DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service
Food and Drug Administration

Memorandum

DEC 26 2000

Date:

7912 '01 JAN 26 P2:37

From: Director, Division of Standards and Labeling Regulations, Office of Nutritional Products, Labeling and Dietary Supplements, HFS-820

Subject: 75-Day Premarket Notification for New Dietary Ingredients

To: Dockets Management Branch, HFA-305

New Dietary Ingredient:	L-5-methyl-THF
Firm:	Merck KGaA
Date Received by FDA:	October 12, 2000
90-Day Date:	January 10, 2001

In accordance with the requirements of section 413(a) of the Federal Food, Drug, and Cosmetic Act, the attached 75-day premarket notification for the aforementioned new dietary ingredient should be placed on pubic display in docket number 95S-0316 after January 10, 2001.

Felicia B. Satchell

95S-0316

RPT 87

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Public Health Service

Food and Drug Administration
Washington, DC

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DEC 26 2000

VIA FACSIMILE AND MAIL

Dr. Najib Sehat
Merck KGaA
CHN-BS
Regulatory Affairs, C11/243
Frankfurter Str. 250
64271 Darmstadt, Germany

Dear Dr. Sehat:

This is in response to your letter to the Food and Drug Administration (FDA) dated September 22, 2000, making a submission for a new dietary ingredient pursuant to 21 U.S.C. 350b(a)(2) (section 413(a)(2) of the Federal Food, Drug, and Cosmetic Act (the act)). Your letter notified FDA of your intent to market a dietary supplement product containing the new dietary ingredient described as "the calcium salt of L-5-methyltetra-hydrofolate ("L-5-methyl-THF"). Your entire submission was received on October 12, 2000.

21 U.S.C. 350b(a)(2) requires that a manufacturer or distributor of a dietary supplement that contains a new dietary ingredient submit to FDA, at least 75 days before the dietary ingredient is introduced or delivered for introduction into interstate commerce, information that is the basis on which the manufacturer or distributor has concluded that a dietary supplement containing such new dietary ingredient will reasonably be expected to be safe. FDA reviews this information to determine whether it provides an adequate basis for such a conclusion. Under section 350b(a)(2), there must be a history of use or other evidence of safety establishing that the dietary ingredient, when used under the conditions recommended or suggested in the labeling of the dietary supplement, will reasonably be expected to be safe. If this requirement is not met, the dietary supplement is deemed to be adulterated under 21 U.S.C. 342(f)(1)(B) because there is inadequate information to provide reasonable assurance that the new dietary ingredient does not present a significant or unreasonable risk of illness and injury.

Your submission contains evidence of history of use and other information that you assert is an adequate basis from which to conclude that the type of dietary supplement product containing the new dietary ingredient will reasonably be expected to be safe. FDA has carefully considered the information in your submission and the agency has significant concerns about the evidence on which

you rely to support your conclusion that the new dietary ingredient above, when used under the conditions recommended or suggested in the labeling of the dietary supplement, will reasonably be expected to be safe. You state in your submission that L-5-methyl-THF is "intended for use in dietary supplements as a 1:1 replacement for folic acid." However, the evidence contained in your submission does not establish that L-5-methyl-THF is nutritionally equivalent to folic acid, only that certain absorption and uptake parameters are similar. The animal studies you submitted were not designed to determine the safety of replacing folic acid with L-5-methyl-THF for long periods of time. Short-term exposure studies in animals cannot predict effects of long-term exposure in humans. Moreover, the studies were not designed to determine whether L-5-methyl-THF will support human fetal growth and development during pregnancy. The scientific evidence in your submission is insufficient to establish that L-5-methyl-THF is safe for use during pregnancy as an alternative to folic acid.

You stated in your notification that although there are no data in the scientific literature on the safety of L-5-methyl-THF, it is considered safe because when used as an alternative to folic acid daily intakes of folate will not be exceeded. We disagree that safety of L-5-methyl-THF can be determined from the safety of related substances, in this case folic acid. The information in your submission has not established whether L-5-methyl-THF and folic acid have the same biological activity and safety.

Additionally, the information in your notification does not meet the requirements of 21 C.F.R. 190.6(b)(4), because an English translation of the article "Mueller, H., 1993. Z Lebensm Unters Forsch 196: 137-141 was not included in the submission.

For the reasons discussed above, the information in your submission does not provide an adequate basis from which to conclude that L-5-methyl-THF, when used under the conditions of use recommended or suggested in the labeling of your product, will reasonably be expected to be safe. Therefore, your product may be adulterated under 21 U.S.C. 342(f)(1)(b) as a dietary supplement that contains a new dietary ingredient specified for which there is inadequate information to provide reasonable assurance that such ingredient does not present a significant or unreasonable risk of illness or injury. Introduction of such products into interstate commerce is prohibited under 21 U.S.C. 331(a) and (v).

Your submission will be kept confidential for 90 days from the date of receipt, October, 12, 2000, and after January 10, 2001, your submission will be placed on public display at Dockets Management Branch (Docket No. 95S-0316). Commercial and confidential information in the notification will not be made available to the public.

Please contact us if you have any questions concerning this matter.

Sincerely yours,

Felicia B. Satchell

Felicia B. Satchell
Director
Division of Standards
and Labeling Regulations
Office of Nutritional Products, Labeling
and Dietary Supplement

Datum September 22, 2000
Bereich/Abt. CHN BS
Zuständig Dr. Najib Sehat
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E-mail najib.sehat@merck.de
Ihr Schr. vom
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Deutschland

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Food and Drug Administration
Center for Food Safety and Applied Nutrition, CFSAN
Office of Nutritional Products, Labeling, and Dietary Supplements
HFS-820
200 C Street, S.W.
Washington, DC 20204
USA

Received 1/25/01

Attention: Roslyn Powers

Dear Sir/Madam:

Pursuant to Section 8 of the Dietary Supplement Health and Education Act of 1994, Merck KGaA ("Merck"), located at Darmstadt, Frankfurter Str. 250, 64271 Darmstadt, Germany, submits this new dietary ingredient notification to the Food and Drug Administration (FDA) for the calcium salt of L-5-methyltetrahydrofolate ("L-5-methyl-THF"), a derivative of folic acid to be manufactured by Merck for use in dietary supplements.

Merck's L-5-methyl-THF is intended for use in dietary supplements as a 1:1 replacement for folic acid, so its use level will mirror the existing folic acid use level in dietary supplements.

Attached is a discussion of the scientific data and information demonstrating that Merck's L-5-me-THF, when used under the conditions suggested in the labeling of the dietary supplements, is reasonably expected to be safe. Included in the attachment are the following:

(1) chemistry, manufacturing, and stability information;

(2) a description of the intended use;

(3) a summary of the biological studies of L-5-methyl-THF calcium salt; and

(4) a conclusion

(5) and a list of references.
Copies of 34 cited references, including English translation of two references.

Sincerely,

Merck KGaA

ppa.

Dr. Roland Martin

i. V.

Dr. Najib Sehat

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New Dietary Ingredient Notification Attachement

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TABLE OF CONTENTS

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1. Chemistry Considerations Concerning L-5-methyl-THF

1.1 Chemical Name

(6S)-N-[4-[[(2-amino-1,4,5,6,7,8-hexahydro-5-methyl-4-oxo-6-pteridinyl)methyl]amino]benzoyl]-L-glutamic acid, calcium salt.

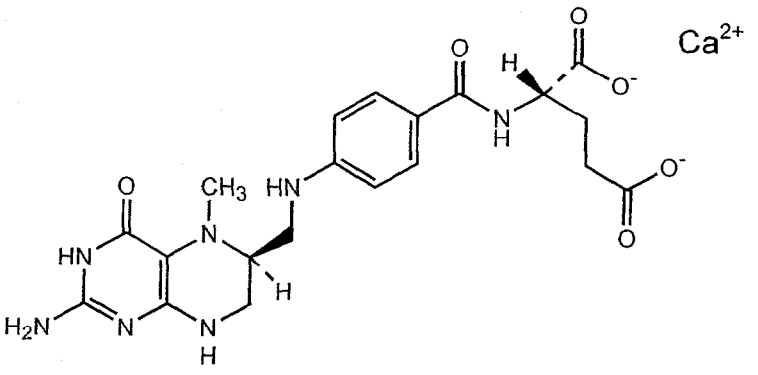
1.2 Chemical Abstract Service (CAS) Registry Number

129025-21-4

1.3 Chemical Synonyms

Calcium-L-mefolinate
L-5-methyl-tetrahydrofolic acid
(6S)-5-methyltetrahydrofolic acid, calcium salt
(6S)-5-methyl-5,6,7,8-tetrahydropteroyl-L-glutamic acid, calcium salt

1.4 Chemical Structure



1.5 Molecular Formula

$C_{20}H_{23}CaN_7O_6$

1.6 Molecular Weight

497.5

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1.7 Physical and Chemical Properties

Physical state, color:	Yellowish crystalline powder
Odor:	Almost odorless
pH (2.5%)	Between 7 and 8
Melting point:	Degradation > 300 ° C
Flash point:	Degradation > 300 ° C
Explosion properties:	Not available, no special properties
Density:	About 0.25 g/cm^3
Solubility:	Soluble in water, but virtually insoluble in organic solvents
Degradation:	Subject to oxidative and nonoxidative modes of degradation (Gregory 1989, 1996)

1.8 Manufacturing Method

The calcium salt of L-5-methyl-THF is manufactured from folic acid in accordance with current good manufacturing practices using both food grade and pharmaceutical grade reagents. In this procedure, folic acid is chemically reduced to

This procedure yields the desired product in high purity and with clear spectral and chromatographic evidence of identity and correct chiral form.

1.9 Specifications

The calcium salt of L-5-methyl-THF is a beige to yellow crystalline powder that has a characteristic infrared absorption spectrum. Merck's specifications for L-5-methyl-THF calcium salt are shown below in Table 1. These specifications indicate a high degree of purity generally consistent with the use of this substance as a nutritional dietary supplement.

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TABLE 1. Specifications for L-5-methyl-THF, calcium salt

1.10 Stability

The stability of 5-methyl-THF has been examined most extensively with regard to both the conditions of thermal processing of foods and the retention of folic acid added in fortification. All folates are subject to chemical deterioration (reviewed by Gregory 1989,1996). Folic acid is very stable under physiological conditions and most conditions of food processing and storage. Tetrahydrofolate is highly susceptible to oxidative cleavage, while substituents at the N-5 position impart improved stability. 5-Methyl-THF is easily oxidized to 5-methyl-dihydrofolate, which retains vitamin activity by virtue of its ability to undergo facile reduction by thiols (e.g., cysteine or glutathione) or ascorbate. Irreversible deterioration of 5-methyl-THF appears to occur mainly by chemical rearrangement of 5-methyl-dihydrofolate to form the pyrazino-s-triazine derivative. Exposure of 5-methyl-dihydrofolate to acidic conditions also yields cleavage of the C9-N10 bond. 5-Methyl-THF is often found to exhibit intermediate stability; it is often significantly less stable than folic acid. The products of 5-methyl-THF breakdown do not appear to be of toxicological significance as they are common constituents of most foods.

The manufacturer has conducted initial tests of the stability of L-5-methyl-THF calcium salt. As seen in many previous studies, the compound degrades completely

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over several days in aqueous solution in the absence of ascorbate or other reductant. Data regarding the stability of the powdered L-5-methyl-THF calcium salt during extended storage at various temperatures and relative humidities indicate excellent stability. Storage stability of the L-5-methyl-THF calcium salt is comparable to or better than that of folic acid. See Tables 2 and 3.

Table 2. Retention of Folic Acid and L-5-Methyl-THF Calcium Salt in Standard Multivitamin Tablets Stored in Blister Packs		
Sample, Storage Time	25°C, 60% Relative Humidity	40 °C, 75% Relative Humidity
Folic acid, 0 months		
Folic acid, 3 months		
Folic acid, 6 months		
L-5-methyl-THF, 0 months		
L-5-methyl-THF, 3 months		
L-5-methyl-THF, 6 months		

Table 3. Stability of L-5-Methyl-THF Calcium Salt During Storage Under Varying Conditions of Temperature and Relative Humidity				
Storage Time in Months	25 °C, 60% Relative Humidity	40 °C, 75% Relative Humidity	4 °C (Humidity Not Specified)	-15°C (Humidity Not Specified)
	----------Assay Percentage (% water-free)----------			
0				
3				
6				
9				
12				
18				

2. Intended Use

2.1 Natural Presence in Diet

Naturally occurring L-5-methyl-THF is a common type of folate in human diets. The (6R)-isomer of 5-methyl-THF does not occur naturally. Naturally occurring folates exist as (poly)glutamate conjugates having from 1 to 8 glutamate residues (Gregory 1989). Although the full distribution of folate forms has not been determined in many

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foods, chromatographic analyses of a wide range of food classes suggests that 5-methyl-THF is the major form of folate in most human diets. The following is a summary of data from representative chromatographic analyses of naturally occurring folates in foods (Table 4). From these data it is clear that all humans consume a high proportion of L-5-methyl-THF chronically from natural food sources.

Table 4. Total Folate and 5-Methyl-THF Content in Nonfortified Foods

Food Type	Total Folate by HPLC Assay	Percent 5-CH_3-THF of Total Folate	Reference
White Bread	21.3±0.69 µg/100g	22.5%	Pfeiffer et al. 1997a
Wheat Bread	29.8±1.94 µg/100g	11%	Pfeiffer et al. 1997a
White Rice	10.8±0.57 µg/100g	34.9%	Pfeiffer et al. 1997a
Spaghetti	22.3±1.77 µg/100g	12.7%	Pfeiffer et al. 1997a
Orange juice	0.23-0.40 µg/ml	100%	Gregory et al. 1984; White et al. 1991
49 Vegetable & fruit products	10-187 µg/100g	mean 70%	Muller 1993a
15 Egg, meat & fish products	1-963 µg/100g	4.5-90.6%	Muller 1993b
10 Dairy products	0.3-398 µg/100g	5.1-36.2%	Muller 1993b
Egg yolk	1.93 nmol/g	100%	Seyoum & Selhub 1993
Cow liver	7.69 nmol/g	19.4%	Seyoum & Selhub 1993
Lima beans	2.27 nmol/g	35.2%	Seyoum & Selhub 1993
Baker's yeast	69.1 nmol/g	86.4%	Seyoum & Selhub 1993

2.2 Intended Use as a Source of Folate

The intended use of L-5-methyl-THF, calcium salt in dietary supplements as a source of folate is based on the compound's vitamin activity, and the physiological and metabolic characteristics that make it advantageous over folic acid, as discussed below. The L-5-methyl-THF calcium salt product will be used as such or microencapsulated in a food grade material, such as a maltodextrin or cellulose-based substance, prior to use. The microencapsulated product is to be used as a 1:1 replacement for folic acid so its use levels would mirror the existing folic acid use levels in dietary supplements.

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3. Biological Studies

3.1 Vitamin Activity of Folic Acid and Folates

Folate is the generic term for the family of pteroylglutamates that exhibit the qualitative vitamin activity of folic acid. As outlined below in Figure 1, folic acid (pteroylglutamic acid) is the form of the vitamin having a fully aromatic ("oxidized") pteridine ring system. This is the chemical form used most frequently for nutritional supplements because of its relative ease of synthesis and chemical stability. Most naturally-occurring dietary folates and folates in mammalian tissues are 5,6,7,8-tetrahydrofolates, primarily as polyglutamyl conjugates (Figure 1). The tetrahydrofolates function in metabolism as acceptors, carriers, and donors of one-carbon units. An additional important function of tetrahydrofolates is to mediate oxidation and reduction of folate-bound one-carbon units (*i.e.*, conversions among formyl, methylene and methyl moieties). The tetrahydrofolates carry one-carbon units as substituents at the N-5 or N-10 positions or as methylene or methenyl carbons bridging from N-5 to N-10 (Figure 1). The tetrahydrofolates have asymmetric centers at the pteridine #6 carbon and at the alpha-carbon of the glutamate residue(s). Enzymatic reduction of folic acid yields only the L-diasteroisomer of tetrahydrofolate, also termed L-tetrahydrofolate, while chemical reduction yields an equimolar mixture of R and L-diasterisomers. The L-isomer has nutritional activity while the R-isomer appears to be metabolically inert in most instances.

3.2 Nutritional Equivalence of L-5-Methyl THF and Folic Acid

Folic acid and 5-methyl-THF exhibit approximate nutritional equivalence with respect to their intestinal absorption, metabolism and in vivo kinetics in biological systems. These compounds give similar response in optimized bacterial assay systems used to measure food folate (Phillips and Wright 1982). All folates are effectively absorbed, with only small differences in transport kinetics reported (Selhub et al. 1983, 1984). Nutritional studies in both chicks (Ristow et al. 1982) and rats (Bills et al. 1991) have shown that diets supplemented with racemic (6R,S)-5-methyl-THF exhibited folate activity similar to that of diets fortified with half the molar concentration of folic acid. These results suggest that the L-isomer of 5-methyl-THF exhibits similar molar activity to folic acid in supporting chick and rat growth and in maintaining blood and tissue folate levels while the R-isomer is inert. This interpretation is confirmed by an additional study in rats that examined the absorption, metabolism and in vivo kinetics of orally administered radiolabeled forms of folic acid, (6S)-5-methyl-THF and (6S)-5-formyl-THF (Bhandari and Gregory 1992). At the tracer doses used, absorption

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of each compound was essentially complete, and there were no significant differences in metabolism or excretion kinetics. A similar study was conducted in humans who were administered a single 400 µg stable isotope labeled bolus dose of either folic acid, (6S)-5-methyl-THF, or several other reduced folates (Gregory et al. 1992). In total, these data strongly support the nutritional equivalence of folic acid and (6S)-5-methyl-THF, as well as (6S)-5-formyl-THF.

Figure 1. Chemical structures of folates.

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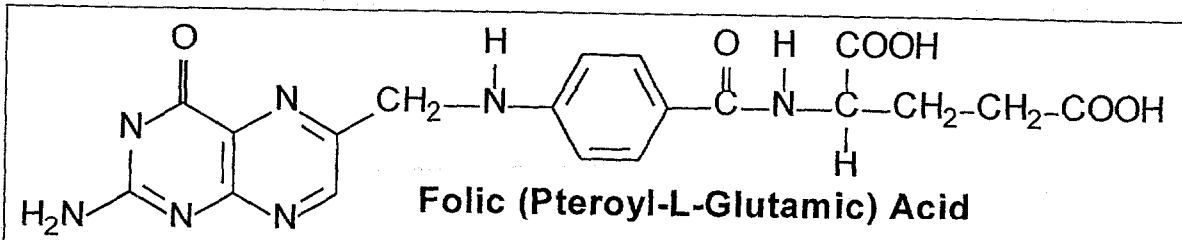
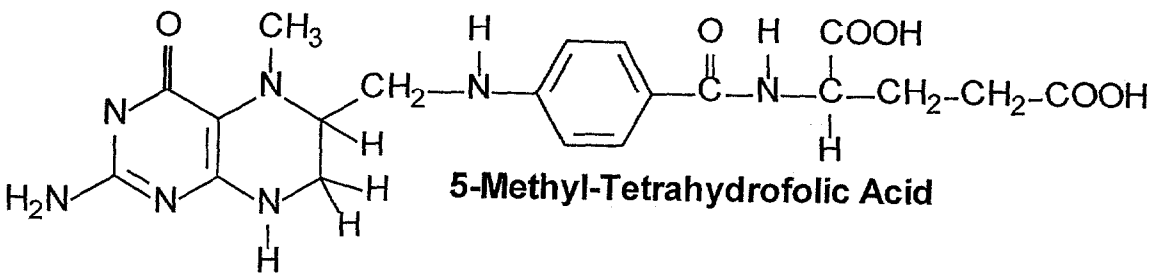
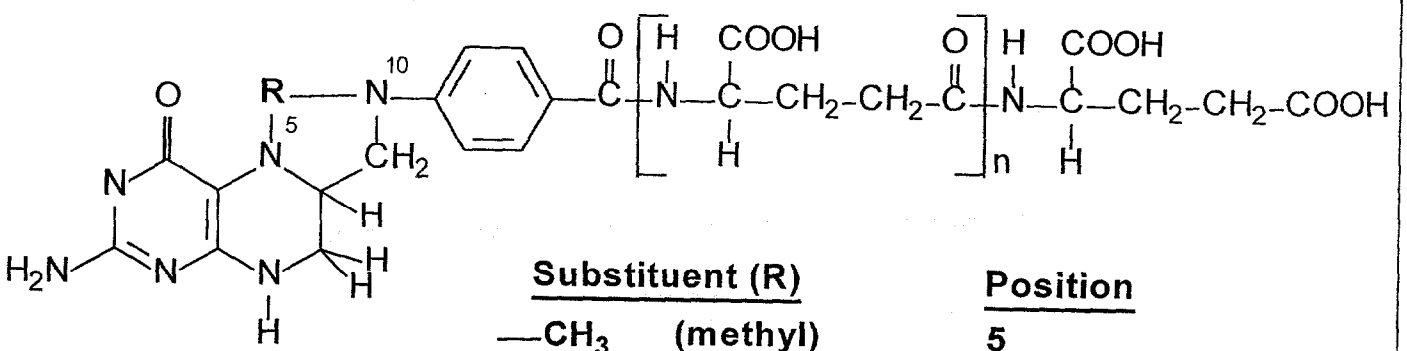


Folic (Pteroyl-L-Glutamic) Acid



5-Methyl-Tetrahydrofolic Acid

Polyglutamyl Tetrahydrofolates



Substituent (R)		Position
—CH_3	(methyl)	5
—CHO	(formyl)	5 or 10
—CH=NH	(formimino)	5
—CH_2—	(methylene)	5 and 10
—CH=	(methenyl)	5 and 10

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3.3 Absorption, Distribution, Metabolism and Excretion

Absorption. Folate absorption occurs mainly in the jejunum. At concentrations associated with most dietary folate intakes, absorption occurs by a carrier-mediated process with a Km in the low micromolar range (Selhub et al. 1983). With higher intakes of folates, associated with intralumenal concentrations of 5-10 µM or above, intestinal transport consistent with passive diffusion predominates. Although absorption of many forms of food folate is inefficient, with a mean bioavailability of ~50% (Gregory 1997), the bioavailability of folate taken as a supplement is high (Pfeiffer et al. 1997b). During the intestinal absorption of folic acid, a substantial dose-dependent fraction undergoes reduction and methylation to form 5-methyl-THF (Selhub et al. 1983). Folate moves from intestinal mucosa to the liver via the portal system. 5-Methyl-THF would undergo effective intestinal absorption, as would all other forms of folate when consumed as a supplement. Studies of intestinal transport indicated only minor kinetic differences among the various folates (Selhub et al. 1983,1984). Early studies in humans suggested effective absorption of 5-methyl-THF (Perry and Chanarin 1970), although the methods used were not sufficiently specific to be conclusive. *In vivo* studies in which human subjects were administered various stable isotopically labeled forms of monoglutamyl folates (including 5-methyl-THF and folic acid) yielded no evidence of a substantial difference in absorption between the forms (Gregory et al. 1992).

Metabolism. Folate (as cellular polyglutamyl tetrahydrofolate, THF) accepts one-carbon units primarily from serine to form 5,10-methylene-THF, which undergoes reduction to form 5-methyl-THF. (See Figure 2 below and Wagner 1995.) 5-Methyl-THF is committed solely to the transfer of its methyl group to homocysteine, yielding methionine and THF. 5,10-Methylene-THF can serve as a carbon donor in nucleotide synthesis in two ways. First, 5,10-methylene-THF can directly transfer its carbon in the thymidylate synthase reaction. Additionally, 5,10-methylene-THF can be converted to 10-formyl-THF, which functions as a carbon donor in two steps of the purine de novo synthesis pathway. Thus, folate is intimately linked to nucleotide synthesis. Folate deficiency is associated with impaired cellular development (including anemia) because of the vital cellular role of these processes.

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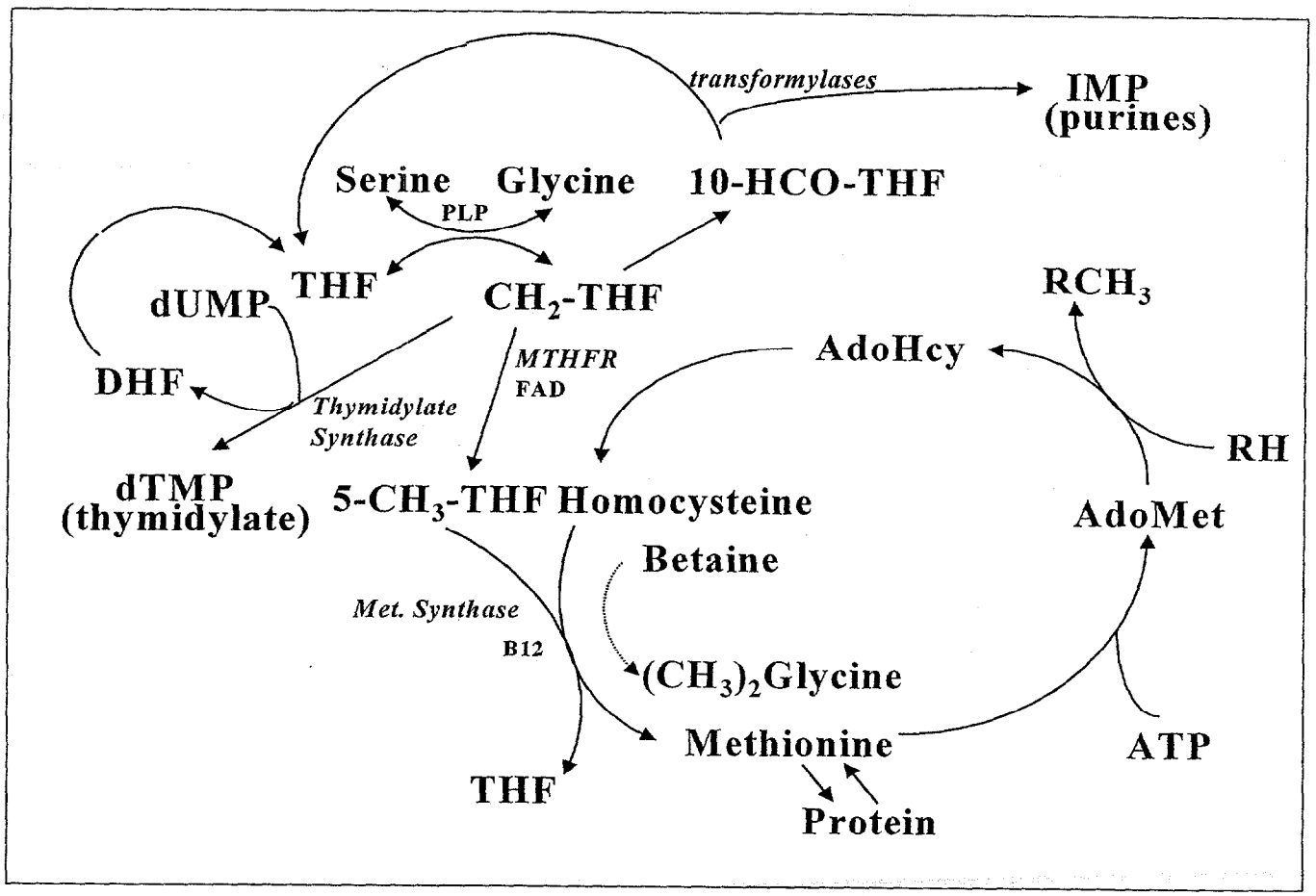
Figure 2. Summary of one-carbon metabolism.



Distribution. When folates are taken up by cells (hepatic or other), several mechanisms serve to achieve their retention. Upon transport into a cell, folates are immediately subject to conversion to polyglutamates (Cook et al. 1987). Introduction of the polyglutamate chain provides a multiple negative charge that serves as a form of metabolic trapping. Several forms of polyglutamyl folates are tightly bound to folate-dependent enzymes, which also increases cellular retention. Retention is further augmented by transport of a significant proportion of folates into mitochondria.

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5-Methyl-THF is a very poor substrate for mammalian folyl polyglutamate synthetase, the enzyme responsible for catalyzing this polyglutamylation (Cichowicz and Shane 1987). As a result, cellular retention of newly absorbed 5-methyl-THF would occur mainly to the extent that it is utilized in the methionine synthase reaction (*i.e.*, transferring its methyl group to homocysteine, yielding methionine). The resulting THF then undergoes elongation to a polyglutamyl form and is strongly retained in the cell, subsequently entering other aspects of folate/one-carbon metabolism. Biochemical data support the view that 5-methyl-THF polyglutamates are formed metabolically from the reduction of 5,10-methylene-THF polyglutamates rather than by direct polyglutamylation of newly absorbed 5-methyl-THF monoglutamate. Two key enzymes, methionine synthase and folyl polyglutamate synthetase, metabolically prevent accumulation of inordinately high levels of 5-methyl-THF in tissues even under conditions of high intake.

Whole body folate physiology, including folate distribution and enterohepatic circulation, has been reviewed in detail. (Steinberg 1984). Steinberg reports that 10-20% of folate administered enterically or intravenously in rats is taken up by the liver during the first pass. Although this has not been determined directly in humans, comparison of oral and intravenous doses of labeled folates indicates large differences in plasma area-under-the-curve (~15-fold), which is attributable to a large hepatic first-pass effect probably along with extensive enterohepatic circulation (Rogers et al. 1997). Enterohepatic circulation of hepatic folates, largely as 5-methyl-THF, is a normal and prominent feature of folate physiology (Steinberg 1984, Steinberg et al. 1979, Gregory et al. 1998b). Folate is distributed among tissues primarily as 5-methyl-THF monoglutamate, mainly bound to several plasma proteins. With oral doses of folic acid approaching 400 µg, the capacity of intestinal and/or hepatic metabolic systems is exceeded and a variable fraction of the dose appears in plasma as unmetabolized folic acid rather than being converted to 5-methyl-THF (Kelly et al. 1997). Based on analysis of urinary folates, little or no unmetabolized folic acid is excreted at doses of up to 400 µg/d (Gregory 1998a, b). The use of 5-methyl-THF in nutritional supplements would eliminate the potential for such metabolic overload.

The kidney plays a vital role in conserving folate by actively reabsorbing folate from the glomerular filtrate (Steinberg 1984). Urinary excretion of folate is typically 1-5 µg/d at typical dietary intakes of 200-300 µg/d (i.e. ~1% of intake). The percentage of folate intake that undergoes urinary excretion increases markedly at or above intakes of 400 µg/d (Gregory et al. 1998a, b). This occurs because plasma folate concentration (and thus folate in the glomerular filtrate) exceeds the capacity for renal tubular reabsorption. In addition, the fraction of newly absorbed folate that is retained in tissues declines with increasing intake. Regardless of the level of folate intake,

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catabolism and fecal excretion remain the primary routes of excretion (Gregory et al. 1998b).

5-Methyl-THF, whether derived from dietary or supplemental 5-methyl-THF or formed during absorption of folic acid, is not well retained in hepatic cells. 5-Methyl-THF can leave liver cells by two main routes. First, it can return to the blood circulation; 5-methyl-THF monoglutamate is the main plasma folate species. Second, it can be secreted back into the intestine via the bile (Steinberg 1984). At nutritionally typical levels of total folate intake (i.e. ≤400 µg/d), kinetic modeling suggests that a large proportion of the secreted biliary folate is reabsorbed (Gregory et al. 1998b). Enterohepatic circulation at higher levels of folate intake has not been studied.

Excretion. The rate and extent of urinary excretion of intact folate is a function of plasma folate concentration. Urinary excretion serves as a buffer in regulating whole-body accumulation during acute or chronic administration of 5-methyl-THF. At plasma concentrations of total folate (which are mainly 5-methyl-THF) above 20 ng/mL, the extent of urinary excretion increases (Gregory et al. 1998a). Kinetic modeling also shows that fecal excretion of folate will increase with high levels of intake (Gregory et al. 1998b), although this has not been evaluated directly.

3.4 Safety Data

There is little or no evidence of toxicity of folic acid that is in any way relevant to conditions of human exposure. Butterworth and Tamura (1989) summarized this topic largely from the viewpoint of chronic supplementation. Several others have suggested that folic acid may reduce zinc absorption, but a specific study to examine this issue indicated that folic acid administered to humans does not alter zinc status (Kauwell et al. 1995).

The FDA has approved folic acid for use in food fortification in 21 C.F.R. §172.345. In its rulemaking procedure, FDA reviewed the available information on folates (scientific evidence), sought guidance from its Folic Acid Subcommittee and other experts, and invited comments from the public. The Agency then concluded that the safe upper limit of folate intake was 1 mg/day (1000 mcg/day). "The agency did not receive any data relating to the safety of long-term intakes of folate at levels above 1 mg per day for any of the groups considered at potential risk from increased intakes." (FDA 1996a, at 8766) And further, "that total folate intake from all sources needs to be considered at arriving at a safe upper limit of daily intake." (FDA 1996a, at 8770) There are reports that humans have ingested 5 mg folic acid/day for months without the appearance of adverse effects. Women who have had a pregnancy resulting in a

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child or fetus with a neural tube defect are advised to elevate their folate intake to 4.0 mg. per day under the care of a physician if they are at risk of another pregnancy (Centers for Disease Control 1992, at 6-7; The American College of Obstetricians and Gynecologists 1996, at 3).

There are no data in the scientific literature on the safety of L-5-methyl-THF. However,
L-5-methyl-THF is considered safe at the anticipated levels of consumption because it will be used as an alternative to folic acid and therefore the daily intake of folates will not be exceeded, and because the metabolites of the synthetic L-5-methyl-THF are identical to the metabolites of the L-5-methyl-THF found naturally in foods and consumed daily without apparent harm. In addition, L-5-methyl-THF is less likely to mask the anemia of vitamin B_{12}.

3.5 Reduced Risk of Masking Anemia in Vitamin B_{12} Deficient Individuals

Metabolic and physiological evidence suggests that 5-methyl-THF is less likely than folic acid to mask anemia in vitamin B_{12} deficient individuals, although direct studies have not been conducted. The concern regarding folate supplementation of vitamin B_{12}-deficient people is that the anemia would be masked while the irreversible neurological deterioration continues. The masking of anemia in a B_{12} deficiency can be detrimental because it may delay diagnosis of the true cause: B_{12} deficiency. The masking effect of 5-methyl-THF would only occur to the extent that it could be processed through the cobalamin-dependent methionine synthase reaction (Green et al. 1988). Thus, the likelihood that supplemental 5-methyl-THF will mask vitamin B_{12} deficiency by reversing the anemia to be much less than with a folic acid supplement.

The main concern with folic acid is indirect, *i.e.*, that it can reverse the anemia associated with vitamin B_{12} deficiency while the irreversible neurological deterioration continues. This anemia mainly occurs due to the "methyl trap" effect, in which methionine synthase activity is low due to lack of the cobalamin coenzyme, in addition to a depletion of tissue folate that occurs concurrently (Cook et al. 1987). If an individual is marginally deficient in vitamin B_{12}, folic acid supplementation can increase nucleotide synthesis to stimulate DNA synthesis and cellular maturation, thus alleviating the B_{12}-deficiency megaloblastic anemia. This is apparent as a reticulocyte response following administration of supplemental folate in patients with pernicious anemia. In view of the role of B_{12}-dependent methionine synthase in this process, it is predicted that 5-methyl-THF used in dietary supplements will have much less of an anemia-masking effect than folic acid.

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4. Conclusion

At the request of Merck, an independent panel of recognized experts, qualified by their scientific and/or medical training and relevant national and international experience to evaluate the safety of food and food ingredients, was convened in order to assess the generally recognized as safe (GRAS) status of L-5-methyl-THF, calcium salt for use as a source of folate in dietary supplements.

A comprehensive search of the scientific literature concerning nutritional, safety, and toxicity information was conducted and made available to the panel. The panel independently and critically evaluated the pertinent articles as well as the other information discussed above and concluded that, under the conditions of intended use, L-5-methyl-THF, meeting appropriate food grade specifications and produced in accordance with current good manufacturing practices, is GRAS based on scientifc procedures (Borzelleca et al. 1999).

Based on the above-described data and information, Merck concludes that its L-5-methyl-THF, calcium salt, when used as a source of folate under the conditions recommended or suggested in the labeling of such dietary supplements, is reasonably expected to be safe.

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