to the insignificant intakes of calcium from consumption of foods fortified with L-MTHF, a more detailed intake assessment of calcium was not required for this Application.

5. Limitations of the dietary intake assessments

Dietary modelling based on 1995 or 1997 NNS food consumption data provides the best estimate currently possible of actual consumption of a food and the resulting estimated dietary intake of a nutrient for the population. However, it should be noted that the NNS data do have limitations. These limitations relate to the age of the data and the changes in eating patterns that may have occurred since the data were collected. Generally, consumption of staple foods such as fruit, vegetables, meat, dairy products and cereal products, which make up the majority of the usual diet, is unlikely to have changed markedly since 1995/1997 (Cook *et al*., 2001).

[9] UL for calcium is 2500 mg for all age and gender groups.

The uncertainty is associated with foods that may have changed in consumption patterns since 1995/1997, or have been introduced to the market since that time.

Over time, there may be changes to the ways in which manufacturers and retailers make and present foods for sale. Since the data were collected for the Australian and New Zealand NNSs, there have been significant changes to the Code to allow more innovation in the food industry. As a consequence, a limitation of the dietary intake assessment is that some of the foods that are currently available in the food supply were either not available or were not as commonly available in 1995/1997. Additionally, since the data were collected for the NNSs, there has been an increase in the range of products that are fortified with nutrients. Therefore, the nutrient databases from the NNSs used for dietary intake assessment may not be entirely representative of the nutrient levels in some foods that are now on the market. FSANZ does update the food composition database through analytical programs, and scans of the marketplace. However, with the marketplace continually changing, it is difficult to account for all fortified products. For the purposes of L-MTHF intake assessments, folic acid concentrations have been assigned to foods to take this into account and therefore should largely reflect current concentrations and foods fortified (e.g. to 15% of breads currently being fortified, as explained under Section 2.5 – Dietary modelling scenarios for assessing L-MTHF intakes).

There are a number of limitations associated with the L-MTHF concentration data. Analytical values used may not fully reflect actual levels due to variation in concentrations between batches of foods and because the technique used to measure folic acid (microbiological assay) is subject to significant uncertainty (Thomson 2005). Data generated from label values has not been adjusted to take into account potential extra addition (overages). For the concentration data, a major limitation is that market share information, used to weight concentration in breads and juices according to the proportion of the category observed to be fortified, may not fully reflect actual fortification practices.

A limitation of estimating dietary intake over a period of time using information from food recalls is that people may over- or under-report food consumption, particularly for certain types of foods. Over- and under-reporting of food consumption has not been accounted for in this dietary intake assessment. However, adjusting intakes based on two days of food consumption data accounts for some variation both within individuals and between individuals.

6. References

Australian Bureau of Statistics. (1998) Technical Paper on the National Nutrition Survey: Confidentialised Unit Record File 1995. Australian Bureau of Statistics, Canberra.

Bakers Delight (2006) *Company Profile*. 21 August 2006.

Cook, T., Rutishauser, I. and Seelig, M. (2001) Comparable data on food and nutrient intake and physical measurements from the 1983, 1985 and 1995 national nutrition surveys.

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

Ministry of Health (MOH). (1999) NZ Food: NZ People: Key results of the 1997 National Nutrition Survey. Ministry of Health, Wellington.

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

National Health and Medical Research Council (2006) *Nutrient Reference Values for Australia and New Zealand Including Recommended Dietary Intakes*. http://www.nhmrc.gov.au/publications/_files/n35.pdf. Accessed on 9 June 2006.

Thomson, B. (2005) *Fortification overages of the food supply. Folate and iron. Report to the New Zealand Food Safety Authority. New Zealand Environmental and Scientific Research.*

Appendix 1

How were the estimated dietary L-MTHF intakes from fortified food calculated?

L-MTHF intakes were calculated for each individual in the NNSs using their individual food consumption records from the dietary survey. The DIAMOND program multiplies the specified concentration of L-MTHF for an individual food by the amount of the food that an individual consumed in order to estimate the intake of L-MTHF from each food. Once this has been completed for all of the foods specified to contain L-MTHF, the total amount of L-MTHF consumed from all foods is summed for each individual. Adjusted nutrient intakes are first calculated (see below) and population statistics (such as mean and high percentile nutrient intakes) are then derived from the individuals' ranked adjusted intakes.

1.1 Adjusting nutrient intakes

Adjusted nutrient intakes, which better reflect 'usual' daily nutrient intakes, were calculated because NRVs such as the UL are based on usual or long term intakes and it is therefore more appropriate to compare adjusted or 'usual' nutrient intakes with NRVs.

1.1.1 Calculating adjusted nutrient intakes

To calculate usual daily nutrient intakes, more than one day of food consumption data are required. Information for a second (non-consecutive) day of food consumption was collected from approximately 10% of Australian NNS respondents and 15% of New Zealand NNS respondents. In order to estimate more usual nutrient intakes using both days of food consumption data, an adjustment is made to each respondent's L-MTHF intake based on the first day of food consumption data from the NNS. The adjustment takes into account several pieces of data including each person's day one nutrient intake, the mean nutrient intake from the group on day one, the standard deviation from the day one sample and the between person standard deviation from the day two sample. This calculation is described in Figure A1.1 below. For more information on the methodology of adjusting for second day nutrient intakes, see the Technical Paper on the National Nutrition Survey: Confidentialised Unit Record File (Australian Bureau of Statistics, 1998).

Adjusted value = $x + (x_1 - x) * (S_b/S_{obs})$

Where:
- x is the group mean for the Day 1 sample
- x_1 is the individual's day 1 intake
- S_b is the between person standard deviation; and
- S_{obs} is the group standard deviation for the Day 1 sample

Source: (Australian Bureau of Statistics, 1998)

Figure A1.1: Calculating adjusted nutrient intakes

Not all foods consumed in the NNSs were assigned a L-MTHF concentration as not all foods are permitted to, or take up, voluntary fortification. Therefore not all NNS respondents are consumers of L-MTHF based on day one food consumption records only.

However, after nutrient intake adjustments have been made based on a second day of food consumption data, all respondents have a L-MTHF intake as a function of how the adjusted intakes are calculated. This doesn't mean that there will be 100% of respondents with L-MTHF intakes for each of the 2 days in reality.

The intake assessments are based on many foods with voluntary fortification permissions, of which some respondents may not have consumed any, and it is simply a function of the second day adjustment methodology.

As a part of the two-day adjustment methodology, each individual below the mean in a L-MTHF intake distribution for day one will have an addition made to their L-MTHF intakes in order to calculate the adjusted intake over two days, as every individual's intakes are brought towards the mean. This applies to the L-MTHF intakes from respondents which are zero for day one. Whilst this may not represent the correct usual intakes at the bottom end of the usual L-MTHF intake distribution, this is unlikely to be a major issue for the risk assessment because the proportion of the population below the Estimated average requirement (EAR) for dietary folate equivalents (DFEs), which uses the lower end of the adjusted nutrient intake distribution, was not required to be determined. For this risk assessment, the concern is related to the proportion of respondents with intakes that exceed the upper safe reference health standard (the UL for folic acid), which would be the L-MTHF intakes at the upper end of the intake distribution. The people in the upper end of the intake distribution would have consumed foods containing L-MTHF. Therefore the adjusted L-MTHF intakes in the upper end of the distribution accurately reflect the usual population intakes.

The benefit in being able to more accurately estimate 'usual intake' by using the two day adjustment factor outweighs the possible over estimation of nutrient intakes for low consumers for risk assessment purposes.

1.1.2 Comparison of one day and usual nutrient intake distributions

The range of nutrient intakes from respondents is broader based on a single day of food consumption data than the range of usual intakes (Figure A1.2) as the latter takes into consideration the day-to-day variation in intakes within each person as well as the difference between each person.

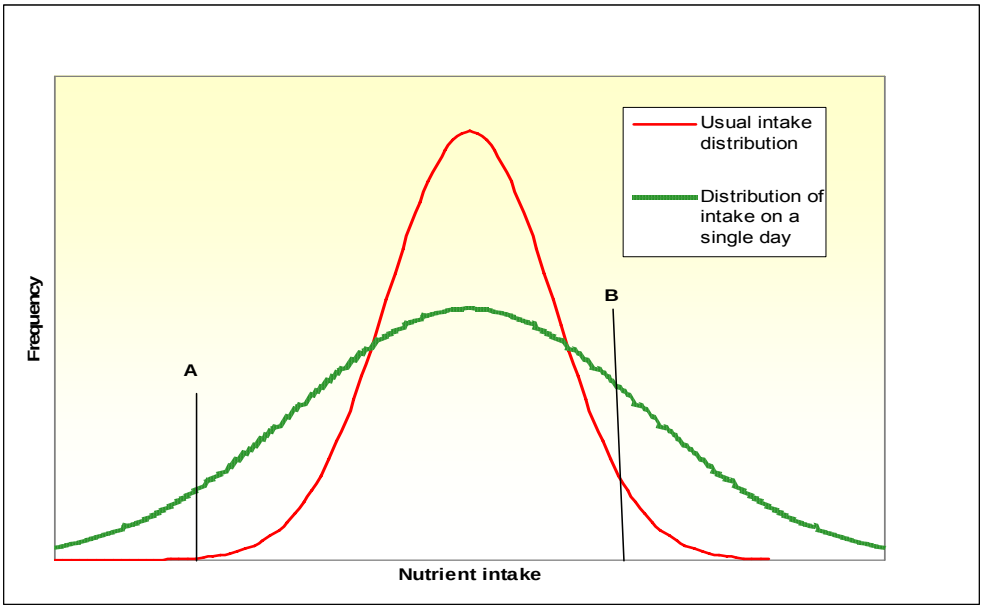


Figure A1.2: Comparison of one day and usual nutrient intake distributions

Using adjusted nutrient intakes provides better information for risk characterisation purposes. Adjusted (or usual) nutrient intakes will have little or no impact on estimated mean nutrient intakes, but would result in an estimated 95th percentile intake that is lower than the 95th percentile intake from a single day only, or a 5th percentile intake that is higher than the 5th percentile intake based on day one intakes only.

1.1.3 Comparison of nutrient intakes with NRVs

Comparison of nutrient intakes based on a single day of food consumption data with NRVs such as the EAR would result in a larger proportion of the population having intakes below a specified level (e.g. Figure A1.2, point A), which may overestimate the level of deficiency or inadequate intakes. A broader distribution from a single day of data also means a greater proportion of a population would exceed an upper cut off level, such as an upper level (e.g. Figure A1.2, point B), which overestimates the level of risk to this group of the population.

Note that where estimated nutrient intakes are expressed as a percentage of the Upper Level (UL), each individual's total adjusted intake is calculated as a percentage of the UL (using the total intakes in units per day) corresponding to their age and gender, the results are then ranked, and population statistics derived.

1.2 Calculation of foods contributing to L-MTHF intakes

L-MTHF intakes were calculated for each individual in the NNSs using their individual food consumption records from the dietary survey. The DIAMOND program multiplies the specified concentration of L-MTHF for an individual food by the amount of the food that an individual consumed in order to estimate the intake of L-MTHF from each food. Once this has been completed for all of the foods specified to contain L-MTHF, the total amount of L-MTHF consumed from all foods is summed for each individual. This is based on a single 24-hour recall only. Percentage contributions from individual foods are then calculated for food groups. Population statistics are then derived from the individuals' results.

APPENDIX 2

Summary of concentration data used for various foods for dietary modelling purposes

Table A2.1: L-MTHF concentration data for main Australian products assumed to contain L-MTHF

Food		Origin of concentration data	L-MTHF concentration (µg/100 g)*
Breads	White bread	Label and analytical, with 15-16% market share weighting.	0-200^
	Multigrain bread	Label and analytical, with 15-20% market share weighting.	0-133^
	Wholemeal bread	Label and analytical, market share weighted.	0-187^
	Rye bread and rolls	Label, with 15% market share weighting.	0-140^
	Fibre-increased bread and rolls	Label, with 15% market share weighting.	0-180^
	Savoury breads (Fancy Breads)	Label, with 15% market share weighting.	0-200^
	Fruit breads	Label, with 15% market share weighting.	0-153^
	Bun, sweet, various types	Label, with 15% market share weighting.	0-147^
	Sandwiches, various	Market share updated as for white bread	0-99^
	Hamburger with meat, bread, other ingredients		0
	Hot dog in bun		0
	Crumbed meat, chicken and fish	Market share updated as for flour	0
	Stuffing, bread based	Market share updated as for bread	0

Food		Origin of concentration data	L-MTHF concentration (µg/100 g)*
White flour	White flour	5% market share. Based on the addition of folic acid to The Healthy Baker flour.	0-250
Other 'wheat flour for making bread' containing foods	Pizza	Market share updated as for flour	0
	Pizza base		0
	Scone, various types	Market share - assumes made with 40% flour	0-100^
	Pancakes and pikelets	Market share updated as for flour	0
	Doughnuts, yeast type	Market share updated as for flour	0
	Croissants	Label and market share updated as for flour	0
Yeast extracts	Yeast-based spreads	Label and analytical	3,250
Breakfast cereals	Bran flakes	Label and analytical depending on brand	330-770^
	Puffed rice-style	Analytical	157-415^
	Wheat biscuits	Analytical	108-411^
	Muesli	Analytical	223
	Grain cereal, with or w/o fruit/nuts	Analytical	108-680^
	Sweetened cereal	Label and analytical depending on brand	140-442^
	Breakfast bars		270

Food		Origin of concentration data	L-MTHF concentration (µg/100 g)*
Juice	Orange juice	Analytical with 50% market share weighting used for commercial orange juice.	0-30^
	Breakfast juice	Mixed fruit juices (breakfast style) assumed to be 100% folic acid fortified.	30
	Fruit juice		0
Soy beverages	Soy beverage, fortified	Analytical, 100% Market share weighting	39-61^
	Soy beverage, fortification not specified	50% Market share weighting	0-60
Other milk substitutes	Infant formula (fortified)		5
Infant food	Infant food		0-75
Meal replacement products	Liquid meal replacements	Label and analytical depending on brand.	20
	Biscuit and bar meal replacements		82
	Supplement powders		117-667^
	Energy drinks		1

* Based on levels of folic acid fortification.
^ Denotes range of values for category - individual products within these broad food categories were assigned a single L-MTHF concentration.
Note: This is not a complete list of L-MTHF concentrations used in the dietary modelling to assess L-MTHF intakes.

Table A2.2: L-MTHF concentration data for main New Zealand products assumed to contain L-MTHF

Food		Origin of concentration data	L-MTHF concentration (µg/100 g)*
Bread	White bread and rolls	Label and analytical, depending on type recorded in NNS	0
	Mixed grain bread and rolls	Analytical depending on type recorded in NNS	0 – 120^
	Wholemeal bread and rolls	Analytical depending on type recorded in NNS	0 – 120^
	Rye bread and rolls	Label and analytical, depending on type recorded in NNS	0
	Fibre-increased bread and rolls	Label and analytical, depending on type recorded in NNS	0 – 120^
	Savoury breads (Fancy Breads)		0
	Fruit breads	Label, with 15% market share weighting	0
	Buns and yeast-based products		0
	Sandwiches, filled rolls		0
	Hamburger with meat, bread, other ingredients		0
	Crumbed meat, chicken and fish		0
	Croutons		0
	Stuffing, bread based		0
Other ‘wheat flour for making bread’ containing foods	Pizza		0
	Scone, various types		0
	Pancakes and pikelets		0

Food		Origin of concentration data	L-MTHF concentration (µg/100 g)*
	Doughnuts, yeast type		0
Yeast extract	Yeast extract	Label and analytical, depending on type recorded in NNS	2,200 – 3,250^
Breakfast cereal	Bran flakes	Analytical depending on type recorded in NNS	69-770
	Wheat biscuits	Analytical	313-450
	Muesli	Analytical	140-680
	Single cereal, puffed flakes or extruded	Label and analytical, depending on type recorded in NNS	157-530
Juices	Orange juice	Analytical with 25% market share weighting	0-44
	Fruit juice		0
Soy beverages	Soy beverage	Analytical	30-85
Other milk substitutes	Rice milk	Analytical	40
Infant food	Infant food		90
Meal replacement products	Liquid meal replacements	Label and analytical depending on brand	40
	Biscuit and bar meal replacements		82
	Supplement powders		40-160

* Based on levels of folic acid fortification.
^ Denotes range of values for category - individual products within these broad food categories were assigned a single L-MTHF concentration.
Note: This is not a complete list of L-MTHF concentrations used in the dietary modelling to assess L-MTHF intakes.

Appendix 3
Complete information on dietary intake assessment results

Table A3.1: Estimated mean and 95th percentile dietary L-MTHF intakes for various Australian population sub-groups

			Mean L-MTHF Intake (µg/day)		95th Percentile L-MTHF Intake (µg/day)	
Age Group	**Gender**	**No. of respondents**	**Market weighted**	**Consumer behaviour range**	**Market weighted**	**Consumer behaviour range**
2 years and above	All	13,858	129	100 - 285	346	306 - 590
2-3 years	All	383	128	106 - 237	232	209 - 412
	M	170	141	117 - 251	250	224 - 454
	F	213	118	97 - 227	210	195 - 408
4-8 years	All	977	140	115 - 270	282	256 - 481
	M	513	157	131 - 295	313	280 - 556
	F	464	121	99 - 242	246	230 - 406
9-13 years	All	913	162	134 - 320	367	341 - 613
	M	474	192	162 - 369	420	386 - 725
	F	439	128	104 - 266	291	257 - 463
14-18 years	All	734	161	129 - 329	378	342 - 659
	M	378	202	166 - 398	539	508 - 766
	F	356	117	90 - 256	273	239 - 477
19-29 years	All	2,203	146	115 - 309	365	329 - 644
	M	1,014	179	142 - 377	449	402- 772
	F	1,189	118	93 - 252	286	244 - 469

Age Group	Gender	No. of respondents	Mean L-MTHF Intake (µg/day)		95th Percentile L-MTHF Intake (µg/day)	
			Market weighted	Consumer behaviour range	Market weighted	Consumer behaviour range
30-49 years	All	4,397	119	90 - 280	342	302 - 602
	M	2,080	140	105 - 330	373	321 - 675
	F	2,317	100	75 - 236	299	274 - 488
50-69 years	All	3,019	114	86 - 270	368	341 - 574
	M	1,442	131	98 - 313	403	364 - 656
	F	1,577	99	76 - 232	325	304 - 494
70+ years	All	1,232	117	91 - 265	357	306 - 530
	M	545	126	97 - 293	331	297 - 551
	F	687	111	87 - 243	369	350 - 498

Table A3.2: Estimated mean and 95th percentile dietary L-MTHF intakes for various New Zealand population sub-groups

Age Group	Gender	No. of respondents	Mean L-MTHF Intake (µg/day)		95th Percentile L-MTHF Intake (µg/day)	
			Market weighted	Consumer behaviour range	Market weighted	Consumer behaviour range
15 years and above	All	4,636	75	73 - 81	214	209 - 229
15-18 years	All	246	81	77 - 90	195	187 - 223
	M	109	113	111 - 119	225	213 - 260
	F	137	54	51 - 67	157	135 - 193
19-29 years	All	804	76	74 - 84	194	180 - 217
	M	286	112	110 - 119	221	205 - 255
	F	518	56	54 - 65	162	159 - 184
30-49 years	All	1,883	75	73 - 80	223	220 - 233
	M	787	86	85 - 91	246	230 - 268
	F	1,096	67	65 - 72	205	200 - 215
50-69 years	All	1,147	76	74 - 81	238	237 - 248
	M	538	84	82 - 89	262	260 - 271
	F	609	69	68 - 73	210	204 - 225
70+ years	All	556	70	69 - 73	187	186 - 189
	M	207	69	68 - 73	188	186 - 191
	F	349	70	69 - 73	179	179 - 185

Appendix 4
Complete information on risk characterisation

Table A4.1: Percentage of respondents with L-MTHF intakes above the Upper Level for various Australian population sub-groups

Age Group	Gender	No. of respondents	% respondents with dietary L-MTHF intakes > Upper Level*
			Baseline (revised)
2-3 years	All	383	2
	M	170	3
	F	213	<1
4-8 years	All	977	1
	M	513	2
	F	464	<1
9-13 years	All	913	1
	M	474	2
	F	439	<1
14-18 years	All	734	<1
	M	378	1
	F	356	0
19-29 years	All	2,203	<1
	M	1,014	<1
	F	1,189	<1
30-49 years	All	4,397	<1
	M	2,080	<1
	F	2,317	<1
50-69 years	All	3,019	<1
	M	1,442	<1
	F	1,577	<1
70+ years	All	1,232	0
	M	545	0
	F	687	0

* Upper levels for folic acid used.

Table A4.2: Percentage of respondents with L-MTHF intakes above the Upper Level for various New Zealand population sub-groups

Age Group	Gender	No. of respondents	% respondents with dietary L-MTHF intakes > Upper Level* Baseline (revised)
15-18 yrs	All	246	0
	M	109	0
	F	137	0
19-29 yrs	All	804	0
	M	286	0
	F	518	0
30-49 yrs	All	1,883	<1
	M	787	0
	F	1,096	<1
50-69 yrs	All	1,147	0
	M	538	0
	F	609	0
70+ yrs	All	556	0
	M	207	0
	F	349	0

* Upper levels for folic acid used.

Attachment 5

Food Technology Report

Application A566 – L-Methyltetrahydrofolate, Calcium as a Permitted Vitamin Form of Folate

Summary

The FSANZ food technology report was based on technical information provided by the Applicant, published studies in scientific journals and other technical references. L-MTHF is a white to light yellowish, almost odourless, crystalline powder which is sparingly soluble in water. It is synthetically produced from folic acid under conditions of Good Manufacturing Practice (GMP) and is intended for use as an alternative form of folate for food fortification purposes.

L-MTHF is reported to be stable in crystalline form during long-term storage (48 months at 40^{o}C and up to 75% relative humidity), after micronising or milled, and when compounded into vitamin and mineral tablets. However, limited information is available (either from the Application or from the literature) on the stability of added L-MTHF during food production. The available data is summarised below.

In a study provided by the Applicant, both crystalline and microencapsulated L-MTHF were shown to be relatively stable in bread baking, with microencapsulation improving stability. Compared to average losses of folic acid during bread baking as reported in the literature, the stability of L-MTHF appears to be similar to, if not greater than, folic acid during bread baking.

Similarly, microencapsulated L-MTHF offered improved stability over crystalline L-MTHF when added during the manufacture of breakfast cereal. The losses reported by the Applicant are comparable with average reported production losses of folic acid in ready-to-eat breakfast cereals of 25%.

As folates are known to be heat labile and sensitive to environmental effects, the stability of added L-MTHF (and folic acid) has been studied in a liquid model food system based on infant formula. In this study, the stability of added L-MTHF was found to be similar to that of folic acid during heat treatment (100-140^{o}C) however the authors considered the results to be primarily due to low oxygen concentrations in the system tested. In this study, stability of L-MTHF was also enhanced by the addition of ascorbic acid and ferrous sulphate.

On the basis of the available data, L-MTHF appears to demonstrate stability under a range of food processing conditions. Manufacturers using L-MTHF in the (folate) fortification of certain foods would need to consider its stability in particular applications and adopt practices that accounted for losses of L-MTHF during production and processing of their product.

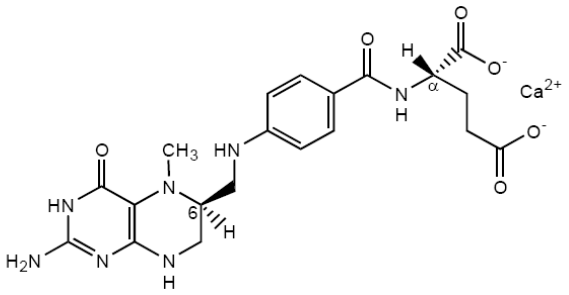
Introduction

The Applicant has developed L-MTHF as an alternative form of folate that is suitable for use in foods and dietary supplements. L-MTHF is synthetically produced from folic acid and is marketed under the trade name Metafolin®. It is available commercially in dry crystalline or microencapsulated form. The proposed use levels of L-MTHF are equivalent to those levels currently permitted for folic acid in the Code. 1.123 units of L-MTHF (anhydrous basis) are equivalent to 1 unit of folic acid (that is, 100 μg folic acid is equivalent to 112 μg L-MTHF).

To supplement the data that was provided as part of the Application, FSANZ sought further information (via a stop clock) on the stability of added L-MTHF during food processing and long-term storage in the food matrices for which permissions are sought. FSANZ also sought comparative information on the stability of added L-MTHF and folic acid in different food matrices.

In response to the stop clock, the Applicant provided a number of studies in relation to the stability of naturally occurring L-MTHF and added folic acid during bread baking. These studies showed variable losses of folic acid and endogenous folates during bread baking, of around 10 to 30%, depending on the baking conditions. However, these studies were of limited use in assessing the stability of added L-MTHF in different foods and under different food processing conditions. Therefore, FSANZ has only been able to assess the stability of added L-MTHF on the basis of relevant, published information.

Physical and Chemical Properties

Chemical Name	N-{4-[[((6S)-2-amino-1,4,5,6,7,8-hexahydro-5-methyl-4-oxo-6-pteridinyl)methyl]amino]benzoyl}-L-glutamic acid, calcium salt
Synonyms	L-5-methyltetrahydrofolic acid, calcium salt; L-methyltetrahydrofolate, calcium salt; L-methylfolate, calcium; and L-5-MTHF.
Trade Name	Metafolin®
C.A.S Registry Number	151533-21-1
Chemical formula	$C_{20}H_{23}CaN_7O_6$ (anhydrous form)
Structural formula	
Molecular weight	497.5 Daltons (anhydrous form)
Description	White to light yellowish, almost odourless, crystalline powder
Solubility	Sparingly soluble in water; very slightly soluble or insoluble in most organic solvents; soluble in alkaline solutions
Melting Point	>300ºC
pH of Aqueous Solution	6.5-7.5 (0.5 g/100 mL)

In comparison with folic acid which is water soluble, L-MTHF has low solubility in water. This difference in solubility may be of significance when determining the most appropriate form of folate for use in different food matrices and under various processing conditions.

Manufacturing Process

L-MTHF is manufactured synthetically from folic acid under conditions of Good Manufacturing Practice (GMP). In the initial step, folic acid is reduced to form tetrahydrofolic acid, which is then reacted with formaldehyde to form 5,10-methylene-tetrahydrofolic acid. This intermediate is then reduced to form L-5-methyltetrahydrofolic acid, followed by crystallisation as the calcium salt of L-5-methyltetrahydrofolic acid.

Specifications

The specification details provided by the Applicant are consistent with the specifications prepared at the 65th meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 2005 and published in the FAO Compendium of Food Additive Specifications Addendum 13 (FNP 52 Add 13, 2005). The JECFA specifications are a primary source of specifications, being reference (a) in clause 2 of Standard 1.3.4 – Identity and Purity. A separate specification for L-MTHF is therefore not required in Standard 1.3.4.

Folates

Folates are water-soluble vitamins. The term folate(s) is used generically to describe the family of B-group vitamins and includes both naturally occurring and synthetic forms of the vitamin (Scientific Advisory Committee on Nutrition, 2006).

Natural forms of folate are found in a wide variety of foods including green leafy vegetables, cereals, fruits, grains, legumes, yeast extract and liver. Naturally occurring folate comprises a group of mono- and polyglutamate derivatives of pteroic acid (4-[(pteridine-6-methyl) amino] benzoic acid). Tetrahydro-, dihydro-, formyltetrahydro-, and methyltetrahydrofolates are the predominant naturally occurring folates in foods. Folate can also be supplied in the form of disodium folate (Ball, 1998).

Folic acid, or pteroylmono-glutamic acid (PGA), is the most common synthetic form of folate and is the form used in food fortification and in the majority of supplements (Ball, 1998). Currently, folic acid is the only approved form of folate in the Code for the purposes of folate fortification of food.

Folate Stability

Folic acid is generally the most stable form of folate. It is resistant to oxidation under most conditions of food processing and storage, although reduced stability occurs in acidic media (Gregory, 1996).

The natural folates, which exist mainly as tetrahydrofolates are the least stable form of the vitamin. However, large differences in stability exist among the tetrahydrofolates, the stability of each folate being influenced by the chemical nature of the pteridine ring system.

Folates are highly susceptible to oxidative degradation and are readily extracted from foods by aqueous media. By either means, large losses of naturally occurring folate can occur during food processing and preparation (Gregory, 1996).

The major naturally occurring form of folate in many foods is L-5-MTHF, which is considered to have intermediate stability. The oxidation of L-5-MTHF initially produces 5-methyldihydrofolate, which can be reduced back to L-5-MTHF by the enzyme 5,10-methylene-tetrahydrofolate reductase (MTHFR). Once the partly oxidised form of the 5-methyldihydrofolate is formed, it becomes susceptible to further chemical oxidation, involving the rearrangement of the pteridine ring to produce a new derivative which lacks vitamin activity (Scott, 2001). In relatively anaerobic conditions, the presence of added components such as ascorbate, ferrous iron and reducing sugars tend to improve the oxidative stability of both L-MTHF and folic acid by reducing the concentration of dissolved oxygen through their own oxidation reactions (Gregory, 1996).

Stability of L-5-Methyltetrahydrofolate, calcium

Stability of Crystalline L-MTHF on Storage

L-MTHF is reported to be stable in crystalline form. The Applicant has provided data in relation to the stability of the dry crystalline powder over a four-year period, which was tested according to the Guidelines of the International Conference of Harmonization (ICH)[10]. Eight batches of crystalline L-MTHF were stored for up to 48 months at varying temperatures (up to 40^{o}C) and relative humidity (up to 75%) and were found to be very stable under these conditions. Storage of crystalline micronised L-MTHF particles (particle size <100 μm) for 12 months at up to 40^{o}C and 75% relative humidity provided essentially identical results, indicating that the larger surface area of the micronised particles did not result in lower stability.

Stability of L-MTHF in Solid Foods

The Applicant has provided data on the stability of L-MTHF in bread and breakfast cereal. Microencapsulated L-MTHF was prepared by absorbing the substance in different inert carriers (maltodextrin, micro-crystalline cellulose, calcium hydrogen phosphate) and coating the mixture with one or two layers of suitable film-forming materials, such as food-grade modified cellulose or shellac.

Stability of L-MTHF in bread and breakfast cereal

Bread was prepared with the addition of pure or microencapsulated L-MTHF yielding a calculated concentration of approximately 8-10 mg/100 g (this is considerably higher than the maximum permitted claim of 200 μg/100 g for folic acid in bread in Standard 1.3.2 – Vitamins and Minerals). Bread without added L-MTHF contained approximately 0.2 mg/100 g.

The addition of pure (crystalline) or microencapsulated L-MTHF to bread (275 g loaves) yielded recoveries of between 89-94% and 86-105%, respectively. In a large loaf (1 kg) with a longer baking time, the recovery was 80% (Biodar, 2000a).

[10] ICH/Q1A Stability Testing of New Drug Substances and Products.

In corn flakes prepared with the addition of pure or microencapsulated L-MTHF using a commercial production line, the recovery of pure L-MTHF was 74% and that of micro-encapsulated L-MTHF was 79-80% (Biodar, 2000a).

From these studies, the authors concluded that L-MTHF is relatively stable in bread baking and extruded corn flakes and that microencapsulation improves the stability (Biodar, 2000a).

Stability of L-MTHF in Liquid Foods

Stability of L-MTHF in solution during UHT treatment

L-MTHF is less stable in aqueous solutions at elevated temperature, forming products with and without folate vitamin activity as a result of oxidative degradation.

In a study by Viberg *et al.* (1997), L-MTHF was dissolved in phosphate buffer (pH 7; 260 micrograms/100 mL). The solutions had either a normal or low oxygen concentration (6.8 and 0.3 ppm, respectively) and were treated for 0.8 to 6.49 minutes at temperatures of 110^{o}C, 120^{o}C, 140^{o}C and 150^{o}C to cover the whole temperature range normally used in UHT processing. The results indicate that the presence of oxygen accelerates degradation only at higher temperatures (140 - 150^{o}C). The authors recommended that degassing of liquid foods be undertaken before thermal processing to reduce the oxygen content and therefore maximise the amount of L-MTHF retained during thermal processing (Viberg *et al.,* 1997).

Stability of L-MTHF in an infant formula model system

The stability of L-MTHF and folic acid were tested in a liquid infant formula model system. L-MTHF and folic acid were added at levels of 10 mg/kg and examined during heat treatment (100-140^{o}C) for different time periods (up to 250 minutes). The stability of L-MTHF was found to be similar to that of folic acid under the applied test conditions. Additions of ascorbic acid and ferrous sulphate exerted a further stabilising effect. The authors concluded however that the apparent stability of L-MTHF in these experiments was likely to be due to the reduced oxygen concentration in the sealed pouches in which the samples were tested (Day and Gregory, 1983).

Summary and Conclusion

Based on the studies provided by the Applicant, crystalline L-MTHF is stable on long-term storage (48 months at 40^{o}C and up to 75% relative humidity) and after micronising. However, neither the Application nor the literature provides information on the stability of L-MTHF fortified foods that have a long shelf-life or have been stored for extended periods of time.

L-MTHF in both crystalline and microencapsulated forms was shown to be relatively stable in bread baking, yielding recoveries of 89-94% and 86-105%, respectively. Based on these data, L-MTHF is at least as stable as folic acid during bread baking.
Furthermore, in corn flakes prepared with both pure and microencapsulated L-MTHF recoveries of L-MTHF were 74% and 79-80%, respectively. These losses are comparable with average production losses of folic acid in ready to eat breakfast cereals of 25%, as reported in the literature.

In a liquid infant formula model system, the stability of L-MTHF was found to be similar to that of folic acid during heat treatment (100-140^{o}C) for different time periods (up to 250 minutes). Stability of L-MTHF was enhanced following the addition of ascorbic acid and ferrous sulphate, however was primarily dependent on low oxygen concentrations in the systems tested.

On the basis of the available information, L-MTHF appears to demonstrate stability in a range of processed foods.

References

Ball, G.F.M. (1998) *Bioavailability and Analysis of Vitamins in Foods*. Chapman and Hall, London, pp 444, 474.

Biodar (2000a) Microencapsulated Ca-Mefolinate Project. Final Report. Unpublished study report of Bio Dar Ltd., Yavne, Israel (provided in the Application).

Day, B.P.F. and Gregory, J.F. (1983) Thermal stability of folic acid and 5-methyltetrahydrofolic acid in liquid model food systems. *J Food Sci*, **48**:581-587, 599.

Gregory, J.F. (1996) Vitamins. In: Fennema, O.R., eds. Food Chemistry, 3rd edn, Marcel Dekker, New York, pp 531-532, 590-599, 610-616.

Joint FAO/WHO Expert Committee on Food Additives (JECFA). (1992) Compendium of Food Additive Specifications, FAO Food and Nutrition Paper 52 Addendum 13 (2005). http://www.fao.org/docrep/008/a0044e/a0044e05.htm. Accessed on 1 June 2007.

Scientific Advisory Committee on Nutrition. (2006) Folate and Disease Prevention. TSO, Norwich. http://www.sacn.gov.uk/pdfs/folate_and_disease_prevention_report.pdf. Accessed on 16 April 2007.

Scott, J.M. (2001) Methyltetrahydrofolate: the superior alternative to folic acid. In: Kramer, K., Hoppe, P.P and Packer, L., eds. *Nutraceuticals in Health and Disease Prevention*. Vol. 6, Marcel Dekker, New York, pp 75-90.

Viberg, U., Jägerstad, M., Oste, R. and Sjöholm, I. (1997) Thermal processing of 5-methyltetrahydrofolic acid in the UHT region in the presence of oxygen. *Food Chemistry*, **59**(3):381-386.

Attachment 6

Summary of second round public submissions

Nine submissions, summarised below, were received following release of the Draft Assessment.

1. Australian Food and Grocery Council

- Supports Option 2, approval of L-MTHF as a permitted form of folate.
- Supports the use of L-MTHF for all fortification purposes, including the mandatory fortification of bread, because it can be readily utilised by the body, is unlikely to mask a vitamin B_{12} deficiency and does not result in plasma folic acid.
- Manufacturers should be able to choose either folic acid or L-MTHF based on the technological needs of the product and its consumer utility.
- L-MTHF may be preferred over folic acid because of the use of overages which is an important consideration when determining the potential for excessive consumption in some vulnerable consumer groups such as children.
- Further research is needed before the adoption of a Nutritional, Health and Related Claims Standard.
- Opposed to the FSANZ assessment that a health claim should be solely limited to foods fortified with folic acid. Published studies show that L-MTHF improves some health parameters connected to an increased risk of neural tube defects and other birth defects. These studies support the use of L-MTHF over folic acid.
- Limiting the use of L-MTHF is an unnecessary restriction on manufacturers and undermines the value of the approval to use L-MTHF in foods as a source of folate.
- Restricting the use of L-MTHF unnecessarily limits the opportunities to improve education in the target consumer group – women of child bearing age.

2. Victorian Department of Human Services

- The approval of L-MTHF as a permitted form of folate is supported.
- Expresses lingering concerns with the [mandatory] fortification of bread with folic acid and considers that L-MTHF would be preferred because it occurs naturally in the body, is metabolised through normal pathways, and does not result in circulating unmetabolised folic acid.
- The current wording of the proposed Nutrition and Health Claims Standard 'greater than 65 μg per serve of folate and/or folic acid' does not appear to preclude the use of L-MTHF as a basis for a claim relating to neural tube defects.

3. Country Women's Association of New South Wales

- Not opposed to the use of L-MTHF as a permitted form of folate.

4. Food Technology Association of Australia

- Supports Option 2, approval of L-MTHF as a permitted form of folate.

5. Professor John Birkbeck, Massey University, NZ

- Supports Option 2, approval of L-MTHF as a permitted form of folate.

6. Queensland Health

- Supports Option 2, approval of L-MTHF as a permitted form of folate, because it offers advantages in that it is a naturally occurring form of folate, unlikely to mask a vitamin B_{12} deficiency, and is bioequivalent to folic acid when added to foods.
- There appears to be no safety concerns with L-MTHF – the upper level of intake is based on the masking of the symptoms of a vitamin B_{12} deficiency (as applies to folic acid) which is unlikely to occur with this compound.
- The current draft Standard 1.2.7 relating to a health claim for folic acid and NTD reduction refers to food containing no less than 65 µg folate and/or folic acid per serving. This appears to provide a loophole for a folate-NTD claim for L-MTHF, although such permission was specifically excluded in the Draft Assessment.
- Given that L-MTHF may not be as stable as folic acid in some foods, is potentially more expensive and a folate-NTD claim cannot be made, it is not clear what if any advantages it offers to manufacturers.

7. George Weston Foods

- Supports Option 2, approval of L-MTHF as a permitted form of folate.
- Fully supports the views expressed by the AFGC that FSANZ permit the use of L-MTHF as an alternative to folic acid in all foods where folic acid is permitted, including for mandatory fortification purposes.
- As major manufacturers of bread products, George Weston Foods would not have access to the use of L-MTHF as an alternative to folic acid because of the limited permission for L-MTHF proposed by FSANZ at Draft Assessment.

8. New South Wales Food Authority

- Supports progressing the Application to Final Assessment.
- FSANZ should provide information relating to stock in trade provisions that would apply to wheaten bread making flour or products made with wheaten bread making flour fortified with L-MTHF after September 2009.
- FSANZ should address the apparent inconsistency with respect to a permission to use L-MTHF and the proposed drafting in Standard 1.2.7 before this application is completed.

9. New Zealand Food Safety Authority

- Supports the approval of L-MTHF as a permitted form of folate.
- Supports the proposed amendment to Standard 1.1A.2 (Transitional Standard for Health Claims) that would disallow a folate-NTD health claim when L-MTHF is used as the fortificant.
- Notes that there is evidence showing L-MTHF is just as effective as folic acid in increasing blood folate levels, and increased blood folate levels have been linked to a reduction in NTDs.

- Also notes that appropriate clinical trials with L-MTHF would be impossible and therefore under the current high level health claims assessment structure, L-MTHF would be unable to be approved for mandatory fortification.
- Given that a folate-NTD claim is not possible for L-MTHF, it will be necessary to consider all permitted dietary forms of folate when monitoring the efficacy of mandatory fortification with folic acid.
- Considers that this Application presents an opportunity to align the recommended dietary intakes of folate listed in the Code with the recently published Nutrient Reference Values, and to standardise the use of the terms folic acid and folate within the Code.