

## EXPERT GROUP ON VITAMINS AND MINERALS

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### REVIEW OF FOLIC ACID

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The attached review of folic acid is a slightly revised version of the paper first presented to the Expert Group on Vitamins and Minerals at the meetings on 1 November 2000, October and 19 December 2001.

The following annexes are also attached:

- Annex 1 Tables and figures referred to throughout the review
- Annex 2 Intakes of folic acid from food and supplements in the UK
- Annex 3 Summary table of selected nutrition related information and existing guidance on intakes

Expert Group on Vitamins and Minerals Secretariat  
August 2002

## FOLIC ACID

### Chemistry and geochemistry

1. Folate is a generic term for compounds that have a common vitamin activity and includes the synthetic form of the vitamin folic acid (pteroyl glutamic acid) and a wide variety of derivatives (Department of Health 2000). Folic acid, the synthetic form of folate, is not present in nature.

2. PGA (CAS Registry Number 59-30-3;  $C_{19}H_{19}N_7O_6$ ; relative molecular mass 441.4) is composed of three major subunits – pteridine, *p*-aminobenzoic acid, and glutamic acid. The pure compound is odourless and tasteless, is virtually insoluble in water (soluble to approximately 1% in boiling water), alcohol, acetone, chloroform and ether; soluble in hydrochloric and sulphuric acids, yielding pale yellow solutions. The disodium salt is freely soluble. Aqueous solutions of PGA are heat sensitive and decompose rapidly in the presence of light. The crystalline form is yellowish-orange in colour, stable at 4°C, and in alkaline solution, but is less stable in acid solution (Davis 1986, Herbert 1999).

### Natural occurrence

3. PGA itself is not present in significant quantities in foods or in the human body (Herbert 1999). The derivatives of PGA which are predominantly present in the human body, and in plant- and animal-derived foods, are reduced folates, mostly 5,6,7,8-tetrahydrofolates (THF), and also 7,8-dihydrofolate (DHF). Other modifications that occur are various one-carbon adducts linked to THF at the N-5 and/or N-10 position, and the presence of multiple (generally up to 7, less commonly up to 11) glutamate residues linked by peptide bonds between the amino group and the  $\gamma$ -carboxyl group of the preceding glutamate residue.

4. Within this report, the term folic acid is used to indicate the parent compound, pteroylglutamic acid, whilst folate is used in a generic sense to indicate one or a mixture of pteroylglutamates, with various levels of reduction of the pteridine ring, one-carbon substitutions, and numbers of glutamate residues. This nomenclature follows guidelines of an IUPAC-IUB advisory panel on folic acid/folate nomenclature (*cited in* Herbert 1999).

### Occurrence in foods, food supplements and medicines

#### *Foods*

5. Folates are ubiquitous in nature, being present in the majority of natural foods. Rich sources of folate (> 100  $\mu\text{g}/\text{serving}$ ) are Brussel sprouts, asparagus, spinach, kale, black eyed beans and liver. Other vegetables and fruits that contain lower but significant amounts of folate (50 - 100  $\mu\text{g}/\text{serving}$ ) include broccoli, spring greens, cabbage, cauliflower, iceberg lettuce, parsnips and oranges. It is also present in yeast, yeast extract and beer (Department of Health 1992). Polyglutamate forms based mainly on 5-methyl-THF account for approximately 90% of the folate forms in fresh foods, with the majority of the remainder in the 10-formyl form (Butterworth *et al*

1963, Stokstad & Koch 1967). These compounds are unstable and undergo oxidation and breakdown to monoglutamates during storage and preparation. A significant proportion of food folates may be lost to cooking water during boiling. It has been estimated that 50 - 95% of food folate may be destroyed and/or lost by extensive cooking or processing, and that the refining of foods removes all folate (*reviewed by Davis 1986, Recommended Dietary Allowances 1989, Herbert 1999*).

6. At present the most widely used and accepted procedure for food folate analysis is the microbiological assay using *Lactobacillus rhamnosus* (formerly *L. casei*) as the test organism. (paragraph 24). This assay is sensitive only to short-chain polyglutamates, and thus analytical accuracy may be aided by first treating foods with conjugase enzymes (paragraph 48) which reduce long-chain polyglutamates. An indication of the folate content of a selection of common foods is given in Table 1.

#### *Fortification of foods*

7. The synthetic pharmaceutical form used for food folic acid fortification and in folic acid-containing supplements is PGA, due to the greater stability of this compound relative to other forms of the vitamin (*cited in Herbert 1999, Rothenberg 1999*).

8. Although folic acid is not naturally present in foods it is widely used as a fortificant. It is readily absorbed and metabolised in the liver to the polyglutamate forms, which are indistinguishable from those derived from naturally occurring folates in food. Breads and breakfast cereals are the foods that manufacturers have customarily fortified with folic acid. It has been estimated that between 80 and 90% of breakfast cereals consumed are fortified with folic acid. Muesli-type products, which account for approximately 11% of the breakfast cereal market are not usually fortified. Most folic acid fortified products contain between 125 and 200 µg/100g. Some brands are fortified at a substantially higher level (333 µg/100 g). Fortification of bread is less widespread and it is mainly the softgrain varieties that are fortified to a level of approximately 120 µg/100 g (Department of Health 2000). Some low-fat spreads have also been fortified to a level of 200µg/20g portion.

9. At present fortification of food products with folic acid in the UK is solely on a voluntary basis. The Committee on the Medical Aspects of Food and Nutrition Policy (COMA) reviewed the role of folic acid in the prevention of disease (Department of Health 2000). They suggested a number of options including universal fortification of flour with folic acid at the following levels 140 µg/100 g of flour, 200 µg/100 g, 240 µg/100 g, 280 µg/100 g or 420 µg/100 g as a means of reducing the number of neural tube defect (NTD) affected pregnancies. The Department of Health and The Food Standards Agency consulted on the COMA report *Folic Acid and the Prevention of Disease* and a decision on the fortification of flour with folic acid is being considered. The issue of folic acid fortification of foods is discussed in a recent review article (Mills 2000).

#### *Licensed medicinal products for oral use*

10. Folic acid products may be sold in supermarkets and other retail outlets without the supervision of a pharmacist for use prior to pregnancy and during the first

trimester to reduce the risk of neural tube defect, where there is no history of this condition in a previous child. Most products contain 400 micrograms, but the maximum permitted under the Medicines (General Sales List) Order, 1984 is 500 micrograms.

11. Multi-nutrient products with maximum daily doses up to 500 µg folic acid are generally only available from pharmacies.

12. Folic acid preparations providing a daily dose of more than 500 µg are only available on prescription. Most products provide a maximum daily dose of 5 mg for the treatment of folate-deficient megaloblastic anaemia, or for prophylaxis against folate deficiency in chronic haemolytic states and renal dialysis, or to prevent neural tube defect where there is a history of this condition in a previous child.

#### *Other uses*

13. Folic acid is widely available as a nutritional supplement, either alone or as a component of B-complex or multivitamin supplements.

#### **Intake/Exposure**

14. Humans are unable to synthesise folate directly and, thus, requirements are provided by the diet, either as the naturally-occurring substance, or *via* fortified food or dietary supplements. Folate is also synthesised by organisms within the human gut, but this does not provide a significant contribution to the host nutrition (*cited in Davis 1986*).

#### *Food*

15. Average daily intakes from food and supplements for adults aged 16-64 years were 321 µg for men and 219 µg for women in 1986/7 (Gregory *et al* 1990). However, intake from folic acid supplements, particularly in women of child-bearing age, and from fortified breakfast cereals and breads, can be expected to have increased since then. Supplements made a substantial contribution to average intakes in some age groups, particularly older adults who were taking prescribed folic acid supplements (see Annex 2).

#### **Recommended amounts**

16. Current dietary reference values for folate in the UK are shown in Table 2 (Department of Health 1991). COMA reviewed the current Dietary Reference Values (DRV) for folate for the report *Folic Acid and the Prevention of Disease* (Department of Health 2000). The current folate DRV is based on its requirement for the avoidance of megaloblastic anaemia. Red cell folate is used as a marker of adequacy. A variety of new data has accumulated since 1991 when the DRVs were established. In particular there is evidence that a modest daily increase in folic acid of around 100 µg to 200 µg/day can significantly reduce the risk of NTDs and also lower plasma homocysteine levels (Department of Health 2000). The Group reviewed the DRVs for folate particularly considering whether raised homocysteine could be used as a functional marker of folate deficiency. COMA concluded that homocysteine is not a

specific marker for folate status and that there is insufficient evidence to revise the DRV at the present time (Department of Health 2000).

#### *Adults*

17. The lower reference nutrient intake (LNRI) is determined as 100 µg/day for adults, based on observational studies of levels of folic acid supplementation required to treat the clinical symptoms of folate-deficiency. A reference nutrient intake (RNI) of 200 µg/day for adults is based upon the assessment of average daily intakes within the UK and other populations, in which clinical or haematological signs of folate deficiency are rare.

#### *Infants and children*

18. Folate-deficiency anaemia has not been identified in breast-fed infants, even in those with folate-deficient mothers. Breast milk is estimated to provide approximately 40 µg/day folate. A formula containing 50 - 60 µg/day produced lower red cell folate levels than breast milk therefore the Reference Nutrient Intake (RNI) for formula-fed infants is set at 50 µg/day (Department of Health 1991). LRNI/RNI values for older children are extrapolated from adult values. A recent study (Moynihan *et al* 2001) has shown that, without flour fortification 7% of adolescent girls fail to reach the UK lower reference nutrient intake for total folate.

#### *Pregnancy and lactation*

19. Recommendations for folate intake to allow for increased requirements during pregnancy and lactation are 100 µg/day and 60 µg/day, respectively, in addition to the normal adult RNI.

20. Health experts recommend peri-conceptional folic acid supplementation in women for the prevention of neural tube defects in developing foetuses. The Department of Health recommends that all women planning a pregnancy take 400 µg of folic acid from when they cease contraception to the twelfth week of pregnancy to reduce the risk of them having a NTD-affected pregnancy. Women who have a family history of NTDs are advised to take a 5 mg supplement (Department of Health 1992). The Health Education Authority recently reported that the proportion of women claiming to take folic acid when planning for a baby rose from 24% in 1997 to 38% in 1998 (Health Education Authority, 1998).

#### *International and US guidelines (data as cited in, and summarised in Shils *et al.*, 1999)*

21. WHO (1990), EC (1992) and previous US (1989) guidelines for folate intake are, on the whole, similar to those in the UK, although recommended intakes are generally higher for pregnant women.

22. However, a recent reassessment of folate requirements by the US Food and Nutrition Board led to significant (2 to 3-fold) increases in the recommended dietary allowance (RDA) values established for this nutrient (Bailey, 1998) (Table 3), as compared with the previous standards (Food and Nutrition Board 1989). These

increases were based upon recent evidence indicating that increased folate intake by women during the peri-conceptual period is associated with reduced incidence of babies born with NTDs, and also on suggestions that increased folate intake may reduce the incidence of vascular disease in the general population (paragraphs 67 - 69).

#### **Analysis of tissue levels** (Chanarin 1990, Tamura 1990)

##### *Serum and red cell folate*

23. Folate status is usually measured by determination of serum and/or red cell folate levels, in which the predominant folate form is 5-methyl-THF.

24. Original methods of analysis were based upon microbiological assays, using *Lactobacillus casei*, *Streptococcus faecalis*, or *Pediococcus cerevisiae*. *L. casei* assay has been most widely used as it is more sensitive, responds well to 5-methyl-THF, and can be used to measure both serum and red cell folate. This assay also measures triglutamate and other short-chain folate forms, but does not respond well to longer chain folates. The *S.faecalis* and *P.cerevisiae* assays do not measure methyl form folates and are, thus, of limited use in assessing body folate status.

25. Radioisotopic assays for folate have also been developed, generally based upon competitive binding of serum and radiolabelled folate to specific folate binding compounds. Such assays can be used for the accurate determination of serum folate levels, but are not satisfactory for the measurement of erythrocyte folate (Davis 1986).

26. High-performance liquid chromatography may also be used for plasma, tissue or food folate analysis (Gregory *et al* 1984, Witthoft & Bitsch 1993).

27. As a consequence of the lability of folate during storage, serum concentrations may be falsely low. To avoid this, serum may be protected against oxidative destruction by the addition of a reducing agent, such as ascorbate, although this may destroy vitamin B<sub>12</sub> within the sample (which is often assayed in parallel with folate) (*cited by* Herbert 1999).

28. Serum folate levels in humans are normally within the range of 5 - 16 ng/ml (11 - 36 nmol/l folic acid activity (PGA equivalents)) (Herbert 1999). Lawrence *et al* (1999) reported that median serum folate values in clinical specimens in the US increased from 12.6 to 18.7 ng/ml during the period 1994 - 1998, presumed to be a consequence of the introduction of mandatory cereal fortification (140 µg folic acid/100 g grain) during 1996 - 1998. Serum folate levels ≤ 3 ng/ml (7 nmol/l) indicate a negative folate balance, which may be due to a transitory reduction in folate intake or to chronic folate deficiency. This can be confirmed by assessment of red cell folate status, for which a level of < 140 ng/ml (317 nmol/l) indicates reduced body stores, whilst levels < 100 ng/ml (226 nmol/l) indicate chronic deficiency (Bailey 1990, Department of Health 1991). (Measurement of erythrocyte folate is actually a measure of folate status at the time the cell was synthesised, as only DNA-synthesising cells within the bone marrow take up folate).

*Liver folate*

29. Liver folate levels may be measured if a biopsy has been taken. The normal mean value is cited as 7.1 µg/g (folate/wet weight liver), with deficiency being indicated by a level < 1 µg/g (Chanarin 1990).

**Bioavailability**

30. The availability of folate from a typical North American diet has been suggested as ≈ 50 - 75% (Herbert 1987a). Sauberlich *et al* (1987) reported that the overall bioavailability of naturally-occurring folate in a mixed diet is ≤ 50% compared with synthetic folic acid given in a formula diet. The results of a long-term, controlled feeding study showed that natural folate resulted in a significantly smaller increase in red blood cell folate concentration, relative to folic acid supplements or folic acid in fortified cereals, whilst the bioavailability of folic acid in fortified food was not significantly different from that of folic acid supplements (Cuskelly *et al* 1996). Neuhouser *et al.* (1998) reported that the serum folate response (area under the curve – AUC) of a single oral dose of 0.4 mg synthetic folic acid (PGA) (as a supplement pill) was significantly higher (approximately 1.5-fold) than that of a single dose of spteroylpolyglutamic acid (as 32 fluid ounces of orange juice) in a group of 18 women with a history of either normal- or NTD-affected pregnancies.

31. Colman and colleagues compared the relative bioavailability, on the basis of short-term change in plasma folate, of synthetic folic acid, added as a food fortificant to cereal grain foods of native South Africans. Folic acid added to maize and rice, or to bread, was approximately 50 - 60% and 40% bioavailable, respectively, as compared with that of the compound given in water (Colman *et al* 1975a, b, Margo *et al* 1975). Studies using a single-dose, dual-label, stable isotope protocol showed ≈ 15% lower bioavailability of <sup>13</sup>C-labelled folic acid consumed with a breakfast meal than that consumed with water, as indicated by the urinary excretion rate in relation to that of a concurrent intravenous dose of <sup>3</sup>H-labelled folic acid (although this difference was not statistically significant) (Pfeiffer *et al* 1997).

32. Priest *et al* (1999) studied the pharmacokinetics of folic acid and its metabolites in humans. Healthy volunteers were given folic acid by oral or intravenous (*i.v.*) administration at doses of 25 or 125 mg/m<sup>2</sup> folic acid (single dose), or daily oral doses of 100 (4 x 25) mg/m<sup>2</sup> or 500 (4 x 125) mg/m<sup>2</sup> for 3 days. Results are shown in Figure 1(a-d). A single 25 mg/m<sup>2</sup> dose produced a maximal folic acid plasma response at approximately 3 hours, with approximately 60% AUC compared with *i.v.* application (6977 ± 747 nmol/l per hour and 11632 ± 817 nmol/l per hour, respectively, for oral and *i.v.* application). The concurrent plasma responses of 5-methyl-THF and methylene-THF were also comparable by oral or *i.v.* application of folic acid at this dose. At the higher dose (125 mg/m<sup>2</sup>) the AUC for oral folic acid was only 17% compared with *i.v.* administration of an equivalent dose (14,127 ± 1790 and 83 066 ± 6663 nmol/l per hour for oral and *i.v.* application, respectively), and the plasma levels of 5-methyl-THF, methylene-THF were similarly limited by oral, compared with *i.v.* application at this dose. Multiple oral dosing at the lower dose resulted in relatively constant levels of folic acid metabolites, approximately 2-fold the maximal response of a single oral dose. Plasma responses of folic acid itself to this regime were less uniform, showing similar patterns of saturable uptake as with single

dose studies. The US Institute of Medicine estimated a bioavailability of 85% for folic acid from fortified foods and that it is 1.7 times more bioavailable than food folate. In order to account for the difference in bioavailability of folates the use of dietary folate equivalents has been adopted in the US (Institute of Medicine 1998).

## Interactions

### *Antifolate drugs*

33. Some antifolate drugs inhibit the absorption of orally ingested folates by competing for the same transport system, and act as antifolate agents by targeting enzymes involved in folate metabolism, with a resultant inhibition in thymidylate and/or *de novo* purine (ie, DNA) synthesis (*reviewed by Calvert 1999*). Such agents may be given at low doses to alleviate the symptoms of conditions such as rheumatoid arthritis (RA), whilst high-dose therapy is used for the treatment of cancer. Methotrexate (MTX) is an anti-folate drug which acts mainly by inhibiting the enzyme dihydrofolate reductase (DHFR), leading to reduced cellular levels of THF and a consequent inhibition of both thymidylate and purine synthesis (paragraphs 55-57). High doses of an active folate, formyl-THF (folinic acid, leucovorin) have been shown to reduce the effectiveness of MTX in patients with RA, leading to concern that folic acid supplementation may also reduce MTX efficacy (Campbell 1996 *and refs therein*). Suzuki *et al* (1999) recently reported that 3/14 patients withdrew from a study of folic acid supplementation (5 mg/week) in MTX-treated RA patients, due to exacerbation of RA symptoms. However, a number of studies of folic acid supplementation (5-27.5 mg/day for up to one year) in (RA or psoriasis) patients receiving low-dose MTX therapy have shown reduced MTX toxicity, with no evidence of impairment of MTX efficacy (Morgan *et al* 1990, 1994, Duhra 1993). Hunt *et al* (1997) reported that 1 mg/day folic acid therapy (6 weeks) did not affect the clinical efficacy of oral weekly MTX therapy in a double-blind, randomised, placebo-controlled trial of 19 MTX-treated children with juvenile rheumatoid arthritis. Ortiz *et al* (1998) published a meta-analysis of double-blind, randomised, controlled trials in which adult patients with RA, were treated concurrently with low doses of MTX and either folic acid (total = 67 patients) or folinic acid (total = 80 patients). Folic acid therapy was associated with a statistically significant reduction in MTX-associated side-effects, whilst the authors reported no consistent differences in disease activity variables between patients receiving folic acid or placebo. High-dose folinic acid supplementation was associated with disease exacerbation.

34. Limited data are available regarding the effects of folic acid supplementation on the efficacy of anti-folate chemotherapy in cancer patients. One (retrospective) study in children receiving high-dose MTX therapy for acute lymphoblastic leukaemia showed an association of folic acid-containing multivitamin use with a reduction in MTX toxicity, but did not address the issue of potentially reduced effectiveness of the chemotherapy in those children taking supplements (Schroder *et al* 1986). A phase I clinical study of the anti-folate agent, lometrexol (which selectively inhibits GARFT –a folate-dependent enzyme involved in purine synthesis) showed that treatment of patients with folic acid (5 mg/day for 7 days prior to, and 7 days following, lometrexol treatment, at 4 week intervals) increased the maximum tolerated dose of lometrexol 10-fold. The authors reported that the mechanism responsible for this reduction in lometrexol toxicity had not been defined, but that

pharmacokinetic studies carried out on the treated patients suggested that folic acid did not enhance lomotrexol plasma clearance (Wedge *et al* 1995, Laohavinij *et al* 1996).

#### *Anticonvulsant drugs*

35. Anticonvulsant drugs (e.g. phenytoin, phenobarbital, carbamazepine) interfere with folate metabolism and may be associated with low folate status and, infrequently, with the development of megaloblastic anaemia in treated patients. The mechanism by which this occurs has not been established. Treatment to correct the folate deficiency has been associated with the precipitation of seizures or increased seizure frequency in some individuals. Ch'ien *et al* (1975) reported a study in which 8 phenytoin-treated epileptic patients were given parenteral (*i.v.*) folic acid. One patient with poorly controlled seizures experienced electroencephalographic (EEG) changes after administration of folic acid (total 7.2 mg) for 3 minutes, followed by a clinical seizure 3 minutes later (total 14.4 mg folic acid), at which point the infusion was stopped. A repeat infusion 30 minutes later (a total of 19.2 mg folic acid during a 12 minute period) induced another seizure, which was preceded by EEG changes. Another patient showed abnormal EEG without clinical manifestations after administration of 150 mg folic acid over a 30 minute period. Conversely, 6 patients given 75 mg folic acid intravenously during a 30 minute period showed no EEG, or other, abnormalities, and the authors of this report commented on the large individual differences in folic acid sensitivity in this study.

36. A few case reports have described increased seizure activity in drug-treated epileptic patients given oral folic acid therapy at doses  $\geq 1$  mg/day for periods of several weeks (Strauss & Bernstein 1974, Berg *et al* 1983, Inoue & Kolabinski 1986). Reynolds & Wales (1967) reported results from an uncontrolled trial in which 26 drug-treated epileptic subjects given 3 x 5 mg/day folic acid for 1-3 years showed an overall increase in seizure frequency (increase in 13 subjects, no change in 12 subjects, decrease in 1 subject). However, a number of (larger, many randomised and double-blind) prospective studies have shown no effect of folic acid supplementation (up to 20 mg/day for periods of several weeks or months) on seizure frequency in epileptic patients treated with anticonvulsant drugs (mostly phenytoin) (Grant & Stores 1970, Baylis *et al* 1971, Norris & Pratt 1971, Gibberd *et al* 1981, Mattson *et al* 1973, Ralston *et al* 1970, Horwitz *et al* 1968, Jensen & Olesen 1970, Brown *et al* 1991). These data are summarised in Table 4.

37. The Folic Acid Subcommittee of the United States Department of Health and Human Services has concluded that 1 mg/day oral folic acid supplementation is safe for individuals with controlled epilepsy (*cited in* Lewis 1998).

#### *Anti-inflammatory drugs*

38. Salicylazosulfapyridine (Azulfidine, sulfasalazine), an anti-inflammatory drug used for the treatment of inflammatory bowel disease, inhibits enzymes involved in folate absorption and metabolism (Reisenauer & Halsted 1981, Selhub *et al* 1978). Other commonly used nonsteroidal anti-inflammatory drugs have anti-folate activity *via* their action as inhibitors of enzymes involved in folate metabolism (Baggott *et al*

1992a). The dose-response relationships with respect to antagonistic effects on folate metabolism have not been established (*cited in* Gregory 1997).

#### *Oral contraceptives*

39. Some investigators have reported an association of oral contraceptive use with marginally reduced folate status, although others have found no effect of these drugs on folate status (*reviewed by* Davis 1986, Sauberlich 1990).

#### *Alcohol*

40. Folate deficiency is common in chronic alcoholic patients (Sauberlich 1990). In addition to low dietary intake in such individuals, studies have shown that folate deconjugation and absorption are impaired by chronic alcohol use. Alcohol ingestion also appears to block the secretion of folate into the bile, interfering with enterohepatic recirculation and re-absorption (Halsted 1990). *In vitro* studies have suggested that free radicals generated during ethanol metabolism may increase folate turnover by enhancing oxidative cleavage (Shaw *et al* 1989).

#### *Vitamin B<sub>12</sub>*

41. Folate and vitamin B<sub>12</sub> (cobalamin) metabolism are linked. Methionine synthase, a vitamin B<sub>12</sub>-dependent enzyme, is required for the conversion of 5-methyl-THF to THF, which is the active substrate for folate polyglutamate synthesis within cells. In vitamin B<sub>12</sub>-deficiency, the cobalamin-dependent conversion of 5-methyl-THF to THF is suppressed, leading to a functional folate deficiency within the cell. Red cell THF levels, and thus polyglutamate synthesis, are reduced, whilst serum folate levels are raised. Unreduced, synthetic folic acid (PGA) is metabolised to polyglutamates within the cell *via* a vitamin B<sub>12</sub>-independent mechanism (*via* DHFR and THFR) (paragraph 55), and may, thus, improve haematological status without correcting the vitamin B<sub>12</sub> deficiency.

42. Some investigators have reported that folic acid supplementation may lower serum vitamin B<sub>12</sub> levels, although others have not observed this effect. Bok *et al* (1958) reported that 10/13 pernicious anaemia patients treated with 15 mg/day folic acid, for periods of 4-8 days, showed reduced serum vitamin B<sub>12</sub> levels after, as compared with prior to, treatment. Hunter & Barnes (1969) reported that treatment of 31 hospitalised epileptic patients, for 3 months, with 15 mg/day folic acid was associated with a significant ( $\approx$  2-fold) reduction in mean serum vitamin B<sub>12</sub> concentrations compared with pre-treatment levels. Conversely, one controlled study (Norris & Pratt 1971) and a number of uncontrolled studies (Hunter *et al* 1970, Cooper & Lowenstain 1966, Herbert & Zalusky 1962, Baylis *et al* 1971, Norris & Pratt 1971) have shown no significant association between vitamin B<sub>12</sub> plasma levels and folic acid therapy at doses up to 15 mg/day.

43. The potential adverse effects of folic acid supplementation in relation to vitamin B<sub>12</sub> deficiency are discussed in paragraphs 79-86.

### Zinc

44. Folic acid and zinc may form insoluble complexes at the low pH present in the stomach, but these complexes should dissolve at the higher pH within the duodenum (Ghishan *et al* 1986). Folic acid complexation may, however, significantly reduce the absorption of zinc from zinc oxide supplements, which are insoluble at the higher pH present in the small intestine (Wolfe *et al* 1994).

45. Folic acid supplementation has been reported to have a negative effect on zinc status, although many studies have not observed this effect. Milne *et al* (1984) noted increased faecal zinc loss in 4 men given supplemental folic acid (0.4 mg every other day for 6 months) compared with 4 men not given supplements, but reported that reduced urinary losses maintained overall zinc balance. In an uncontrolled study of 20 healthy women, 0.35 mg/day oral "folate" (presumably folic acid) supplementation for 2 weeks, either with (10 pregnant women) or without (10 non-pregnant volunteers) concurrent iron supplementation, significantly reduced the subsequent bioavailability of a single dose of 200 mg ZnSO<sub>4</sub>, but did not affect fasting serum zinc levels (Simmer *et al* 1987). A negative correlation between serum folate and zinc levels was reported in a retrospective study of 60 preterm infants supplemented with 1 mg/day folic acid for various periods during the first 16 weeks of life (Fuller *et al* 1992). Some authors have suggested that correlations between low plasma zinc concentrations, high plasma folate concentrations and pregnancy complications or foetal distress may be due to impairment of zinc absorption by folic acid supplementation during pregnancy (Mukherjee *et al* 1984).

46. Other studies have shown no adverse effects of folic acid supplementation, at doses up to 10 mg/day for several weeks or months, on serum or red cell zinc status in adults (Butterworth *et al* 1988, Tamura *et al* 1992, Hambidge *et al* 1993, Kauwell *et al* 1995) (*data from these studies are summarised in Table 5*). Keating *et al* (1987) reported that ingestion, in water, of 25 mg zinc (as ZnSO<sub>4</sub>) with or without 10 mg folic acid, produced similar changes in serum zinc concentrations in 6 men over a 4 hour period, with peak levels 2 hours post-ingestion, whilst Arnaud *et al* (1992) found no effect of 200 mg folic acid on serum or urinary zinc levels in response to a concurrent dose of zinc gluconate (30 mg elemental zinc) in 10 subjects. It has been suggested that, as zinc levels naturally decline during pregnancy, the association of folic acid supplementation with reduced zinc levels in the later stages of pregnancy is not necessarily causal (Tamura & Goldenberg 1996).

### Iron

47. Iron and folate deficiencies commonly occur concurrently in humans. It is generally assumed that these deficiencies develop independently, however some studies in animals have shown that iron deficiency may cause altered folate utilisation (folate depletion), particularly during the reproductive and neonatal stages of the life cycle (*reviewed by O'Connor 1991*).

**Absorption** (reviewed by Davis 1986, Herbert 1999).

*Human*

48. The majority of dietary folate is absorbed within the proximal region of the small intestine (Halsted 1990). Hydrolysis of polyglutamates to monoglutamates is necessary before they can be absorbed, a process which is carried out by conjugase (hydrolase) enzymes in the brush border of the enterocytes of the small intestine (Chandler *et al* 1986). (Hydrolysis may also occur by conjugase enzymes which occur naturally in some vegetables). Hence, monoglutamate food folate sources are usually more bioavailable than polyglutamates. Some components of food (for example, factors in yeast and beans), as well as low pH, inhibit the action of the brush border conjugase enzymes and thus impair the absorption of polyglutamate food folates. The activities of these enzymes may also be reduced in some disease states, or by exposure to certain drugs (eg, salicylazosulfapyridine) or alcohol.

49. Folate monoglutamates are transported across the brush border membrane by energy-dependent, carrier-mediated mechanisms, involving membrane-associated folate-binding proteins. Non-saturable, passive diffusion also occurs, and is predominant at high intraluminal folate concentrations (Kelly *et al* 1997, McPartlin *et al* 1997).

50. Ingested folic acid (PGA) is mostly converted to reduced forms and then methylated or formylated within the intestinal lumen and enterocytes. The rate-limiting enzyme for the conversion of PGA is thought to be DHFR (Strum 1979). Ingestion of high concentrations ( $> \approx 200\text{-}300 \mu\text{g}/\text{meal}$ ) of folic acid leads to the direct appearance of this form of the compound, unmodified, in the plasma (Strum 1979, Kelly *et al* 1997, McPartlin *et al* 1997). The specific mechanism(s) by which folates are transferred from the enterocytes to the portal blood has not been established.

*Animal*

51. The pig is reported to be a more appropriate animal model than the rodent for the study of folate absorption in humans. Only humans and pigs have been shown to have folate conjugase activity associated with the jejunal brush border membrane, and these enzymes are active at a similar pH range in the two species (Wang *et al* 1985). Folate conjugase enzymes in rats are secreted mostly in pancreatic juice (Kesavan & Noronha 1992), a route which appears to be minor in humans and pigs (Bhandari *et al* 1990). Because of the differences in the properties of these enzymes, it has been noted that conclusions from animal bioassays may not be useful in predicting dietary folate bioavailability in humans (Gregory 1997).

**Distribution and metabolism** (reviewed by Chanarin 1990, Herbert 1999)

*Distribution & storage*

52. Folate monoglutamates, mainly 5-methyl-THF, are the major circulating and transport folate forms. The majority of serum folate exists either free (unbound), or

bound to low-affinity, non-specific binders (eg, albumin). A small amount is bound to high-affinity folate-binding proteins, which are thought to be synthesised and secreted by granulocytes (Colman & Herbert 1976, Fernandez-Costa & Metz 1979). These glycoproteins (MWt  $\approx$  40 000), have binding constants of  $\approx 10^{-10}$  and  $10^{-8}$  mol/l for folic acid and 5-methyl-THF, respectively. Saturation is reported as  $\approx 67\%$  in normal human serum, but accounting for only  $\approx 5\%$  of total serum folate, due to the low levels of these proteins within the serum. The specific function(s) of these proteins has not yet been established (Herbert 1999). Absorbed folate is carried, *via* the portal blood, to the liver, whereby a proportion may be excreted into the bile and undergo enterohepatic circulation and reabsorption.

53. Total body folate stores in adult humans are generally in the range of 5 - 10 mg, of which approximately half is within the liver (*cited by* Herbert 1999). Polyglutamates, synthesised by polyglutamate synthetase enzymes, are the main intracellular storage forms of the folate vitamin group, and are the preferred substrates and active coenzyme forms in a number of one-carbon metabolism pathways. Polyglutamates require subsequent hydrolysis by conjugase enzymes before they can be transported out of the cell, and thus polyglutamate synthetase/conjugase enzymes play a role in regulating folate storage within the body.

#### *Cellular uptake/transport*

54. Due to the anionic, lipophilic nature of folates, transport across biological membranes (into, out of and within cells) occurs mostly by energy-dependent, carrier- or receptor-mediated processes, the mechanisms of which are being elucidated in large part due to the study of the kinetics and metabolism of antifolate drugs, such as methotrexate. The most extensively characterised is the reduced folate carrier (RFC1), an energy-dependent, anion exchange, concentrative process which mediates the bidirectional transport of reduced folates. Other energy-dependent mechanisms, distinct from RFC1, also exist to pump folates out of cells. Membrane folate receptors (MFR) transport both folic acid and reduced folates into cells *via* a unidirectional, receptor-mediated, endocytic mechanism, and are important for cells which require high folate levels, such as kidney, placenta, and breast. There is also a separate, energy-requiring process, optimal at low pH, which transports folates into cells and sustains transmembrane gradients. More detailed accounts of these processes can be found in recent review articles (Wolf 1998, Sierra & Goldman 1999, Sirotiak & Tolner 1999).

#### *Metabolism (Figure 2) (reviewed by Shane, 1990; Rothenberg, 1999)*

55. Within cells, folate is retained in the cytoplasm by polyglutamation. Plasma folate is present mainly as 5-methyl-THF monoglutamate (5-methyl-THFGlu<sub>1</sub>). Upon entry into the cell this species is either demethylated *via* a homocysteine and vitamin B<sub>12</sub>-dependent methionine synthase enzyme, producing THF monoglutamate (THFGlu<sub>1</sub>) (the reduced, metabolically active form), or subsequently exits the cell. (5-methyl-THFGlu<sub>1</sub>, unlike THFGlu<sub>1</sub>, is a poor substrate for polyglutamate synthetase enzymes). Folic acid, however, on entering the cell is reduced *via* DHFGlu<sub>1</sub>, to THFGlu<sub>1</sub>, which is then polyglutamated (i.e. does not have to pass through a vitamin B<sub>12</sub>-dependent pathway).

56. Various THF-polyglutamates function metabolically as coenzymes and substrates in one-carbon metabolism. Transfer of a one-carbon unit from serine to THF *via* pyridoxal phosphate (PLP)-dependent serine hydroxymethyltransferase (SHMT), in the coupled serine to glycine conversion pathway, produces 5,10 methylene-THF. This reduced folate cofactor serves as the substrate to generate 5,10 methenyl-THF (5, 10 formyl-THF, anhydroleucovorin) (dehydrogenase reaction) and 10-formyl-THF (cyclohydrolase reaction), which are required for synthesis of the purine ring. 5,10 Methylene-THF also provides the methyl group for methylation of deoxyuridine monophosphate (dUMP, deoxyuridylic acid) for the *de novo* synthesis of deoxythymidine monophosphate (dTMP, thymidylic acid) catalysed by thymidylate synthase. This reaction generates DHF, which is reduced to THF by the enzyme dihydrofolate reductase (DHFR). Some anti-folate chemotherapeutic agents, (e.g. methotrexate), act by inhibiting DHFR, leading to cellular accumulation of DHF and deoxyuridine, reduced levels of THF and a consequent inhibition of both thymidylate and purine synthesis. Other, more-specific anti-folates (e.g. raltitrexed), exert their main antiproliferative action *via* the direct inhibition of thymidylate synthase (TS), thus reducing thymidylate, but not purine, synthesis (Calvert 1999).

57. The other pathway that requires 5,10 methylene-THF is the biosynthesis of 5-methyl-THF, catalysed by the enzyme methylene THF reductase (MTHFR), using NADPH as a cofactor. This pathway is:- 1] an absolute requirement for the *de novo* synthesis of 5-methyl-THF, the predominant form of intracellular folate, and 2] irreversible under normal physiological conditions. The N-5 methyl group of 5-methyl-THF can be used metabolically only for transfer to homocysteine, resulting in the regeneration of methionine. In addition to its role as an amino acid, methionine serves as a methyl group donor *via* conversion to S-adenosyl methionine (SAM), an important biological methylating agent involved in many methyltransferase reactions. The conversion of homocysteine to methionine, *via* methyl transfer from 5-methyl-THF, is catalysed by the enzyme methionine synthase (homocysteine-methyltransferase) and requires methyl-Cobalamin (vitamin B<sub>12</sub>) as a cofactor. Deficiency of either folate or vitamin B<sub>12</sub>, therefore, results in increased cellular and plasma concentrations of homocysteine (Figure 3).

**Excretion** (reviewed by Herbert, 1999).

58. Approximately 5-40 µg/day folate is excreted in the urine, either in the metabolically active form, or as breakdown products (Herbert 1968, *cited in* Basu & Dickerson 1996). Free, metabolically active folate within the serum is filtered within the glomeruli of the kidneys, with some active reabsorption. The main breakdown product of folate within the urine is acetamidobenzoylglutamate, which is presumed to be formed by the oxidative cleavage of the folate molecule at the 9-10 bond, with acetylation of the *p*-aminobenzyl moiety in the liver before excretion (Murphy *et al* 1976). It has been suggested that scorbutic patients may lose large amounts of folate *via* irreversible oxidation of 10-formylfolate, with subsequent excretion in the urine (Stokes *et al* 1975). In normal subjects urinary losses of folate are < 10 µg/day (Chanarin 1990).

59. Approximately 0.1 mg/day folate is excreted into the bile, and recirculated *via* the enterohepatic circulation.

## Function

60. Folate coenzymes within the cell are involved in one-carbon transfer reactions, including those involved in phases of amino acid metabolism, purine and pyrimidine synthesis, and the formation of the primary methylating agent, S-adenosylmethionine (SAM). The roles of folate in these metabolic processes are described in paragraphs 56 and 57.

## Deficiency

61. Deficiency of folate results in a reduction in *de novo* DNA biosynthesis and, thus, impairment of cell replication, with the most obvious effects apparent in rapidly dividing cell-types, such as red blood cells and other cells generated by the bone marrow, enterocytes, and skin cells. This condition is recognised, haematologically, as a macrocytic anaemia, with characteristic red cell precursors (megaloblasts) present in bone marrow aspirates, and the clinical manifestation of megaloblastic anaemia. Chronic, severe folate deficiency has also, rarely, been associated with neurological changes and depression (*cited in* Weir & Scott 1999).

62. Sequential stages in the development of folate deficiency are; 1] negative folate balance; 2] folate depletion; 3] folate-deficient erythropoiesis and 4] folate-deficiency anaemia. Negative folate balance is indicated by a serum folate concentration < 3 ng/ml (6.8 nmol/l), although such a value does not give an indication of depleted tissue stores or inadequate tissue folate available for biochemical function, but simply indicates greater utilisation than absorption in the short term. An indication of depleted tissue folate stores may be seen by an abnormal deoxyuridine (dU) suppression test (a biochemical test showing a reduced rate of *de novo* DNA synthesis), morphologically abnormal cells (hypersegmentation in the neutrophils and larger than usual reticulocytes and platelets), decreased liver folate (< 1.6 µg/g) and a red cell folate level < 120 ng/ml (280 nmol/l) (Herbert 1987b).

63. Common causes of folate deficiency are pregnancy, infection or malignant disease (due to increased requirement), malabsorption syndromes (coeliac disease, tropical sprue, congenital folate malabsorption), alcoholism (folate deficiency attributed to general malnutrition, and possibly also to reduced absorption and increased renal loss of folate) and certain drug therapies (anticonvulsant drugs, chemotherapies such as methotrexate). Nutritional folate deficiency *per se* is not common in developed countries, and is associated with generally poor diets, particularly those low in fresh fruits and vegetables (Davis 1986, Scott 1997).

64. Folate deficiency is easily treated with folic acid therapy. However, it is recommended that, where possible, a clear diagnosis of folate-deficiency should be established prior to treatment as the haematological signs and symptoms are identical to those of vitamin B<sub>12</sub> deficiency.

## Overview of reported, non-nutritional, beneficial effects

### *Prevention of neural tube defects in the developing foetus*

65. A number of randomised, controlled trials, as well as observational studies, have indicated that peri-conceptional folic acid supplementation in women is associated with a significant reduction in the incidence of neural tube defects (NTDs) in the babies born to these women (MRC Vitamin Study Research Group 1991, Czeizel & Dudas 1992, *see also reviews by Botto et al 1999, Kadir et al 1999*). Many health experts now recommend that all women of childbearing age take a supplement of 400 µg folic acid daily for the prevention of NTDs, and that women who have either given birth previously to a baby with NTDs, or those who have immediate relatives with NTDs, should take 5 mg/day folic acid before and during pregnancy. Within the UK, an expert advisory group for the Department of Health has recommended that women who are trying to conceive should take 400 µg folic acid per day, in addition to normal dietary intake, until the 12<sup>th</sup> week of pregnancy (Department of Health 1992). These recommended supplementation levels substantially exceed levels of intake which are considered necessary to meet normal metabolic requirements for the vitamin. It has been suggested that the beneficial effects of such supplementation are due to the reduction of homocysteine levels (see below, paragraphs 68 and 69), an amino acid metabolite which has been associated with the (folic acid-preventable) development of neural tube and heart defects in avian embryo models (Rosenquist *et al* 1996). Fleming & Copp (1998) have recently shown that the development of NTDs in genetically-predisposed (homozygous *plotch*) mice can be prevented by the application of either folic acid or thymidine to embryos in culture, or *in utero*, suggesting that abnormal pyrimidine biosynthesis may be functionally important in preventing closure of the neural tube.

66. Following the publication of the Medical Research Council's Vitamin Study in 1991, an expert advisory group was set up by the Chief Medical Officer of the UK to review the available evidence relating to folic acid and its role in preventing NTDs. Despite widespread dissemination of this report and its recommendations, Department of Health research in 1995 found that women's knowledge of folic acid remained very low. This prompted the Department of Health to commission the Health Education Authority to run a national integrated campaign aimed at increasing the average intakes of folates and folic acid in women who might become pregnant by at least 400 µg (Department of Health 1999).

### *Hyperhomocysteinaemia*

67. Homocysteinuria, the excretion of homocysteine disulphide in the urine, results from inborn errors of metabolism in pathways for homocysteine removal, and is associated clinically with early onset vascular disease with thromboembolism. Homocysteine is a sulphhydryl-containing amino acid which is derived from the metabolic demethylation of dietary methionine. This compound is highly reactive and many studies have suggested an association between high serum levels (hyperhomocysteinaemia) and vascular endothelial cell damage and cardiovascular disease in the general population, although the evidence for this association is not, as yet, conclusive (*reviewed by Hankey & Eikelboom 1999, McDowell & Lang 2000*).

68. Removal of homocysteine occurs *via* two separate metabolic pathways – remethylation to methionine using folic acid (5-methyl-folate) as a one-carbon donor or metabolism *via* a B6-dependent enzyme (Figure 6). Randomised, controlled trials have shown that folic acid supplementation is associated with a reduction of serum levels of homocysteine (paragraphs 100 - 110). The minimum daily dose to achieve maximal homocysteine-lowering efficacy has been determined as approximately 400 µg folic acid, with higher doses reportedly no more effective, although it is noted that efficacy may vary between individuals. A number of large-scale, randomised, controlled trials of folic acid supplementation in the prevention of cardiovascular disease are currently underway (*summarised by* Hankey & Eikelboom 1999).

69. COMA concluded that fortification of food with folic acid would lower plasma homocysteine levels in at least some sections of the general population. Observational studies suggest that this might in turn reduce the incidence of cardiovascular disease. No randomised controlled studies investigating the link between folate intake and cardiovascular disease have been completed (Department of Health 2000).

#### *Malignancy*

70. It has been suggested that low folate intakes might increase the risk of cancer in humans. A long-term study in 90,000 nurses reported that the relative risk for colon cancer was markedly lower after 15 years in those who reported using multivitamin supplements containing folic acid (Giovanucci *et al* 1998). However, a 1998 COMA committee concluded that there was insufficient evidence for a specific association between folate intake and cancer prevention to be assumed (Department of Health, 1998).

### **Toxicity**

#### *Human toxicity*

71. Folic acid is generally considered as safe, even at extremely high doses, as excesses of the compound are mostly excreted in the urine, rather than being stored in the tissues. The consequences of long-term excessive intakes are not, however, clearly established, and certain groups may be particularly susceptible to potential adverse effects. Intakes > 1 mg/day total folate are contraindicated by many healthcare experts as this level is considered to be sufficient to reverse the megaloblastic anaemia caused by vitamin B<sub>12</sub> deficiency, complicating the diagnosis and increasing the risk of neurological damage which can be associated with this deficiency. Some drugs interfere with folate metabolism and may, theoretically, be less effective in subjects taking folic acid supplements (paragraphs 33 - 37). Some researchers have suggested that folic acid interferes with zinc homeostasis (paragraphs 44 - 46). A small number of hypersensitivity reactions to folic acid have been reported.

*Acute toxicity**Hypersensitivity reactions*

72. A small number of case reports have described hypersensitivity reactions to folic acid, administered orally or parenterally. Chanarin *et al* (1957) reported the case of a healthy male volunteer who developed symptoms of general malaise, aching pain in the lower thoracic region, respiratory difficulty, itching and generalised pruritus after taking 20 mg folic acid orally. The subject had been given 3 mg oral folic acid 6 weeks previously with no adverse effects. Mitchell *et al* (1949), reported a patient who developed maculopapular dermatitis during a course of oral folic acid treatment (15 mg/day for 2 weeks) and a subsequent, severe anaphylactoid reaction following *i.v.* administration of 50 mg folic acid. Sparling & Abela (1985) described a case of severe hypersensitivity reaction (bronchospasm, generalised itchy rash) in a 62-year-old man shortly after taking one 5 mg folic acid tablet, with a similar subsequent reaction to 5 mg folic acid given in a sugar base, and a positive reaction to intradermal folic acid challenge. Mathur (1966) reported the case of a 9 month-old infant who displayed allergic reactions to therapy with 5 mg folic acid tablets on 2 separate occasions, followed by a positive intradermal test for folic acid sensitivity. Sesin & Kirschenbaum (1979) described the case of a 36-year old anephric man who experienced pruritus upon beginning oral folic acid supplementation (1 mg/day). Symptoms disappeared on discontinuation of the therapy, but returned when supplementation was given again 3 months later, with a subsequent positive reaction to intradermal folic acid challenge. Woodliff & Davis (1966) described allergic reactions to *i.v.* folic acid in 2 patients.

73. It has, however, been noted that in some cases hypersensitivity reactions attributed to folic acid may have been caused by other components of the therapy (e.g. tartrazine) (Gotz & Lauper 1980, Butterworth & Tamura 1989).

*General adverse effects of short-term supplementation*

74. One report described adverse effects of 15 mg/day folic acid therapy in an uncontrolled, unblinded trial in which 14 healthy volunteers (6 men, 8 women) were given oral folic acid supplements (3 x 5 mg/day), with the aim of assessing the effects of this therapy on serum vitamin B<sub>12</sub> levels over a period of 3 months. The trial was ended prematurely after 1 month, after the majority (13) of participants reported mental changes, sleep disturbances and gastrointestinal symptoms (Hunter *et al* 1970) (Table 6).

75. Other authors, however, claim that 15 mg/day folic acid is not generally associated with adverse side effects, and that the toxicity reported by Hunter and colleagues may have been due to toxic contamination of the folic acid supplements used, or to non-specific symptoms which were not accounted for by the inclusion of a placebo group within the study (Davis & Woodliff 1970, Salter 1971). Several reports have described a lack of toxicity of short-term, high-level daily folic acid supplementation in normal subjects. These are considered below.

76. Zettner *et al* (1981) carried out a study of the absorption of folic acid in 4 hyperuricaemic men given megadose supplements (up to 1000 mg/day for periods of

approximately 1-3 weeks). The authors reported that these large daily doses of oral folic acid were mostly not retained within the body, and that supplementation was well tolerated with no evidence of toxic effects (haematological, liver function and renal function tests were normal in all patients throughout the study). Czeizel & Tomcsik (1999) reported no acute, adverse effects associated with the ingestion of folic acid in attempted suicide cases in 4 pregnant women (3 women each ingested 120 mg folic acid in combination with other compounds; 1 woman ingested 150 mg folic acid alone). Suarez *et al* (1947) reported that no toxic effects were observed in subjects treated with oral folic acid at doses of 100-500 mg (single dose) or 5-100 mg/day (for periods of  $\approx$  10-14 days) for the treatment of tropical sprue. Weissberg *et al* (1950) reported no ill effects associated with 20 mg/day folic acid supplementation, for 6-12 months, in 26 adult volunteers. Harvey *et al* (1950) described a total of 40 healthy subjects, given 20 mg/day folic acid supplements for periods of 3 months to 1 year, with no ill effects noted. Hellstrom (1971) reported no treatment-related adverse effects in 15 healthy volunteers given 15 mg/day (3 x 5 mg/day) oral folic acid therapy for 1 month in a randomised, double-blind, placebo-controlled study. Richens (1971) similarly reported a lack of toxicity of 15 mg/day oral folic acid therapy given for 1 month to 12 healthy volunteers, as compared to a similar period of placebo treatment within the same group (*data summarised in Table 6*).

77. Individual case reports also suggest that high-level folic acid therapy is without toxicity. Richens (1971) described one subject who took 30 mg/day folic acid for 10 weeks with no adverse effects, whilst Sheehy (1973) reported no ill effects of 60 mg/day (45 mg orally, 15 mg parenterally) folic acid therapy, for 3 years, in a healthy male subject.

78. More-recent trials, carried out to assess the potential efficacy of folic acid supplementation in various population groups have shown no evidence of toxicity associated with oral folic acid therapy alone, or in combination with other vitamins, at levels of approximately 0.4 - 10 mg/day for periods of a several weeks or months (paragraphs 93 - 113).

### *Neurotoxicity*

#### *Vitamin B<sub>12</sub>-deficiency-associated neuropathy*

79. A major concern regarding the adverse effects of folic acid food fortification and/or vitamin supplementation is the potential for this to mask the haematologic signs and symptoms of vitamin B<sub>12</sub> deficiency (see also paragraphs 41 and 42). Deficiency of vitamin B<sub>12</sub> can cause neurologic damage (subacute combined degeneration of the spinal cord (SACD), which is thought to occur due to interruption of the methylation cycle, and reduced ability to methylate myelin basic protein. Delayed haematological recognition of vitamin B<sub>12</sub> deficiency allows the associated neurologic deterioration to progress, and may ultimately result in permanent damage to the nervous system (Bower & Wald 1995, Campbell 1996, Weir & Scott 1999). Pharmaceutical folic acid (at high doses, generally  $\geq$  10 mg/day), rather than liver extract, was first used to treat patients with pernicious anaemia in the mid-1940s. However, from around 1947 onwards, there were a number of reports that this therapy corrected the anaemia, but did not prevent the development of progression of the signs

and symptoms of SACD from developing or progressing (*reviewed by Dickinson 1995*).

80. Few systematic data exist regarding the level of folate intake required to mask vitamin B<sub>12</sub> deficiency (Koehler *et al* 1997). The majority of available information relates to early case reports of (mostly high dose, for example  $\geq 5$  mg/day) folic acid therapy for the treatment of pernicious anaemia (Heinle *et al* 1947, Vilter *et al* 1947, Bethell *et al* 1948, Ross *et al* 1948, Vilter *et al* 1950, Will *et al* 1959, Schwartz *et al* 1950, Ellison 1960, Marshall *et al* 1960, Baldwin *et al* 1961, Hansen & Weinfeld 1962, Vilter *et al* 1963). In general, data taken from these reports has suggested that supplementation with  $\leq 1$  mg/day folic acid is safe in this respect (i.e. does not alleviate vitamin B<sub>12</sub>-associated anaemia in the majority of subjects). The effects of doses between 1-5 mg/day are unclear, whilst (*cited by Chanarin 1994, Bower & Wald 1995*) supplementation with  $\geq 5$  mg/day folic acid is reported to reverse the haematological signs of vitamin B<sub>12</sub>-deficiency in at least 50% of subjects (*discussed by Chanarin 1994, Bower & Wald 1995, Savage & Lindenbaum 1995*).

81. As a precaution against the potential adverse effects of this phenomenon, the US Food and Drugs Agency (FDA) has set an upper safety limit of 1 mg/day for folic acid intake in relation to safe levels of food fortification with this vitamin (Kessler & Shalala 1993). The following passage, regarding the establishment of this upper safe limit, is taken from the US FDA Department of Health and Human Services document regarding food fortification with folic acid (Kessler & Shalala 1993) [reference citations have been substituted for reference numbers in the original text, and are included in the reference list accompanying this report].

82. *“... in the presence of excess folate and inadequate vitamin B<sub>12</sub>, the megaloblastic anemia of vitamin B<sub>12</sub> deficiency may not develop, but severe and irreversible nerve damage may continue (Herbert *et al* 1980). This interaction between the functions of folate and vitamin B<sub>12</sub> has been recognized for many years (Herbert *et al* 1980). Because the anemia of vitamin B<sub>12</sub> deficiency is often the first clinical symptom to appear, and one that requires further tests to accurately identify its cause, the activity of folate to “mask” the development of the anemia of vitamin B<sub>12</sub> deficiency is the basis for the requirement for the precautionary statement on oral and parenteral preparations of folic acid used for treating folate-deficiency anemias.*

*Following the identification and chemical synthesis of folic acid in 1946, but before the isolation of vitamin B<sub>12</sub>, folic acid, usually in doses of 5 mg or higher, was used to treat pernicious anemia. A number of studies reported that the anemia in many pernicious anemia patients is correctable, at least temporarily, by administration of folic acid (Spies *et al* 1945, Vilter *et al* 1950, Will *et al* 1959, Ellison & Curry 1960, Baldwin & Dalessio 1961, Vilter *et al* 1963). However, a number of other studies showed that, while doses of 5 mg of folic acid daily can reverse the hematologic abnormalities of vitamin B<sub>12</sub> deficiency (Hall & Watkins 1947, Heinle & Welch 1947, Schwartz *et al* 1950, Challenger & Korst 1960, Katz 1973), neurologic damage progresses. Hall and Watkins (1947) reported neurological degeneration within 2 to 5 months in 14 patients with vitamin B<sub>12</sub> deficiency who were treated with oral doses of 5 to 20 mg folic acid. In another study, 55 of 98 patients (56 percent) in a clinical study in which patients with pernicious anemia were treated with 5 mg folic acid orally for up to 3.5 years suffered hematologic relapses, neurologic relapses, or combined system relapses (Schwartz *et**

al 1950). Although the neurologic degeneration was not caused by the folic acid treatment, their data clearly demonstrate the potential of folic acid to mask the anemia of vitamin B<sub>12</sub> deficiency without stopping degenerative results of this deficiency.

The first demonstration of dramatic beneficial effects of vitamin B<sub>12</sub> in treating pernicious anemia occurred in 1948. The advent of the specific therapy for the pernicious anemia of vitamin B<sub>12</sub> deficiency during the late 1940's and early 1950's diminished the use of high levels (5 mg or 5,000 µg) of folic acid in patients with this condition. Because 1 mg of folic acid became the more common therapeutic dose for folate deficiency, there are limited data available on the effects of doses of folic acid lower than 5 mg in persons with pernicious anemia.

Despite the lack of systematic evaluation of the effect of folic acid on the anemia of vitamin B<sub>12</sub> deficiency at intakes less than 5 mg/day, several case reports have described hematologic improvement in pernicious anemia with doses of folic acid lower than 1 mg (e.g. 200 to 500 µg) (Baldwin & Dalessio 1961, Vilter et al 1963, Marshall & Jandl 1960, Chosy et al 1962, Hansen & Weinfeld 1962). Chosy et al (1962) reported that daily injections of 400 µg (0.4 mg) of folic acid caused hematologic responses in three of five patients with pernicious anemia. Other investigators have reported suboptimal responses to 0.5 mg of folic acid administered intramuscularly or orally to patients with pernicious anemia (Baldwin & Dalessio 1961; Vilter et al., 1963). On the basis of the reticulocyte response, 0.2 mg (200 µg) of folic acid has been used to differentiate between the megaloblastic anemias caused by folate deficiency and vitamin B<sub>12</sub> deficiency (Herbert et al 1980). Some investigators, however, have not been convinced that amounts of folic acid within the range of 200 to 500 µg/day (0.2-0.5 mg) would mask pernicious anemia (Marshall & Jandl 1960, Cooper & Abe 1976). Marshall and Jandl (1960) concluded that from 200 to 500 µg of folic acid daily should suffice for the prevention of folic acid deficiency without endangering patients with undiagnosed pernicious anemia, while larger doses should be reserved for prescription use in patients with abnormal absorption or utilization of folic acid. The results of these studies show, in general, that responses of doses of folic acid below 1 mg have been less predictable than those to doses of 5 mg and higher.

*In summary, the available evidence shows that as many as 50 percent or more of patients with pernicious anemia will show a normalization of their anemias with doses of 5 mg of folic acid and higher. Although there are no systematic studies to evaluate the effect of folate on masking the pernicious anemia of vitamin B<sub>12</sub> deficiency between intakes of 1 and 5 mg daily, several case reports suggest that some patients with pernicious anemia will respond to folate in doses of less than 1 mg/day. Results at these low levels are often suboptimal and less predictable than those occurring at higher intakes."*

83. The potential for folic-acid masking of vitamin B<sub>12</sub> deficiency is considered to be greatest in the elderly, in whom the risk of vitamin B<sub>12</sub> deficiency (usually in the absence of overt clinical signs) is most prevalent (Stabler 1995, Carmel 1996, Weir & Scott 1999). Some authors have, thus, recommended that elderly subjects taking folic acid supplements should also take 400-1000 µg/day vitamin B<sub>12</sub> (Boushey et al 1995, Oakley et al 1996). Clinically-manifest vitamin B<sub>12</sub> deficiency may be caused by specific gastrointestinal tract disease (for example, pernicious anaemia), leading to reduced absorption of the vitamin, or may be secondary to dietary deficiency (e.g. in strict vegetarians). The majority of subjects with clinically apparent vitamin B<sub>12</sub> deficiency have a reduced ability to absorb this vitamin from the gastrointestinal tract

and so may not respond well to oral vitamin B<sub>12</sub> supplements, unless these are given in high doses (Savage & Lindenbaum 1995). Treatment of vitamin B<sub>12</sub> deficiency is usually by means of intramuscular injections. In a comment regarding the case of a young female patient with sickle cell anaemia, who was subsequently also diagnosed with pernicious anaemia, Sinow *et al.* (1987) noted that these two conditions may co-exist, even in young patients, and that care should be taken when given folate supplementation in such cases.

84. A study of the prevalence of clinically overt vitamin B<sub>12</sub> deficiency in the US showed incidences of approximately 0-0.004 % in 25-35 year olds, increasing to around 0.15% in those > 85 years old. The yearly prevalence of vitamin B<sub>12</sub> deficiency in Sweden has been reported as approximately 0.009-0.017%, whilst the overall lifetime risk has been estimated as 0.1-1.4% (*data cited by* Campbell 1996).

85. In addition to masking signs and symptoms of vitamin-B<sub>12</sub>-deficiency, some authors have suggested that folic acid supplementation may have a more direct role in either precipitating, or enhancing, vitamin B<sub>12</sub>-deficiency-associated neuropathy. Data from a few uncontrolled trials have suggested that supplementation with folic acid can reduce serum vitamin B<sub>12</sub> levels, although others have not observed this effect (paragraph 42). Some early case reports also intimated that folic acid supplementation, in the absence of co-supplementation with vitamin B<sub>12</sub>, may enhance the neurologic complications of vitamin B<sub>12</sub> deficiency. Conversely, some patients with known cobalamin deficiency have shown no development of neurologic complications when treated with high (10 - 100 mg) daily doses of folic acid for a number of years (Campbell 1996 *and refs therein*). There is a lack of data from randomised, controlled studies to determine whether the progression of neurologic deterioration associated with folic acid supplementation is more severe than that which would occur in the absence of such supplementation. However, in general, it has been concluded that anecdotal information suggests that < 5 mg/day folic acid supplementation is rarely associated with a direct adverse effect on vitamin B<sub>12</sub>-deficiency-associated neurological damage (Butterworth & Tamura 1989, Campbell 1996).

86. In summary, folic acid supplementation, at levels up to 5 mg/day is not likely to be associated with decreased blood vitamin B<sub>12</sub> levels or worsening of the vitamin B<sub>12</sub>-deficiency-associated neuropathy. Folic acid supplementation, at levels > 1 mg/day may, however, cause a reversal of haematological abnormalities associated with vitamin B<sub>12</sub> deficiency, leading to a reduced detection of this deficiency and allowing the associated neurological damage to progress.

#### *Direct neurotoxicity*

87. Studies in animals have shown that folic acid is neurotoxic and epileptogenic when applied directly to the brain (paragraph 122). However, there are few data indicating that oral folic acid is directly neurotoxic in humans and supplementation studies in non-pernicious anaemia subjects have not shown evidence of associated neurotoxicity. Weissberg *et al* (1950) reported an uncontrolled study of the neurological effects of 20 mg/day folic acid supplementation, for 6-12 months, in 26 normal volunteers and 22 (non-pernicious) anaemia patients. Prior to therapy, 6 of the normal subjects and 7 of the anaemic subjects showed some abnormal neurological

signs (but not those of subacute combined spinal cord degeneration), which were not significantly altered during the therapy. Four subjects (1 normal, 3 anaemic) developed central nervous system (CNS) changes during the folic acid treatment, but these changes were not considered to be related to the therapy. Harvey *et al.* (1950) reported that oral folic acid supplementation (20 mg/day for 3-12 months) produced no indications of spinal cord or peripheral nerve damage in 40 healthy subjects without pernicious anaemia (13 subjects had mild hypochromic anaemia). Folic acid supplementation was not associated with signs or symptoms of neurotoxicity in a study of 18 patients with Parkinson disease who were treated with 15 mg/day folic acid therapy for periods of 14-182 days (McGeer *et al* 1972) (Table 6).

### *Carcinogenicity*

88. One large epidemiological study, designed to screen for potentially carcinogenic prescription drugs (143 574 subjects assessed for the incidence of cancers at 56 specific sites over a period of  $\geq 11$  years, with regard to drugs dispensed prior to the study) found significant positive associations between folic acid intake and the incidences of oropharynx, hypopharynx and total cancers. However, the authors of this report suggested, anecdotally, that this was probably due to confounding by alcohol and smoking (possible confounding factors were not included in the statistical analyses) as most folic acid recipients were diagnosed alcoholics (Selby *et al* 1989). Moreover, it is well known that alcohol intake is associated with the incidence of oropharyngeal cancers.

89. Conversely, many epidemiological studies have shown an inverse relationship between body folate levels, dietary folate intake (including folic acid supplementation) and certain cancers (particularly colorectal cancer and also, less conclusively, cervical neoplasia, squamous metaplasia of the lung, pancreatic and breast cancers) (*reviewed by* Kim 1999).

90. The UK Committee on Medical Aspects of Food and Nutrition Policy (COMA), however, recently concluded that there was insufficient evidence for linking folate intake to cancer prevention (Department of Health 1998).

### *Genotoxicity*

91. *In vitro* studies using human lymphocyte cultures have indicated that the frequency of spontaneous chromosome abnormalities is increased when cells are cultured in folic-acid-deficient medium (Reidy *et al* 1983, 1984, Dai *et al* 1986).

92. Jainkittivong *et al* (1989) reported that culturing human buccal mucosal epithelial cells in medium containing high concentrations of folic acid (50-200  $\mu\text{g/ml}$ ) did not induce significant cytotoxicity, but stimulated differentiation of the cells. Cultures exposed to medium containing 1 or 10  $\mu\text{g/ml}$  folic acid showed no significant differences from those grown in standard culture medium.

### **Human supplementation studies**

93. Many studies have been carried out to assess the efficacy of folic acid supplementation, either alone or in combination with other vitamins, in the prevention

of disease. Most studies fall into one of two categories; 1] peri-conceptual supplementation in women for the prevention of foetal neural tube defects (NTDs), and 2] possible prevention of cardiovascular disease *via* reduction of plasma homocysteine levels. Of these, the majority of studies have been carried out in groups considered to be at high risk for the incidence/recurrence of specific pathologies (e.g. women with a previous NTD-affected pregnancy, subjects with hyperhomocysteinaemia or established cardiovascular disease), although a few have also been carried out in general population groups. Many of these trials have shown beneficial effects, with little or no evidence of adverse effects or toxicity, associated with oral folic acid supplementation, at levels up to  $\approx 10$  mg/day, for periods of several weeks or months. The majority of studies have not, however, specifically addressed the issue of adverse effects of the therapy. These studies are described in the paragraphs below, and data are summarised in Table 6.

#### *Peri-conceptual supplementation for the prevention of NTDs*

##### *Open trials*

94. Smithells *et al* (1981) carried out an open study to assess the beneficial effects of peri-conceptual vitamin supplementation in women with previous NTD pregnancies. Women planning a pregnancy were offered supplementation with “Pregnavite Forte F”, containing a daily dose of 0.36 mg folic acid, 4000 IU vitamin A, 400 IU vitamin D, 1.5 mg thiamine, 1.5 mg riboflavin, 1 mg pyridoxine, 15 mg nicotinamide, 40 mg ascorbic acid, 75.6 mg Fe equivalent (as ferrous sulphate), 480 mg calcium phosphate. Two hundred women complied with “full” supplementation ( $\geq 28$  days pre-pregnancy to the date of the 2<sup>nd</sup> missed period), with another 50 “partially” supplemented. The incidence of NTDs (total = 1) was substantially lower, as compared with a control group of 305 women who did not participate in the trial (total NTD incidence = 13). The authors reported that 13 subjects withdrew due to the “alleged side effects” (not-specified) of supplementation.

95. Vergel *et al* (1990) carried out a similar study, in which 101 women with a history of previous NTD-affected pregnancy were given 5 mg/day folic acid, without any other vitamins, for a period of  $\geq$  one menstrual period before conception until the 10<sup>th</sup> week of pregnancy (81 fully-supplemented) or a shorter duration (20 partially supplemented). Comparison with a similar group of 114 women not involved in the trial showed a substantially lower incidence of NTDs associated with folic acid supplementation (0 in the folic acid groups, 4 in the comparison group). Adverse effects of the therapy were not reported.

##### *Randomised, controlled trials*

96. Laurence *et al* (1981) carried out a randomised, double-blind, placebo controlled trial of folic acid supplementation in a total of 111 Australian women with a history of a previous NTD-affected pregnancy. Participants were assigned to receive 4 mg/day folic acid or placebo, from the time of stopping contraceptive precautions (the general duration of the therapy was not clearly stated by the authors, but appears to be until  $\approx 6$  weeks’ gestation). The study showed a significant reduction in the incidence of NTD-affected pregnancies in the folic acid-treated group (NTD

incidence = 0 and 2 in folic acid “compliers” and “non-compliers” respectively; 4 in the placebo group). Side effects of the therapy were not reported.

97. The British Medical Research Council carried out a multicentre, randomised, double-blind, placebo-controlled trial, in which a total of 1817 women with a previous NTD-affected pregnancy were assigned to 1 of 4 supplementation groups; A] 4 mg/day folic acid; B] 4 mg/day folic acid + multivitamins (daily – 4000 IU vitamin A, 400 U vitamin D, 1.5 mg each vitamins B<sub>1</sub> and B<sub>2</sub>, 1.0 mg vitamin B<sub>6</sub>, 40 mg vitamin C, 15 mg nicotinamide); C] placebo; D] multivitamins (as B], without folic acid). The duration of therapy was from the date of randomisation until the 12<sup>th</sup> week of pregnancy. Statistical analysis showed a significantly reduced relative risk for NTDs associated with folic acid supplementation compared with no folic acid supplementation (RR = 0.28; 95% CI, 0.12 - 0.71). The authors also reported that possible adverse effects of folic acid to the foetus and the mother were examined. Reported congenital abnormalities, other than NTDs, are shown in Table 6. The authors concluded that there was no demonstrable harm from the folic acid supplementation, although the ability of the study to detect rare or slight adverse effects was limited. The incidences of general side-effects (e.g. infertility, irregular menses, vomiting in pregnancy, upper respiratory illness) reported by women taking part in the trial were similar in all 4 supplementation groups (MRC Vitamin Study Research Group 1991).

98. Czeizel & Dudas (1992) carried out a randomised, controlled primary prevention trial to assess the effectiveness of peri-conceptual multivitamin (including 0.8 mg/day folic acid) supplementation in reducing the incidence of NTD-affected pregnancies in a group of 7540 (mostly nulliparous) Hungarian women. Participants were assigned to one of two supplementation groups;

1] Daily – multivitamin supplement (0.8 mg folic acid, 6000 or 4000 IU vitamin A, 1.6 mg vitamin B<sub>1</sub>, 1.8 mg vitamin B<sub>2</sub>, 19 mg nicotinamide, 2.6 mg vitamin B<sub>6</sub>, 4 µg vitamin B<sub>12</sub>, 100 mg vitamin C, 500 IU vitamin D, 15 mg vitamin E, 10 mg calcium pantothenate, 0.2 mg biotin, 125 mg calcium, 125 mg phosphorus, 100 mg magnesium, 60 mg iron, 1 mg copper, 1 mg manganese, 7.5 mg zinc), or

2] Daily – trace-element supplement (1 mg copper, 1 mg manganese, 7.5 mg zinc, 7.5 mg vitamin C).

Supplementation was given for a period of ≥ 1 month before conception until the date of the 2<sup>nd</sup> missed menstrual period. Side-effects of the therapy in women participating in the trial were not reported. Pregnancy was confirmed in 4753 women, with the outcomes known in 4704. Multivitamin supplementation was associated with a significant reduction in NTD-affected pregnancies (0/2394 in the vitamin-supplemented group, 6/2310 in the trace-element group) (p = 0.029). The overall incidences of congenital malformations (including NTDs) were 1.13% (vitamin supplemented) and 2.29% (trace-element supplemented). These data are summarised in Table 7. The authors noted that the rate of cleft lip, with or without cleft palate, in the vitamin-supplemented group was nearly twice the rate in the Hungarian population, but that this finding is not in agreement with a previous report of a protective effect of vitamin supplementation. In a subsequent report regarding the overall incidence of congenital malformations in this trial, Czeizel and colleagues noted that multivitamin supplementation was also associated with significant increases in both fecundity and the prevalence of spontaneous abortion (embryonic and early foetal death) (Czeizel *et al* 1994). Hook & Czeizel (1997) suggested that

this increase in the incidence of spontaneous abortions may be a terathanasic effect of folic acid i.e. folic acid increases the rate of death or abortion of affected fetuses, although, in fact, such effects can not be specifically attributed to folic acid, as the compound was given in combination with a multivitamin supplement. It is most likely to be due to an increased fertility and multiple pregnancies among women taking supplements and to an effect of folate in allowing the survival of non-viable conceptuses to a stage where their loss is recognised as an abortion (Department of Health 2000). Additionally recent experiments in mouse models; administration of folic acid to genetically-predisposed mouse embryos *in utero* was observed to prevent the development of NTDs by normalising the neurulation process, and did not cause the abortion of affected foetuses (Fleming & Cop 1998).

99. Kirke *et al* (1992) reported a randomised trial carried out in Ireland to assess the effect of folic acid and/or multivitamin supplementation in 354 women with a previous NTD-affected pregnancy. Participants were randomised to supplementation providing daily doses of either;

1] 0.36 mg folic acid,

2] Multivitamins (4000 IU vitamin A, 400 IU calciferol, 1.5 mg thiamin hydrochloride, 1.5 mg riboflavine, 1 mg pyridoxine hydrochloride, 15 mg nicotinamide, 40 mg ascorbic acid, 480 mg calcium phosphate, 252 mg ferrous sulphate), or

3] 0.36 mg folic acid + Multivitamins (as 2]).

The duration of supplementation was from  $\geq 2$  months before conception until the date for the 3<sup>rd</sup> missed period. Foetal NTD occurrence was very low and the reduction in the folic acid supplemented group (1 NTD occurred in the multivitamin-only group, compared with 0 in the folic acid-treated groups) was not significantly significant. Side-effects of the therapy were not specifically addressed. The authors reported that 26 women withdrew from the trial, with no specific reason, whilst 2 women mentioned side effects of the study treatments. With respect to non-NTD foetal and neonatal outcomes, 13 instances of congenital malformations (classed as "major" or "minor" were noted. These occurred in the 3 supplementation groups as follows:-

*Group 1* (folic acid only)] – 1 major (bilateral corneal actasia with agenesis of the corpus callosum), 2 minor (congenital dislocation of the hip, talipes)

*Group 2* (multivitamin only)] – 4 major (polycystic kidneys with cleft palate, congenital mitral insufficiency, polydactyly, pyloric stenosis), 1 minor (scaphocephaly)

*Group 3* (folic acid + multivitamin)] – 2 major (urethral obstruction, cystic fibrosis), 3 minor (congenital dislocation of the hip).

The overall incidence of congenital malformation within this trial was 4.6%, as compared with a general incidence of 2.8% of babies in the Dublin Eurocat register during the same period. The authors suggested that this may have been due to more complete ascertainment within the trial.

#### *Prevention/treatment of hyperhomocysteinaemia and cardiovascular disease*

100. Bostom *et al* (1996) conducted an 8-week trial into the effects of B-vitamin supplementation on plasma homocysteine levels in a group of 27 renal dialysis patients. Participants were randomised to daily supplementation with either 1] 15 mg folic acid, 100 mg vitamin B<sub>6</sub> + 1 mg vitamin B<sub>12</sub>, or 2] placebo. All subjects also

continued taking a pre-prescribed daily supplementation of 1 mg folic acid, 12 µg vitamin B<sub>12</sub> and, in some cases, 10 mg vitamin B<sub>6</sub>. Plasma homocysteine was significantly reduced after 4 and 8 weeks in the active treatment, as compared to the placebo, group (mean concentrations after 8 weeks were ≈ 22 µM and 30 µM in the supplemented and placebo groups, respectively [ $P = 0.0009$ ], with baseline levels of ≈ 30 µM in both groups). To assess the potential side-effects of the therapy, participants were asked to complete a symptoms questionnaire at the end of the study. Active treatment was not associated with an increased frequency of any specific symptoms (nausea, heartburn, diarrhoea, constipation, rash, itching, muscle aches or spasms, tiredness/weakness, fainting, nervousness, headaches, sleep problems, tingling in digits, chest pain, rapid heart beat, nightmares). Biochemical analyses showed no adverse changes in liver transaminases (ALT, AST), creatinine or haematocrit associated with the therapy.

101. Wilmink *et al* (2000) carried out a randomised, double-blind, placebo-controlled crossover trial to assess the effects of pre-treatment with folic acid supplements (10 mg/day for 2 weeks) on endothelial function in a group of 20 healthy volunteers. Folic acid supplementation was associated with a significant improvement in markers of endothelial function after fat loading and a reduction in urinary malondialdehyde excretion. Adverse effects of the therapy were not reported.

102. Landgren *et al* (1995) assessed the effects of treatment with 2.5 mg/day (17 patients) or 10 mg/day (16 patients) folic acid on plasma homocysteine levels in acute myocardial infarction (AMI) patients treated for 6 weeks either immediately following, or during weeks 6-12 after, AMI. Treatment with both doses of folic acid was associated with a significant reduction in homocysteine levels, as compared with an untreated group (mean decrease of 4.4 µmol/l,  $P < 0.001$  in treated subjects; mean increase of 1.7 µmol/l,  $P < 0.001$ , in the untreated group). Adverse effects of the therapy were not reported.

103. Chao *et al* (1999) carried out an open study into the effects of short-term B-vitamin supplementation on post-methionine load hyperhomocysteinaemia and endothelial function in 16 healthy volunteers. Supplementation (5 mg/day folic acid, 100 mg/day vitamin B<sub>6</sub> and 0.5 mg/day vitamin B<sub>12</sub>, for 5 weeks) significantly reduced methionine-loading-associated increases in plasma homocysteine as compared with pre-treatment assessment. (Mean increase in plasma homocysteine concentrations 4 hours post-methionine loading = 15.7 µmol/l [pre-treatment] and 11.8 µmol/l [post-treatment]; reduction = 25% [ $P < 0.001$ ]). Adverse effects of the therapy were not reported.

104. A randomised, double-blind, placebo-controlled, crossover study by Verhaar *et al* (1999) also showed beneficial effects of folic acid supplementation (5 mg/day for 4 weeks) on endothelial function in 20 subjects with familial hypercholesterolaemia. Adverse effects of the therapy were not reported.

105. den Heijer *et al* (1998) carried out an 8-week randomised, placebo-controlled trial including 89 patients with a history of recurrent venous thrombosis and 227 healthy volunteers (a sub-group of whom was classed as hyperhomocysteinaemic if plasma homocysteine levels were > 16 µmol/l). Patients and hyperhomocysteinaemic

volunteers were randomised to placebo or high-dose combined B-vitamin supplement (5 mg/day folic acid, 0.4 mg/day hydroxycobalamin, 50 mg/day pyridoxine). Non-hyperhomocysteinaemic volunteers were randomised to placebo, combined B-vitamins (as above) or single B-vitamins (5 mg/day folic acid, 0.5 mg/day folic acid or 0.4 mg/day hydroxycobalamin). Median plasma homocysteine concentrations in all five treatment-type groups were  $\approx 12 \mu\text{M/l}$ . Both combined B-vitamin (all groups, median reduction 30%), and folic acid (median reduction for both doses  $\approx 25\%$ ), but not cobalamin, supplementation significantly ( $P < 0.001$ ) lowered plasma homocysteine levels (median pre-treatment levels were  $\approx 12 \mu\text{M/l}$  in all groups), as compared to placebo treatment (median reduction, 3%). Adverse effects of the treatment were not reported. Six subjects withdrew from the trial (reasons not stated).

106. Lobo *et al* (1999) carried out a 3-month non-randomised, single-blind, placebo-controlled trial to assess the effects of folic acid (+ B vitamin) supplementation on plasma homocysteine levels in 95 subjects with coronary artery disease. Subjects were assigned to 1 of 4 groups; 1] placebo, 2] 0.4 mg/day folic acid, 3] 1 mg/day folic acid, 4] 5 mg/day folic acid. Subjects in the folic acid treatment groups were also supplemented with vitamins B<sub>6</sub> (12.5 mg/day) and B<sub>12</sub> (500  $\mu\text{g/day}$ ). All doses of folic acid were associated with a significant reduction in plasma homocysteine levels after 30 and 90 days treatment, as compared with baseline values (mean concentrations were reduced from  $\approx 14$ , 13 and 15  $\mu\text{mol/l}$  in the 0.4, 1.0 and 5.0 mg/day folic acid groups, to  $\approx 10 \mu\text{mol/l}$  after 90 days in all groups,  $P \leq 0.001$ ). This effect was not seen in the placebo group (pre- and post-treatment level  $\approx 12 \mu\text{mol/l}$ ). Seven subjects reported adverse reactions to the therapy (1 in the placebo group; 4 in the 0.4 mg/day, 1 in the 1 mg/day, and 1 in the 5 mg/day folic acid groups).

107. Bealieu *et al* (1999) reported a 12-week trial to assess the effect of folic acid supplementation on total homocysteine levels in 60 chronic, stable renal transplant recipients. Participants were randomised to 1 of 3 groups; 1] 0.4 mg/day folic acid, 2] 2.4 mg/day folic acid, 3] placebo. All subjects also received 50 mg/day vitamin B<sub>6</sub> and 0.4 mg/day vitamin B<sub>12</sub>. All groups (including the placebo group) showed statistically-significant reductions in plasma homocysteine concentrations during the study (mean values reduced from  $\approx 17 \mu\text{mol/l}$  in all groups to  $\approx 11$ , 13 and 14  $\mu\text{mol/l}$  in groups 1, 2 and 3, respectively). As compared with the placebo group, the percentage reduction in plasma homocysteine levels was significantly greater in group 1 ( $P = 0.001$ ), but not group 2 ( $P = 0.153$ ). Adverse effects of the therapy were not reported.

108. Malinow *et al* (1997) reported that folic acid supplementation (1 or 2 mg/day, for 3 weeks) was associated with a reduction in plasma homocysteine levels in a randomised, non-placebo-controlled study including 242 participants (102 healthy volunteers; 140 subjects with coronary heart disease). Adverse effects of the therapy were not reported.

109. Ubbink *et al* (1994) carried out a randomised, placebo-controlled study to assess the effects of supplementation with folic acid and vitamins B<sub>6</sub> and B<sub>12</sub>, alone or in combination, on plasma homocysteine levels in a group of 100 hyperhomocysteinaemic men. Participants were randomised to daily supplementation,

for 6 weeks, with either; 1] 0.65 mg folic acid, 2] 0.4 mg vitamin B<sub>12</sub>, 3] 10 mg vitamin B<sub>6</sub>, 4] 0.65 mg folic acid + 0.4 mg vitamin B<sub>12</sub> + 10 mg vitamin B<sub>6</sub>, or 5] placebo. Folic acid supplementation, either alone, or in combined supplement, was associated with the most substantial, significant reduction in plasma homocysteine levels (40 - 50% reduction compared with baseline levels,  $P < 0.001$ ). Vitamin B<sub>6</sub> or placebo treatment did not significantly alter plasma homocysteine concentrations. Adverse effects of the therapy were not reported.

110. Brouwer *et al* (1999) assessed the effect of folic acid supplementation on plasma homocysteine levels in 144 healthy female volunteers. Participants were randomised to supplementation, for 4 weeks, with either; 1] 0.5 mg/day folic acid, 2] 0.5 mg folic acid every 2<sup>nd</sup> day, 3] placebo. Folic acid supplementation (both groups) was associated with a significant reduction in plasma homocysteine levels compared with placebo treatment ( $\approx 11$  and 22 % reduction compared with baseline values,  $P < 0.001$ , in groups 1 and 2, respectively). Adverse effects of the therapy were not reported.

### Cancer

111. Heimburger *et al* (1988) reported results from a randomised, double-blind, placebo-controlled trial to assess the efficacy of folic acid + vitamin B<sub>12</sub> supplementation in reducing bronchial squamous metaplasia in smokers. A total of 73 men were randomised to 4 months' supplementation with either 10 mg folic acid + 0.5 mg hydroxocobalamin, or placebo. Supplementation was associated with a significant reduction in atypical squamous metaplasia, but not metaplasia *per se*. Adverse effects of the therapy were not reported.

112. Butterworth *et al* (1992) evaluated the effect of folic acid supplementation on the course of cervical dysplasia. A total of 235 subjects with grade 1 or 2 cervical intraepithelial neoplasia (CIN) were randomly assigned to supplementation with either 10 mg/day folic acid or placebo, for a period of 6 months. Active therapy was not associated with significant differences in dysplasia status, biopsy results or prevalence of human papillomavirus type 16 infection. Adverse effects of the therapy were not reported.

113. The effect of folic acid supplementation on the natural history of CIN was also evaluated in a multicentre, randomised, double-blind, placebo-controlled trial. A total of 331 women with biopsy-proven koilocytic atypia, mild CIN, or moderate CIN, were randomised to receive oral folic acid supplementation (5 mg/day) or placebo, for 6 months. Primary endpoints were Papanicolaou smear and colposcopy cytology. Supplementation was not associated with significantly different outcomes as compared to placebo treatment. Two participants from each of the groups were removed from the trial due to progression of cervical dysplasia. Folic acid therapy did not affect serum retinol, retinyl palmitate,  $\alpha$ -tocopherol or  $\beta$ -carotene levels. Adverse effects of the therapy were not reported (Childers *et al* 1995).

### Others

114. Juhlin & Olsson (1997) carried out a 2-year open study to assess the efficacy of folic acid and vitamin B<sub>12</sub> supplementation in the treatment of vitiligo

(characterised by destruction of melanocytes in, and hence depigmentation of, the skin). One hundred patients were treated with 10 mg/day folic acid + 2 mg/day vitamin B<sub>12</sub>, for ≥ 3 months. Treatment was associated with skin repigmentation, particularly if combined with exposure to the sun. Adverse effects of the therapy were not reported.

115. Mackey & Picciano (1999) reported results from a randomised, double-blind, placebo-controlled trial to assess the effects of supplemental folic acid on maternal folate status and infant growth rate. A total of 42 lactating women was randomised to therapy with either 1 mg/day folic acid, or placebo. All women were also given a daily multivitamin and mineral supplement. Supplementation was for 3 months, from 3 months postpartum. Analyses at 6 months postpartum showed significantly higher mean erythrocyte folate concentrations, haemoglobin and haematocrit values in the folic acid treated women. A decline in milk folate levels with time was noted in the unsupplemented group, but not in the folic acid treated group. Anthropometric indices of infant growth showed no significant differences between the groups. Adverse effects of the therapy were not reported.

116. Daly *et al* (1997) carried out a randomised, double-blind, placebo-controlled study, with the aim to establish a minimum effective dose for folic acid supplementation in the prevention of NTDs. The measured endpoint was erythrocyte folate levels, the increase in which was taken as a marker for adequate supplementation. A total of 121 women, with base-line red-cell folate levels between 150-400 µg/l, were assigned to 6 months supplementation with either 100, 200, 400 µg/day folic acid, or placebo. Ninety-five participants completed the study. All three treatment groups, but not the placebo group, showed significant increases in median erythrocyte folate levels associated with the treatment, and the authors concluded that a supplemental daily dose of 100 µg folic acid (ie, *via* food fortification), would produce an important decrease in NTD. Adverse effects of the therapy were not reported.

#### *Adverse drug reactions*

117. Suspected adverse reactions to medicinal products are reported to the Committee on Safety of Medicines/Medicines Control Agency. Many factors influence the number of reports received, and in most situations there is considerable “under-reporting” of reactions. Most of the adverse reactions reported for products containing folic acid relate to multiconstituent products, and may not, therefore, be directly attributable to the vitamin. Single constituent folic acid products are associated with a low number of adverse reactions, with no trend or pattern to indicate a particular problem.

#### *Vulnerable groups*

118. Groups vulnerable to adverse effects associated with folic acid supplementation include;

1] Individuals at risk of vitamin B<sub>12</sub> deficiency (most prevalent in the elderly), in whom folic acid supplementation may treat the haematological signs and symptoms of this deficiency, allowing associated CNS neuropathy to develop.

2] Patients treated with drugs that interfere with folate metabolism, in whom folic acid supplementation may be associated with reduced effectiveness of these therapies.

#### *Genetic variation*

119. Subjects homozygous for a variant of the enzyme, 5,10-methylene tetrahydrofolate reductase (MTHFR), show reduced activity of this enzyme, resulting in altered cellular distribution of one-carbon units, and associated with low plasma folate status and hyperhomocysteinaemia (*reviewed by* Bailey & Gregory 1999). Defects of other enzymes involved in homocysteine metabolism (e.g. cystathionine  $\beta$ -synthase, methionine synthase) may also be associated with pathology related to low folate intake/status (*cited by* Department of Health 2000). In addition, congenital errors of various enzymes involved in folate metabolism have been described, associated with functional folate-deficiency (*reviewed by* Zittoun 1995). Population groups with a genetically-determined increase in susceptibility to folic acid toxicity have not been identified. It has been suggested that increased plasma homocysteine levels arising from mutations in MTHFR activity may be associated increased risk of cardiovascular disease. For instance, Nakai *et al* (2000) reported that mutations in MTHFR indicated an increased genetic predisposition towards myocardial infarction in Japanese men; the study population consisted of 199 healthy Japanese men (aged  $60 \pm 8$  years) and 230 male Japanese patients (aged  $59 \pm 9$ ) who had experienced their first myocardial infarction. Güleç *et al* (2001) also reported that polymorphism in the MTHFR gene constituted a risk factor for premature myocardial infarctions in Turkish men. However, other larger scale studies involving American men (age 40 to 75 years) failed to identify an increased risk of coronary heart disease in men with the +/+ genotype for 677T mutation in MTHFR gene, even when intake of folate or other B vitamins was low (Verhoef *et al* 1998).

#### **Animal toxicity**

##### *Acute toxicity*

120. LD<sub>50</sub> values of 305 mg/kg (*i.v.* injection) and > 10 000 mg/kg (*per os*) have been reported for folic acid in mice (Beliles 1972). Parchure *et al* (1985) reported mean LD<sub>50</sub> values for intraperitoneal (*i.p.*) administered folic acid in the range of 85-330 mg/kg bw for different mouse strains tested. Animals showed varying symptoms, such as convulsions, ataxia and muscular weakness prior to death, which generally occurred on day 3 or 4 after treatment. Histopathological examination showed acute renal necrosis in many animals. Other studies have also shown that the administration of large doses of folic acid, parenterally (by intravenous, intraperitoneal or subcutaneous injection), into rats (100 - 400 mg/kg bw) or mice (75 mg/kg bw) produces precipitation of the compound in the renal tubules and renal hyperplasia, hypertrophy and necrosis (Byrnes *et al* 1972, Searle & Blair 1973, Klingler *et al* 1980, Gaddis *et al* 1982, Kavlock *et al* 1985). Hence the renal effects appear to be non-specific.

*Sub-chronic toxicity*

121. Data from sub-chronic toxicity studies of folic acid in experimental animals, other than those described in specific sub-sections later in this report, have not been identified.

*Neurotoxicity*

122. Direct injection of high doses of folic acid or folates into the brain or spinal fluid causes seizures in rats (Obbens & Hommes 1973, Olney *et al* 1981, Snodgrass 1992). Baxter *et al* (1973) reported that intravenous injection of very high levels of folic acid also caused convulsions in mice although, compared with intracerebroventricular injection, the dose required to produce this effect in 50% of animals (EC<sub>50</sub>) ( $\approx$  1000-fold), and the latency time to seizure, were greatly increased.

123. Hommes *et al* (1977) reported that changes in dietary folate content produced inverse effects on the pentylenetetrazol (PT) seizure thresholds of rats. Groups of Wistar rats were fed diets containing folic acid at concentrations of either 0.4 mg/kg ( $\approx$  0.02 mg/kg bw/day) (FA-deficient), 2.7 mg/kg ( $\approx$  0.14 mg/kg bw/day) (standard) or 50 g/kg ( $\approx$  2500 mg/kg bw/day) (FA-supplemented) folic acid for 8 (normal and FA-supplemented animals<sup>1</sup>) or 11 (FA-deficient animals) months, at which time PT threshold levels were assessed. Folate-deficient animals showed reduced weight gain compared with the other groups. PT thresholds were 19% lower ( $p < 0.02$ ) in the supplemented group compared with the control group.

124. Carl & Smith (1983) reported that oral folic acid supplementation (20 mg/kg diet,  $\approx$  1 mg/kg bw/day) did not affect phenytoin levels in phenytoin-treated rats, but caused an increase in post-seizure recovery time. Equivalent folic acid supplementation in non-phenytoin-treated animals significantly increased folate concentrations in all tissues examined, except the brain. Chou & Levy (1984) observed no effects of folic acid treatment (0.1 - 0.4 mg/kg bw/day, for 19 days in drinking water) on the pharmacokinetics of an acute *i.v.* dose of phenytoin in female pregnant or non-pregnant rats.

*Carcinogenicity**Tumour promotion studies*

125. Shirai *et al* (1984) reported that high (nephrotoxic) dose parenteral administration of folic acid promotes 2-(ethylnitrosamino)ethanol (EHEN)-induced renal carcinogenesis in F344 rats. A test group of animals was treated with 0.1% EHEN in drinking water for 1 week and then injected subcutaneously with folic acid once per week (300 mg/kg bw/day for the first 8 weeks and thereafter at 100 mg/kg bw/day) for 35 weeks. Control groups were treated with either EHEN, or folic acid, according to the same regime. At week 3 a right-sided nephrectomy was performed on all animals to enhance renal neoplasia. In EHEN-treated animals, renal cell tumour incidence was significantly ( $p < 0.001$ ) increased by folic acid treatment. Animals

<sup>1</sup> Supplementation at 50 g/kg diet, as stated by the authors, is likely to be a typographical error for 50 mg/kg diet, which would provide  $\approx$  2.5 mg/kg bw/day folic acid.

treated with folic-acid alone showed reduced weight gain and interstitial nephritis, but no tumours occurred in this group.

126. Baggott *et al* (1992b) investigated the effects of nutritional folate deficiency and supplementation on the initiation and early promotion of methylnitrosourea (MNU)-induced mammary cancer in female Fischer 344 rats. Animals were fed diets containing 0 (FA-), 2 (control), 40 (FA+) mg/kg diet ( $\approx$  0, 0.1 or 2 mg/kg bw/day) folic acid, or 20 mg/kg diet ( $\approx$  1 mg/kg bw/day) folinic acid, for 30 days, injected with MNU, and then fed the control diet for 180 days. The incidence of mammary cancer was not significantly different amongst the groups. However, the number of cancers per tumour-bearing animal (1.32, 1.90, 2.14 and 273 in FA-, control, FA+ and folinic acid group, respectively) was significantly lower in the FA-, as compared with the other groups, whilst the time to tumour occurrence (170, 142, 100 and 85 days, in the FA-, control, FA+ and folinic acid groups, respectively) was significantly greater, in animals fed the folate deficient diet, as compared with the folic- or folinic-acid supplemented groups. This relative enhancement of tumour growth by folic acid supplementation presumably relates to the inhibitory effect of folate deprivation on rapidly growing tumour cells, rather than a specific tumour-promotional effect of folic acid supplementation.

#### *Tumour inhibition studies*

127. Maru & Bhide (1982) reported that administration of 1.1 mg/day ( $\approx$  55 mg/kg bw/day) folic acid (by gavage, starting 1 week prior to administration of carcinogen, followed by co-administration for approximately 12 months), had a marginal ( $p < 0.1$ ) inhibitory effect on the incidence of hydrazine sulphate-induced lung tumours in Swiss mice.

128. Kim *et al* (1996) reported that feeding of Sprague-Dawley rats with diets containing 8 mg/kg diet ( $\approx$  0.4 mg/kg bw/day) folate<sup>2</sup> for 20 weeks was associated with a significant reduction in the incidence of macroscopic, but not microscopic, dimethylhydrazine-induced colonic neoplasms, compared with animals fed a basal diet (2 mg/kg, or  $\approx$  0.1 mg/kg bw/day, folate). However, higher-level folate supplementation (40 mg/kg diet,  $\approx$  2 mg/kg bw/day) was not significantly protective compared with the control, or a folate-deficient, diet.

129. The same group also investigated the effects of dietary folate supplementation in a murine model of intestinal tumorigenesis (Apc<sup>+/+</sup>-Msh2<sup>-/-</sup> mice). Animals were fed diets containing either 0 or 8 mg/kg diet (approximately 0 or 1.2 mg/kg bw/day) folate<sup>2</sup>, from 3- or 6- weeks (before or after the establishment of neoplastic foci, respectively), until 11- weeks of age. As compared with the folate-deficient diet, folate supplementation, when given from 3-weeks, was associated with significantly decreased incidence of adenomas in the small intestine and aberrant crypt foci (ACF) in the colon (with no difference in colonic adenoma incidence). Conversely, folate supplementation from 6-weeks was associated with a significantly greater number of small intestinal adenomas, compared with the folate-deficient diet, although there were no significant differences in the frequencies of colonic ACFs or adenomas (Song *et al* 2000).

<sup>2</sup> Formulation not stated, but assumed to be folic acid

*Genotoxicity*

130. Evidence from animal models and *in vitro* studies suggests that folate deficiency is associated with DNA damage, abnormal DNA methylation, and impaired DNA repair (Kim 1999).

*In vitro*

131. Folic acid was not mutagenic in the Ames *S.typhimurium* test, strains TA97A, TA102 (0.01 - 0.5 µg/plate, with or without metabolic activation) (Fujita & Sasaki 1986, cited by CCRIS database, accessed at <http://toxnet.nlm.nih.gov/cgi-bin/sis/search>, August 2000 ). The compound was not effective as an anti-mutagen against the effects of various heterocyclic amines in *S.typhimurium* reversion assays (Edenharder *et al* 1999).

*In vivo*

132. Subcutaneous injection of 25 mg folic acid in adult male rats resulted in an approximately 4-fold increase in mean number of mitotic counts in the sections taken from the kidneys of treated rats, as compared with adults injected with a control solution, although there was no alteration in differential mitotic counts (distribution through the 4 phases of mitosis) (Hollis 1969). Klingler *et al* (1980) also reported dose-related increases in the mitotic activity of renal tubular cells in rats treated parentally with high levels of folic acid (100 - 400 mg/kg bw), in association with alterations in renal tubular morphologic features and renal function.

133. Kim *et al* (2000) investigated the effects of dietary folic acid supplementation on DNA strand breaks in the p53 and Apc genes in the rat. Three groups of animals were fed diets containing 0 (deficient), 2 (basal) or 8 (supplemented) mg/kg diet (approximately 0, 0.1 or 0.4 mg/kg bw/day) folic acid for 5 weeks. Folic acid deficiency was associated with a significant incidence of DNA strand breaks within exons of 5-8 of the colonic p53 gene, whilst supplementation was significantly correlated with increased p53 integrity compared with the deficient and basal diets. The same group previously reported that dietary supplementation with the same levels of folic acid, had no significant effect on *Apc* (exons 5-9) or *p53* (hotspot) mutations in tumours isolated from dimethylhydrazine-treated rats (Sohn *et al* 1999).

*Reproductive toxicity (data summarised in Table 8)*

134. Chung *et al* (1993), in a study of the synergistic effects of folic acid and the anti-malarial drug, pyrimethamine, reported that folic acid treatment alone showed no embryotoxicity. Groups of 10 pregnant female rats were supplemented from days 7-17 of gestation, by gavage, as follows; 1] PYM I (1.2 mg/kg bw/day pyrimethamine – cited as the approximate equivalent dose used to treat malaria in humans and considered to be a subtoxic dose); 2] PYM II (2.7 mg/kg bw/day pyrimethamine) 3] FA (50 mg/kg bw/day folic acid); 4] PYM I + FA; 5] PYM II + folic acid (12 mg/kg bw/day); 6] vehicle control. Animals were killed on day 20 of gestation. Results are summarised in Tables 10a-c. No treatment-related effects or pathologic findings were observed in the dams of any group. Analysis of foetuses showed statistically-

significant incidences of external malformation in the PYM II group, and of visceral and skeletal anomalies in the PYM II and PYM I + FA groups. Folic acid treatment alone was not associated with adverse foetal outcomes. Hence, 50 mg/kg bw/day folic acid supplementation, whilst without effect itself, was associated with an enhancement of pyrimethamine embryotoxicity, and the authors of the study suggested that synergistic embryotoxicity by the coadministration of these 2 compounds may pose some risk in humans.

135. Quinn *et al* (1990) investigated the effects of dietary folic acid supplementation on teratogenesis in zinc-deficient rats. Pregnant Wistar rats, fed zinc-deficient (< 0.5 mg/kg) or zinc-supplemented (75 or 95 mg/kg) diets from mating until day 18 of gestation, were concomitantly fed either a basal (0.56 mg/kg diet, approximately 0.03 mg/kg bw/day) or supplementary (30 - 200 mg/kg diet,  $\approx$  1.5-10 mg/kg bw/day) level of folic acid. Zinc deficiency was associated with a high incidence of teratogenesis, which was not alleviated by folic acid supplementation. Zinc-deficient rats supplemented with  $\approx$  10 mg/kg bw/day folic acid showed a significantly increased incidence of clubbed foot, compared with an equivalent (i.e. zinc-deficient) group fed the basal level of folic acid. Folic acid supplementation was not associated with reduced maternal or foetal zinc status.

136. Fuller *et al* (1988) studied the effects of dietary folic acid and zinc supplementation on the outcome of pregnancy and early growth in Norwegian Hooded rats. Four groups of animals were fed diets containing combinations of 0 or 100 mg/kg diet (0 or  $\approx$  5 mg/kg bw/day) folic acid with 6.6 or 20.2 mg/kg diet ( $\approx$  0.3 or 1.0 mg/kg bw/day) zinc, during pregnancy and lactation. Pups and dams were killed on day 20 of gestation or day 20 postpartum. Body weights, food intakes, blood folate and tissue zinc levels were assessed. Folic acid supplementation was associated with increased erythrocyte and plasma folate levels, but not with decreased tissue zinc concentrations of dams or pups.

137. Achon and colleagues have reported experiments in which the effects of dietary folic acid supplementation (approximately 20-fold the normal requirement) during pregnancy were assessed in Wistar rats.

138. In one study, 38 female rats (dams and virgin rats) were fed diets containing 2 mg/kg ( $\approx$  0.1 mg/kg bw/day – control), or 40 mg/kg ( $\approx$  2 mg/kg bw/day – supplemented) folic acid, resulting in 4 test groups;

- 1] Virgin rats/ control diet
- 2] Virgin rats/ folic acid supplemented diet
- 3] Dams/ control diet
- 4] Dams/ folic acid supplemented diet

Diets were fed for 3 weeks, corresponding to a complete pregnancy. Metabolic balance studies were carried out during days 1-5, and 17-21 of the experiment. Animals were killed on day 21 and embryonic development was evaluated by measuring foetal length and weight and total number of resorptions. Folic acid was not associated with significant alterations of dietary intake, body weight, or fat or nitrogen digestibility coefficients in the supplemented rats. Protein utilisation was significantly reduced in folic-acid supplemented rats during the second metabolic measurement period (days 17 - 21) in both dams and virgin rats. Urinary nitrogen was also significantly higher in virgin rats fed the folic acid-supplemented diet during the

second metabolic period. There was no significant association between folic acid supplementation and gestational development (number of live foetuses). Statistical analysis showed significantly lower body weight and vertex-coccyx length in foetuses from supplemented dams compared with those from control dams (Achon *et al* 1999) (Table 10). However, the validity of the statistical analysis carried out is questionable.

139. In a separate report, the same group described data from the same or an identical experiment. Animals and diets were as described in paragraph 138, and again the authors reported that gestational development was considered adequate and similar in both folic acid supplemented and unsupplemented groups, but that folic acid supplementation was associated with significant reductions in foetal body weight and vertex-coccyx length. Analyses of samples collected from each animal termination showed that folic acid supplementation (in both dams and virgin animals) was associated with significantly increased serum folate, decreased serum homocysteine, and increased liver SAM, but not SAH (S-adenosyl-homocysteine), levels (Achon *et al* 2000).

140. Hansen *et al* (1995) studied the effects of dietary folic acid supplementation on valproic acid-induced NTDs in CD-1 mice and Nctr:SDN strain rats. Female animals were fed diets containing 2 mg/kg (control) or 12 mg/kg (FA+) folic acid, equivalent to  $\approx$  0.3 or 1.8 mg/kg bw/day (mice), and 0.1 or 0.6 mg/kg bw/day (rats). Diets were given for 3 weeks prior to breeding and throughout gestation. Each dietary group was also divided in valproic acid (VPA)- or vehicle-treated groups (*s.c.* injection on day 8 of gestation), resulting in 4 treatment groups;

1] control/vehicle

2] control/VPA

3] FA+/vehicle

4] FA+/VPA

Assessed outcomes are summarised in Tables 12 a&b. In mice, VPA treatment (groups 2 and 4, as compared with groups 1 and 3, respectively) was associated with a significant increase in the number of non-live implants and of pups with exencephaly. Folic acid supplementation (group 4 compared with group 2) reduced the number of VPA-associated non-live implants, but had no significant effect on the incidence of exencephaly. VPA treatment was also associated with an increase in the number of non-live implants in rats (groups 2 and 4, compared with groups 1 and 3, respectively), and this effect was significantly increased by folic acid supplementation (group 4 compared with group 2). Folic acid supplementation alone (group 3) was not associated with significant differences in assessed maternal (weight or weight gain, number of litters, number of live/non-live implants, zinc status) or foetal (weight, incidence of exencephaly, zinc status) outcomes compared with the unsupplemented controls (group 1) in mice or rats. Folic acid supplementation (group 3 *vs.* group 1) also had no significant effect on maternal liver, kidney or brain zinc concentrations in mice and rats, or on embryonic zinc concentrations in rats (these data not reported for mice).

141. Matte *et al* (1993) reported that dietary folic acid supplementation in pigs, before and during pregnancy, was associated with significantly increased foetal folate concentrations. Three groups of 34 gilts were fed diets supplemented with either 0, 5, or 15 mg/kg [ $\approx$ 0, 0.2, or 0.6 mg/kg bw/day] folic acid, from 9 weeks of age until slaughter at 7 weeks' gestation. Serum folate concentrations increased linearly with

the level of added folic acid. Growth performances were not influenced by the treatments during the overall growing period, but feed intake and body weight gain from 17 to 21 wk of age increased linearly as folic acid level in the diet increased. Age and body weight at puberty as well as body weight gain during gestation were not influenced by treatments. Dietary folic acid addition did not affect either total weight and empty weight of uterine horns or ovarian total weight, stroma weight, and number and weights of corpora lutea. No treatment effect was observed on placental surface, number of placental areolae, litter size, foetus weight, or total litter weight or on foetal DNA, RNA, and protein. Foetal folate concentrations, however, showed significant, dose-related increases associated with addition of folic acid to the diet.

142. Shin & Shiota (1999) assessed the effects of folic acid supplementation in suppressing heat-induced neural tube defects in mice. Groups of 12 - 14 ICR mice were fed diets supplemented with 3 mg/kg (approximately 0.45 mg/kg bw/day) folic acid, from day 0.5 to 9.5 of gestation, with or without heat treatment on day 8.5. A control group received heat treatment but not folic acid supplementation. Folic acid supplementation was not associated with teratogenic or embryolethal effects, but significantly reduced the incidence of malformations and resorptions associated with heat treatment.

143. Morgan & Winick (1978) studied the effects of folic acid supplementation during pregnancy in the rat. A total of 24 female Sprague-Dawley rats was randomly assigned to one of 3 groups; A] folic acid-free diet, B] folic acid supplementation at 1.8 mg/kg diet (approximately 0.09 mg/kg bw/day), C] dietary supplementation (as B]) + 1 mg/day folic acid by *i.p.* injection. Animals were mated on day 14 and diets were continued throughout gestation. Animals were killed on day 21 of gestation. Foetuses, placentas and livers were significantly larger, with higher RNA, DNA and protein content, in group C, compared with groups A and B. THFR enzyme activity was significantly increased in the livers of foetuses from folic acid supplemented rats, as compared with the other two groups.

### **Mechanisms of toxicity**

#### *Experimental neurotoxicity in animals*

144. Seizures caused by intracerebral folic acid injection in experimental animals show similarities to those produced by the neurotoxin, kainic acid (Olney *et al* 1981) and it was originally proposed that folate may be the endogenous ligand for the kainate receptor. Subsequent studies have, however, shown that the toxicity of folates does not correlate well with their affinity for the kainate receptor, and that folate-mediated neurotoxicity probably occurs *via* a different mechanism. Ajit *et al* (1999) have recently reported results from *in vitro* studies suggesting that the neurotoxic effects of folates may be due to the liberation of glutamate residues from the polyglutamate tail of folate polyglutamate species.

#### *Precipitation of seizures in drug-treated epileptics*

145. Anticonvulsant drugs interfere with folate metabolism and may be associated with low folate status in treated patients. Treatment to correct the folate deficiency has been associated with the precipitation of seizures or increased seizure frequency in

some individuals. The specific mechanisms by which these interactive effects occur have not been established.

*Potential to reduce the efficacy of anti-folate drugs*

146. Folic acid supplementation may, theoretically, reduce the effectiveness of anti-folate drugs by interfering with the absorption and/or metabolic effects of these compounds.

**Regulatory considerations**

147. Folic acid in doses of 500 µg or greater requires medical prescription (*cited by* Department of Health, 2000). The Recommended Daily Allowance in the Food Labelling Regulations for folacin is 200 µg. The Processed Cereal-based Foods and Baby Foods for Infants and Young Children Regulations recommend a maximum folic acid content of 50 µg/100 kcal. The Foods Intended for use in Energy Restricted Diets for Weight Reduction Regulations (1997) recommend that whole diet products should provide 200 µg folic acid and meal replacements 60 µg.

**Existing recommendations on maximum intake levels**

148. The British National Formulary (BNF) notes that folic acid should not be prescribed alone in the presence of vitamin B<sub>12</sub> deficiency. Clinicians in practice 40 years ago were convinced that patients with vitamin B<sub>12</sub> deficiency were at increased risk of SCD because:

- i) the incidence of vitamin B<sub>12</sub> neuropathy was high (up to 80%) within one year of commencing folic acid in untreated vitamin B<sub>12</sub> deficiency;
- ii) there appeared to be a particularly high possibility of severe neurological symptoms developing at about three months after starting folic acid in these patients;
- iii) there was some evidence of a higher incidence of neuropathy as the dose of folic acid increased from 1.0mg to 15 mg or more daily.

**Existing recommendations on maximum supplementation levels**

149. The European Federation of Health Product Manufacturers Associations (EHPM) recommends a long-term upper safe level of 1000 µg/day (EHPM 1997). The Council for Responsible Nutrition, a UK Trade Association recommends an upper safe level of 400 µg/day folic acid for long term supplementation and 700 µg/day for short term supplementation (CRN, 1999).

## Summary

150. The term “folate” is used generically to describe the various derivatives of pteroylglutamic acid (PGA, folic acid), the reference, common pharmaceutical and most stable form of the folate vitamins group. Food and body folates generally exist as reduced, di- or tetra-hydrofolates (DHF or THF), which may also be polyglutamated and contain additional one-carbon adducts. Polyglutamate forms, based mainly on 5-methyl-THF account for approximately 90% of food folates, but the compounds are readily oxidised and degraded to monoglutamate forms during storage and preparation. Average daily folate intakes in the UK population are cited as 300 µg and 209 µg for men and women, respectively. The RNI for adults and children ≥ 11 years old is 200 µg/day. Folate supplements and medicines are available as folic acid (PGA), the oxidised, synthetic form of the folate vitamins group, which is not normally present in significant quantities in foods or the human body. The UK Department of Health recommends that women who are trying to conceive should take 400 µg folic acid supplement per day until the 12<sup>th</sup> week of pregnancy, in addition to normal dietary intake, for the prevention of neural tube defects (NTDs). In addition, trials are underway to assess the beneficial effects of folic acid supplementation in the prevention of cardiovascular disease.

151. The majority of dietary folate is absorbed within the proximal region of the small intestine by active, carrier-dependent mechanisms. Polyglutamate forms must first be hydrolysed to monoglutamates, which is carried out in humans by conjugase enzymes within the enterocyte brush border. Conjugase enzymes are inhibited in some disease states and by certain drugs or alcohol, thus reducing folate polyglutamate bioavailability. Ingested folic acid is enzymatically reduced and methylated within the intestinal lumen and enterocytes, although ingestion of high concentrations (> 200 - 300 µg/meal) results in the direct appearance of the compound, unmodified, in the plasma. Absorbed folate is carried *via* the portal blood, to the liver, whereby a proportion (≈ 0.1 mg/day) is excreted into the bile and undergoes an enterohepatic circulation and reabsorption. The liver is also the main storage site, containing approximately half of the total (5 - 10 mg) body folate. Folate is excreted in the urine and faeces, either as the metabolically active form, or as breakdown products.

152. The majority of plasma folate is present as 5-methyl-THF-monoglutamate. Within cells, folate is retained in the cytoplasm by polyglutamation. 5-methyl-THF is not a good substrate for polyglutamation, and must be first converted, *via* a vitamin B<sub>12</sub>-dependent reaction, to THF. Conversely, folic acid can be converted to polyglutamate (i.e., metabolically active) forms *via* a vitamin B<sub>12</sub>-independent pathway. Folate coenzymes within the cell are involved in one-carbon transfer reactions, including those involved in phases of amino acid metabolism, purine and pyrimidine synthesis, and the formation of the primary methylating agent, S-adenosylmethionine (SAM).

153. Folate deficiency results in reduced *de novo* DNA biosynthesis and, thus, impairment of cell replication, with the most obvious effects relating to rapidly dividing cell-types, such as erythrocytes and other cells generated by the bone marrow, enterocytes and skin cells. This condition is recognised, haematologically, as a macrocytic anaemia, and clinically as megaloblastic anaemia. Negative folate

balance is indicated by a serum folate concentration  $< 3$  ng/ml, whilst folate depletion or deficiency is indicated by erythrocyte and liver folate levels  $< 120$  ng/ml or  $1.6$   $\mu$ g/g (wet weight), respectively.

154. Oral folic acid therapy is generally considered to be safe, even at high doses, as the compound is water-soluble, with excesses excreted in the urine. However, potential adverse effects may occur in specific population groups. A small number of case reports have described hypersensitivity reactions to relatively high-dose oral folic acid therapy (generally  $\geq 1$  mg/day). One short-term, uncontrolled supplementation trial reported adverse symptoms in healthy volunteers given high doses of folic acid (15 mg/day) for 1 month. However, many other reports have described a lack of adverse effects associated with very high dose folic acid therapy (e.g. 1000 mg/day for  $\approx 1$ -3 weeks; 20 mg/day for 6-12 months). A major concern of general folic acid supplementation (particularly in relation to food fortification) is the potential for this to mask the haematological signs and symptoms of vitamin B<sub>12</sub> deficiency, allowing the associated central nervous system (CNS) neuropathy to develop untreated. This risk is considered to be greatest in the elderly, in whom vitamin B<sub>12</sub> deficiency is most prevalent. There are few data regarding the specific level of folate intake required to mask vitamin B<sub>12</sub>-deficiency-associated anaemia, however supplementation with  $\leq 1$  mg/day is generally assumed to be safe in this respect. A number of early case reports have intimated that folic acid supplementation may play an active role in reducing anaemia resulting from vitamin B<sub>12</sub> deficiency and/or directly enhancing the associated neuropathy. Data from randomised, controlled trials regarding this aspect are lacking. However it has been generally concluded from anecdotal reports that  $< 5$  mg/day oral folic acid supplementation is rarely associated with a direct adverse effect on vitamin B<sub>12</sub>- associated neurological damage.

155. Other potential adverse effects of folic acid therapy relate to possible interactions of the compound with other nutrients or drugs. Some authors have reported a negative effect of folate on zinc status, although many studies have not observed this effect. Some early case reports and supplementation trials have shown an association between folic acid therapy and the precipitation of seizures in drug-treated epileptics, although again, other studies have not observed such effects. It is also, theoretically, possible that folic acid therapy could reduce the efficacy of anti-folate drug treatments, although evidence from most studies suggests that folic acid supplementation can reduce the associated toxicity, without reducing the efficacy, of such treatments.

156. A substantial number of supplementation studies have been/are being carried out to assess the effectiveness of folic acid therapy in disease prevention (generally, either for the prevention of NTD-pregnancies, or cardiovascular disease, in high-risk groups). Many of these trials have shown beneficial effects associated with folic acid supplementation at levels up to  $\approx 10$  mg/day for periods of several weeks or months. In general, treatment-related adverse effects or toxicities have not been reported, although the majority of studies have not specifically addressed this issue.

157. Data regarding toxicological studies of folic acid in experimental animals are limited. A number of reports have described nephrotoxicity associated with the parenteral administration of extremely high doses ( $\geq 75$  mg/kg bw) of folic acid in rodents. Additionally, direct injection of high doses of folic acid or folates into the

brain or spinal fluid has been shown to produce seizures in rats and mice. One study has reported an inverse effect of high-dose (stated by the authors as 2500 mg/kg bw/day, but likely to be a typographical error for 2.5 mg/kg bw/day) dietary folic acid supplementation on pentylenetetrazol-seizure-threshold in rats. There are limited data to suggest that folic acid supplementation, in comparison with deficiency, may be associated with the promotion of tumours in animals which produce spontaneous tumours or are exposed to chemical carcinogens. However, this relative difference presumably relates to the inhibitory effect of folate deprivation on rapidly growing tumour cells, rather than a specific tumour-promotional effect of folic acid supplementation. Available data suggest that folic acid is not genotoxic.

158. Oral folic acid supplementation, alone, has generally not shown reproductive or embryotoxic effects in animal models. One report described reduced body weight and vertex-coccyx length in the foetuses of rats fed diets containing approximately 2 mg/kg bw/day folic acid for 3 weeks during pregnancy as compared with a control group of animals given a basal diet, but the statistics reported appear to be flawed. Folic acid treatment has been reported to enhance the embryotoxic effects of certain drugs (pyrimethamine, valproic acid), and of zinc-deficiency. However, this is thought to be due to an effect on the glycine clearance system for which folate is a co-factor rather than due to an effect on folate metabolism *per se*.

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## ANNEX 1 TO EVM/00/18

**Table 1.** Folate content of selected foods (adapted from Davis 1986)

	Total folate range ( $\mu\text{g}/100\text{ g}$ )
Bread (white)	15-30
Whole wheat flour	32-50
Beans white, dry	129-290
Asparagus	64-109
Potatoes	7-36
Cabbage	22-30
Broccoli	4-56
Pumpkin	5-19
Brussels sprouts	14-49
Spinach	91-240
Oranges	4-46
Orange juice	2-53
Tomatoes	1-39
Grapefruit juice	2-21
Bananas	22-30
Liver (cooked)	145-240
Peanuts	44-106

**Table 2.** Dietary Reference Values for Folate (UK) ( $\mu\text{g}/\text{day}$ ) (adapted, Department of Health, 1991)

Age	Lower Reference Nutrient Intake	Estimated Average Requirement	Reference Nutrient Intake
0-12 months	30	40	50
1-3 years	35	50	70
4-6 years	50	75	100
7-10 years	75	110	150
11 years onwards	100	150	200
Pregnancy			+100
Lactation			+60

**Table 3.** US Food and Nutrition Board RDA (Recommended Dietary Allowance) values for Folate, 1989 and 1998 (*data from Shils et al., 1998*).

	1989		1998	
	Age (years)	RDA for folate ( $\mu\text{g/day}$ )	Age (years)	RDA for folate ( $\mu\text{g/day}$ ) <sup>a</sup>
Infants	0-0.5	25	0-0.5	65 <sup>b</sup>
	0.5-1.0	35	0.5-1.0	80 <sup>b</sup>
Children	1-3	50	1-3	150
	4-6	75	4-8	200
	7-10	100		
Males	11-14	150	9-13	300
	15-18	200	14-18	400
	19-24	200	19-30	400
	25-50	200	31-50	400
	51+	200	51-70	400
			>70	400
Females	11-14	150	9-13	300
	15-18	180	14-18	400
	19-24	180	19-30	400
	25-50	180	31-50	400
	51+	180	51-70	400
			>70	400
Pregnancy (all ages)		400		600
Lactation (all ages)		260-280		500

a] As dietary folate equivalents (DFE). 1 DFE = 1  $\mu\text{g}$  food folate = 0.6  $\mu\text{g}$  folic acid (from fortified food or supplement) consumed with food = 0.5  $\mu\text{g}$  synthetic (supplemental) folic acid taken on an empty stomach.

b] Adequate daily intake (ADI)

**Table 4.** Case reports and supplementation studies of folic acid in epileptic subjects receiving treatment with anti-seizure medications.

Reference	Report/study type	Subject(s)	Folic acid therapy	Duration of therapy	Comments
Strauss & Bernstein (1974)	Case report	Pregnant female	10 mg/day (+ multivitamin)	From 9 <sup>th</sup> week of gestation	Increased seizure frequency from 15 weeks' gestation
Strauss & Bernstein (1974)	Case report	Pregnant female	6 mg/day (+ multivitamin)	weeks 11-18 of gestation	Increased seizure frequency from 15 weeks' gestation; no further seizures upon termination of folic acid therapy
Inoue & Kolabinski (1986)	Case report	Female epileptic with gingival hyperplasia	5 mg/day (3 or 7 times per week)	9 weeks +	Increased seizure frequency 9 weeks after beginning folic acid therapy
Inoue & Kolabinski (1986)	Case report	Male epileptic with gingival hyperplasia	5 mg, 1-3 times per week	17 weeks +	Increased seizure frequency 17 weeks after beginning folic acid therapy
Berg <i>et al.</i> (1983)	Case report	Male epileptic with low erythrocyte folate	1 mg/day	2 weeks +	Increased seizure frequency 5 days after beginning folic acid therapy
Jensen & Olesen (1970)	Double-blind, placebo-controlled, crossover	24 epileptics with low serum folate and poor mental function	20 mg/day	11 weeks	No significant changes in mental state or seizure frequency
Reynolds & Wales (1967)	Open study	26 folate-deficient epileptics	15 mg/day	1-3 years	Improved mental state. Increased seizure frequency or severity in 50% of subjects
Gibberd <i>et al.</i> (1981)	Double-blind, placebo-controlled	30 epileptics	15 mg/day	≤ 1 year	No adverse effects observed
Mattson <i>et al.</i> (1973)	Double-blind, placebo-controlled, crossover	41 epileptics with low serum folate	15 mg/day	6 months	No significant or consistent alteration in seizure control between test and placebo groups. Increased serum, but not cerebrospinal fluid, folate levels in active-treatment group
Grant & Stores (1970)	Double-blind, placebo-controlled trial	51 epileptics with low serum folate	15 mg/day	26 weeks	No significant changes in seizure frequency, behaviour and personality or cognitive functions
Ralston <i>et al.</i> (1970)	Double-blind, placebo-controlled	27 epileptics with low serum folate	15 mg/day	3 months	No change in behaviour, personality or seizure frequency
Baylis <i>et al.</i> , (1971)	Open trial	50 male epileptics	15 mg/day (40 subjects), 5 mg/day (10 subjects)	1 month	Significant, treatment-related reduction in plasma phenobarbitone levels; no significant overall change in seizure frequency (although 1 subject showed a marked increase in fit frequency and severity)
Norris & Pratt (1971)	Single-blind, placebo-controlled, "crossover" (a period of placebo, followed by active, treatment in both groups)	39 epileptics	5 mg/day	2 x 3 months	No effect of therapy on seizure frequency (the defined endpoint of the trial)
Horwitz <i>et al.</i>	Double-blind,		5 mg/day		

(1968)	placebo-controlled, crossover	12 chronic epileptics, with peripheral neuropathy		2 x 3 months	Seizure frequency reportedly unaffected by folic acid therapy
Brown <i>et al.</i> (1991)	Randomised, double-blind, placebo-controlled	20 epileptic patients with phenytoin-induced gingival hyperplasia	3 mg/day	16 weeks	No effect of supplementation on gingival hyperplasia. No increase in seizure activity.

**Table 7.** Folic acid supplementation studies in humans without apparent vitamin B<sub>12</sub> deficiency

Reference	Subjects	<i>n</i>	Study aim	Study design	Folic acid dose(s) (mg/day)	Duration of treatment	Reported adverse, or potential adverse, effects associated with supplementation
Suarez <i>et al.</i> (1947)	patients with tropical sprue	50	treatment of tropical sprue	open	100	2 weeks	no adverse effects observed
Weissberg <i>et al.</i> (1950)	adults, 6 of whom showed neurological symptoms (but not subacute combined spinal cord degeneration)	26	assessment of the potential neurotoxic effects of supplementation	open	20	9-12 months	no adverse effects observed
Harvey <i>et al.</i> (1950)	healthy volunteers	40	assessment of the potential neurotoxic effects of supplementation	open	20	3-12 months	no adverse effects observed
Hellstrom (1971)	healthy volunteers	15	assessment of the side effects of therapy	randomised, double-blind, placebo-controlled	15	1 month	no adverse effects observed
Hunter <i>et al.</i> (1970)	healthy volunteers	14	to assess the effects of folic acid supplementation on serum vitamin B <sub>12</sub> levels	open	15	1 month	trial ended prematurely (intended duration – 3 months) after 13 participants reported mental changes, sleep disturbances and gastrointestinal symptoms
Richens (1971)	healthy volunteers	12	assessment of the side effects of the therapy	double-blind, placebo-controlled	15	1 month	no adverse effects observed
Bostom <i>et al.</i> (1996)	dialysis patients	27	assessment of the effects of supplementation on plasma	randomised, placebo-controlled	15 (+ vitamins B <sub>6</sub> /B <sub>12</sub> ) (all subjects also)	8 weeks	active treatment was not associated with an increased frequency of any specific symptoms or adverse changes in haematocrit, liver transaminases or creatinine.

			homocysteine levels		continued taking a pre-prescribed dose of 1 mg/day folic acid + vitamins B <sub>6</sub> /B <sub>12</sub> )		
Butterworth <i>et al.</i> (1992)	women with cervical intraepithelia neoplasia (CIN)	235	assessment of the effect of supplementation on the natural history of cervical dysplasia	randomised, placebo-controlled	10 mg/day	6 months	not reported
Heimburger <i>et al.</i> (1988)	male smokers with bronchial squamous metaplasia	73	to assess the effects of supplementation on squamous metaplasia and atypia	randomised, double-blind, placebo-controlled	10 (+ vitamin B <sub>12</sub> )	4 months	not reported
Juhlin & Olsen (1997)	vitiligo patients	100	to assess the effects of supplementation on skin re-pigmentation	open	10 (+ vitamin B <sub>12</sub> )	≥ 3 months	not reported
Butterworth <i>et al.</i> (1988)	women with cervical dysplasia	50	assessment of the effects of supplementation on plasma and red cell zinc levels	randomised, placebo-controlled	10	2-4 months	the authors reported that there were no untoward clinical symptoms attributable to medication, and no significant association between folic acid supplementation and zinc levels
Landgren <i>et al.</i> (1995)	acute myocardial infarction patients	33	to assess the effects of supplementation on plasma homocysteine levels	randomised	2.5, 10	6 weeks	not reported
Wilmink <i>et al.</i> (2000)	healthy volunteers	20	to assess the effects of supplementation on endothelial function	randomised, double-blind, placebo-controlled	10	2 weeks	not reported

Childers <i>et al.</i> (1995)	women with a cervical intraepithelial neoplasia (CIN)	331	to assess the potential of folic acid supplementation to improve cervical neoplasia	multicentre, randomised, double-blind, placebo-controlled	5	6 months	not reported
Vergel <i>et al.</i> (1990)	women with a history of a previous NTD birth	101	to assess the potential of periconceptual supplementation to reduce the incidence of foetal NTDs	open	5	≥ 1 menstrual period pre-conception until week 10 of pregnancy	not reported
Lobo <i>et al.</i> (1999)	subjects with coronary artery disease	95	to assess the effects of supplementation on plasma homocysteine levels	non-randomised, single-blind, placebo-controlled	0.4, 1, 5 (all doses + vitamins B <sub>6</sub> and B <sub>12</sub> )	3 months	7 subjects reported adverse reactions (5 in the 0.4 mg group; 1 in the 1 mg group; 1 in the 5 mg group)
den Heijer <i>et al.</i> (1998)	healthy or hyperhomocysteinaemic volunteers (227) and subjects with venous thrombosis (89)	316	to assess the effects of supplementation on blood homocysteine levels	randomised, placebo-controlled	0.5 (alone), 5 (alone), 5 (+ vitamins B <sub>6</sub> /B <sub>12</sub> ),	8 weeks	6 subjects withdrew from the trial (reasons not stated)
Chao <i>et al.</i> (1999)	healthy volunteers	16	to assess the effects of supplementation on endothelial dysfunction	open	5 (+ vitamins B <sub>6</sub> and B <sub>12</sub> )	5 weeks	not reported
Verhaar <i>et al.</i> (1999)	subjects with familial hypercholesterolaemia	20	to assess the effects of supplementation on endothelial function	randomised, double-blind, placebo-controlled	5	4 weeks	not reported
MRC Vitamin Study Research	women with a previous NTD pregnancy	1817	assessment of the potential for	multicentre, randomised, double-blind, placebo-	4 (and/or MV)	from the date of randomisation	similar incidence of non-specific ailments reported in all groups of women;

Group (1991)			periconceptual supplementation to reduce the incidence of foetal NTDs	controlled		until week 12 of pregnancy	slightly greater number of reported (non-NTD) foetal congenital abnormalities in vitamin-treated groups (the authors noted that the power of the study to detect rare or slight adverse effects was limited)
Laurence <i>et al.</i> (1981)	women with a history of a previous NTD birth	111	assessment of the potential for periconceptual supplementation to reduce the incidence of foetal NTDs	randomised, double-blind, placebo-controlled	4	from the time of stopping contraception (duration not stated)	no adverse effects reported (27% non-compliance in the treatment group; one non-complier who had taken a “large number” of folic acid tablets at 7 weeks gestation, spontaneously aborted an anencephalic foetus at 3 months)
Hambidge <i>et al.</i> (1993)	serum samples from women in each of the 4 randomised groups from the MRC Vitamin trial (1991) (see above)	27 cases; 108 matched controls	assessment of the effects of supplementation on serum zinc levels	case-control (affected or unaffected NTD pregnancy)	4 (and/or MV) (or placebo)	from date of randomisation (see above) until week 12 of pregnancy	no significant association between folic acid supplementation and serum zinc levels at 12 weeks gestation
Beaulieu <i>et al.</i> (1999)	chronic, stable renal transplant recipients	60	assessment of the effects of supplementation on total homocysteine levels	randomised	0, 0.4, 2.4 (all doses + vitamins B <sub>6</sub> and B <sub>12</sub> )	12 weeks	not reported
Malinow <i>et al.</i> (1997)	healthy volunteers and subjects with coronary heart disease	242	assessment of the effect of supplementation on plasma homocysteine levels	randomised	1, 2	3 weeks	not reported
Tamura <i>et al.</i> (1992)	multiparous, pregnant women	285	assessment of the effects of supplementation on serum zinc concentrations	open	1 (+ iron)	“during pregnancy”	no significant effect of folic acid supplementation on serum zinc levels at 18 and 30 weeks gestation; adverse effects not reported.
Mackey &	healthy, lactating women	42	assessment of the	randomised, double-blind,	1	3 months (from 3	not reported

Picciano (1999)			effects of supplementation on maternal folate status and infant growth rate	placebo-controlled	(+ MV)	to 6 months post-partum)	
Fuller <i>et al.</i> (1992)	enterally-fed, pre-term infants	60	assessment of the effects of supplementation on zinc status	open	1	From the 8.7 <sup>th</sup> (median) (range 2 <sup>nd</sup> -42 <sup>nd</sup> ) day after birth, for periods up to 16 <sup>th</sup> week of life	significant inverse relationship between maximum attained serum folate level and minimum attained serum zinc level for each subject
Czeizel & Dudas (1992)	women planning a pregnancy	4753	to assess the potential of periconceptional supplementation to reduce the incidence of foetal NTDs	randomised	0.8 (+ MV), (or trace-element supplement [Zn, Cu, Mn, vitamin C])	from $\geq$ 1 month pre-conception until $\geq$ the date of the 2 <sup>nd</sup> missed menstrual period	not reported
Kauwell <i>et al.</i> (1995)	healthy male volunteers, consuming zinc-adequate, or zinc-deficient, diets	12	to assess the effects of short-term acid supplementation on zinc status	open	0.8	28 days	no significant effect of folic acid supplementation on serum, erythrocyte, or urinary zinc levels, serum alkaline phosphatase activity, erythrocyte metallothionein or serum ferritin, either between zinc-restricted and zinc-adequate groups, or within each group during study period
Ubbink <i>et al.</i> (1994)	hyperhomocysteinaemic men	100	to assess the effects of supplementation on plasma homocysteine levels	randomised, placebo-controlled	0.65 (+ vitamins B <sub>6</sub> /B <sub>12</sub> ), (or vitamin B <sub>6</sub> ), (or vitamin B <sub>12</sub> )	6 weeks	not reported
Brouwer <i>et al.</i> (1999)	healthy female volunteers	144	to assess the effects of supplementation on plasma homocysteine levels	randomised, placebo-controlled	0.25, 0.5	4 weeks	not reported

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Daly <i>et al.</i> (1997)	female volunteers	172	to assess the effectiveness of folic acid doses in raising erythrocyte folate levels (as a marker for determining an effective level for food fortification regarding NTD prevention)	randomised, double-blind, placebo-controlled	0.1, 0.2, 0.4	6 months	not reported
Kirke <i>et al.</i> (1992)	women with a previous NTD pregnancy	354	to assess the potential of periconceptional supplementation to reduce the incidence of foetal NTDs	randomised, double-blind, not placebo-controlled	0.36 (and/or MV)	from $\geq$ 2 months prior to conception until the date of the 3 <sup>rd</sup> missed period	2 women reported side affects of the treatment
Smithells <i>et al.</i> (1981)	women with a history of a previous NTD birth	250	to assess the potential of periconceptional supplementation to reduce the incidence of foetal NTDs	open	0.36 (+ MV)	from $\geq$ 28 days pre-conception (mean 110 days) to the date of the 2 <sup>nd</sup> missed period	not reported; (13 subjects withdrew, citing side effects of the therapy)

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MV = multivitamin, or multivitamin and mineral preparation

**Table 6.** Informative pregnancies: abnormalities other than neural tube defects according to randomisation group in the MRC Vitamin Study Trial (MRC Vitamin Study Research Group, 1991).

Randomisation group	Folic acid	Other vitamins	Total non-NTD	Number with abnormal outcomes other than NTD	All reported abnormalities as notified (T = termination of pregnancy; M = miscarriage)
A	+	-	296	7	Agenesis of corpus callosum and hydrocephalus (T); Down's syndrome; tetralogy of Fallot; severe asphyxia with low birthweight and cleft palate; pes varus; intrauterine growth retardation; polydactyly
B	+	+	291	12	Lethal multiple pterygium syndrome (M); trisomy 15 (M); Adams-Oliver syndrome; Turner's syndrome (T); talipes; hypospadias; pyloric stenosis; dislocatable hips; persistent foetal circulation; pectus excavatum; purple birthmark/lump between eyes; unexplained neutropoenia
C	-	-	287	5	Partial deletion chromosome 18 (M); Down's syndrome; bilateral talipes (2); cardiac murmur
D	-	+	294	8	Hydropic foetus with cervical cystic lymphangioma, complex cardia malformation, and ganglioneuroblastic hamartoma of adrenals; hydropic foetus and cervical hygroma (T); Klinefelter's syndrome (T); congenital nystagmus and dilated ventricles; arthrogryposis; pes equinovarus; mongolian blue spot; skin tag at base of spine
Total			1168	32	-

**Table 7.** Congenital malformations according to study group in the Hungarian periconceptional vitamin supplementation study (Czeizel & Dudas 1992)

Malformation	Vitamin Group (number of malformations)	Trace Element Group (number of malformations)
Neural tube defect	0	6
Congenital hydrocephalus	0	2
Cardiovascular malformation	6	9
Cleft palate	0	2
Cleft lip (with or without cleft palate)	4	3
Hypospadias	1	1
Obstructive defects of urinary system	1	2
Congenital postural deformity	2	0
Limb-reduction defect	1	5
Foramina parietale permagna	0	2
Exomphalos and gastroschisis	1	1
Large hemangioma on face	3	1
Down's syndrome	2	3
Unidentified multiple malformations	3	3
Other*	4	7
Total	28	47

\* Each congenital malformation occurred only once in either group

**Table 8.** Studies of the reproductive and developmental toxicity of folic acid in animals.

Reference	Species	Supplementary folic acid dose (mg/kg bw/day)	Duration	Comments
Chung <i>et al.</i> (1993)	rat	50 (+/- 2.7 mg/kg bw/day pyrimethamine [PYM])	days 7-17 of gestation	no treatment-related effects in dams; enhancement of PYM-related embryotoxicity (visceral and skeletal abnormalities, but not external malformations) by folic acid supplementation
Quinn <i>et al.</i> (1988)	rat	1.5-10	from mating until day 18 of gestation	significantly increased incidence of clubbed foot in 10 mg/kg bw/day folic acid-supplemented, as compared with non-folic acid-supplemented, zinc-deficient rats; no effect of folic acid supplementation on foetal or maternal zinc status
Fuller <i>et al.</i> (1988)	rat	5.0 (+ 0.3 or 1.0 mg/kg bw/day zinc)	during pregnancy and lactation (until day 20 of gestation or day 20 postpartum)	no effect of folic acid supplementation on zinc levels of pups or dams
Achon <i>et al.</i> (1999, 2000)	rat	2	3 weeks (complete pregnancy)	no association between folic acid supplementation and gestational development (number of live foetuses); significantly reduced body weights and vertex-coccyx lengths of foetuses from folic acid supplemented dams, as compared with those fed a basal diet (although the validity of the reported statistical analysis is unclear)
Hansen <i>et al.</i> (1995)	mouse	1.8 (+/- treatment with valproic acid (VPA))	from 3 weeks prior to breeding throughout gestation	folic acid supplementation was associated with a reduced number of non-live implants, but did not significantly alter the teratogenic effects of VPA (exencephaly); no effects of folic acid supplementation, alone, on foetal or maternal outcomes, or on maternal zinc status
Hansen <i>et al.</i> (1995)	rat	0.6 (+/- treatment with valproic acid (VPA))	from 3 weeks prior to breeding throughout gestation	folic acid supplementation was associated with a significant increase in the number of non-live implants in VPA-treated rats; no effects of folic acid supplementation, alone, on foetal or maternal outcomes or zinc status
Matte <i>et al.</i> (1993)	pig	0.6	from 9 weeks' age through puberty, until slaughter at 7 weeks' gestation	folic acid supplementation was associated with significantly increased foetal folate concentrations; no maternal or foetal treatment-related effects
Shin & Shiota (1999)	mouse	0.45 (+/- heat treatment of day 8.5 of gestation)	days 0.5-9.5 of gestation	folic acid supplementation was associated with a significant reduction in the incidence of malformations and resorptions associated with heat treatment
Morgan & Winick (1978)	rat	0.09 (+/- 1mg/day by <i>i.p.</i> injection)	from 14 days prior to, until day 21 of, gestation	folic acid supplementation (dietary + intra-peritoneal injection) was associated with a significantly increase in foetal, placental and liver, RNA, DNA and protein content, and increased liver tetrahydrofolate reductase activity

**Table 9 [a-c].** Studies of the embryotoxicity of pyrimethamine and/or folic acid in rats. a) foetal effects of treatment, b) visceral findings in foetuses, c) skeletal findings in foetuses (*from Chung et al.*, 1993; treatment groups and regimes are described in paragraph 131 of the report). These figures cannot be reproduced for copyright reasons.

**Table 10.** Gestation outcome in Wistar rat dams fed folic acid-supplemented or control diets<sup>1</sup> (adapted from Achon *et al* 1999).

Dams	n	Live foetuses per litter	Foetal body weight (g)	Foetal vertex-coccyx length (cm)
Supplemented	11	11.4 ± 1.16	3.15 ± 0.10**	3.5 ± 0.05**
Control	9	11.6 ± 0.74	3.49 ± 0.22	3.7 ± 0.09

<sup>1</sup> Mean value ± SEM.

\*\* Reported by the authors as significantly different from the control ( $P < 0.001$ )

**Table 11a.** Developmental toxicant effects of VPA in mice on normal or folic acid-supplemented chow (adapted from Hansen *et al* 1995)

	Control + vehicle	Control + VPA	Folic acid supplemented + vehicle	Folic acid supplemented + VPA
No. litters	18	26	22	33
No. implants	205	291	224	378
No. live implants	197	207	215	309
No. non-live implants (%)	8 (4)	84 (29)	9 (4)	69 (18)
Mean proportion of litter that was non-live	1.5	26.5*	1.1	14.9*, <sup>s</sup>
No. live pups with exencephaly (%)	0 (0)	108 (52)	1 (0.5)	129 (42)
Mean proportion of pups in litter with exencephaly	0.0	46.3*	0.02	39.8*
Foetal weight (mean ± SEM) (g)	0.84 ± 0.02	0.71 ± 0.04*	0.91 ± 0.02	0.77 ± 0.03*
Maternal weight gain (g)	16.3 ± 1.15	15.4 ± 1.03	16.6 ± 1.03	17.4 ± 0.60

\* Different from corresponding control group,  $P < 0.05$  by ANOVA.

<sup>s</sup> Different from control + VPA group,  $P < 0.05$  by ANOVA.

**Table 11b.** Developmental toxicant effects of VPA in rats on normal or folic acid-supplemented chow.

	Control + vehicle	Control + VPA	Folic acid supplemented + vehicle	Folic acid supplemented + VPA
No. litters	21	36	22	38
No. implants	316	544	327	540
No. live implants	284	397	291	303
No. non-live implants (%)	32 (10)	147 (27)	36 (11)	237 (44)
Mean proportion of litter that was non-live	5.9	25.9*	6.4	46.0*, <sup>s</sup>
No. live pups with exencephaly (%)	0 (0)	116 (29)	0 (0)	63 (21)
Mean proportion of pups in litter with exencephaly	0.0	19.8*	0.0	13.7*
Maternal weight gain (g)	45.8 ± 5.17	47.2 ± 2.58	39.2 ± 2.57	37.3 ± 2.36 <sup>s</sup>

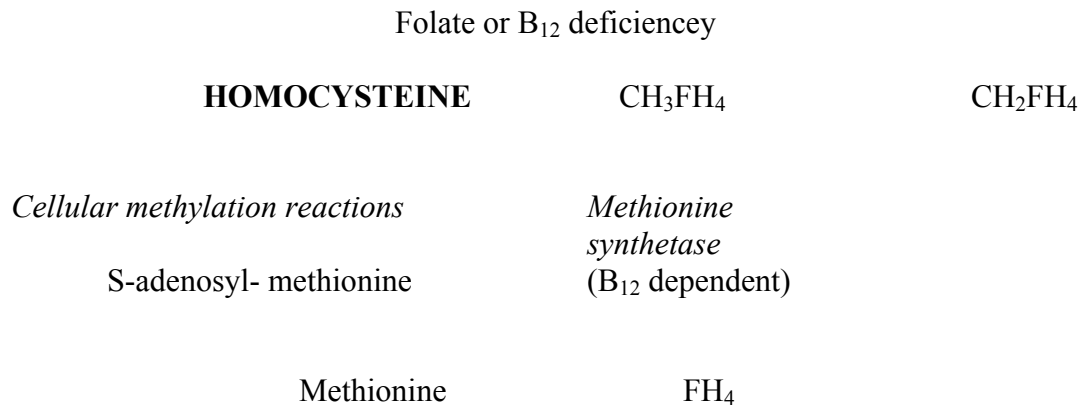
\* Different from corresponding control group,  $P < 0.05$  by ANOVA.

<sup>s</sup> Different from control + VPA group,  $P < 0.05$  by ANOVA.

**Figure 1 [a-d].** Plasma pharmacokinetics of folic acid. These figures cannot be reproduced for copyright reasons but can be consulted in Priest *et al.* (1999)

**Figure 2.** Folate Metabolism. This figure cannot be reproduced for copyright reasons but can be consulted in Herbert, 1999.

**Figure 3.** Role of 5-methyl tetrahydrofolic acid: a reduction in function folate increases plasma homocysteine levels. (adapted *from* Calvert 1999).





## ANNEX 2 TO EVM/00/18

INTAKES OF FOLATE<sup>3</sup> FROM FOOD AND SUPPLEMENTS

The data presented on folate intakes are obtained from dietary surveys of specific population age groups in Britain carried out over the last 15 years<sup>4,5,6,7,8</sup>. In each survey food consumption data were collected by means of a dietary record (usually weighed) kept for 4 or 7 consecutive days. Nutrient intakes were calculated using a set of nutrient composition data contemporaneous with the time of the survey. Therefore some apparent differences in intakes between population age groups may be due to changes in the nutrient composition data and reflect changes in the nutrient composition of manufactured foods over time.

**Total intakes of folate**

Table 1 provides information on the absolute intakes of folate by the British population, classified by age and sex. Mean and median intake, and the upper and lower end of the intake distribution (defined as upper and lower 2.5 percentiles, respectively), are given. In addition, intakes of folate from food and supplements for older people are presented both including and excluding prescribed folate supplements, for comparison. Although these prescribed preparations were only taken by a small minority of participants within this group, intakes from them were found to have a disproportionate impact on mean daily intakes of folate.

Average intakes of folate were lowest for young children aged up to 4½ years. Average intakes from food were highest for males aged 16-64 years, and intakes from food and supplements (including those prescribed) were highest in males aged 85 years and over. Average folate intakes increased significantly with age for pre-school children and young people aged 4-18 years, and decreased significantly with age for older people free-living in the community.

Mean folate intakes (from all sources, including prescribed supplements) were above Reference Nutrient Intakes (RNIs) in all age/gender groups. Median intakes were also above RNIs in all groups except for females aged 15 to 18 and 16 to 34 years, older females aged 75 years and above free-living in the community, and females aged 85 years and above living in institutions. Excluding the contribution from supplements, mean intakes from food only were below the RNI for females aged 16-24 years and 85 years and over, both free-living and in institutions.

Intakes from food and supplements (including prescribed folate) at the 97.5<sup>th</sup>ile were about twice the median in all groups except for males aged 85 years and over, which

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<sup>3</sup> Folate is a generic term for compounds that have a common vitamin activity, and includes the synthetic form of the vitamin folic acid (pteroyl glutamic acid) and a wide variety of derivatives.

<sup>4</sup> Food and nutrient intakes of British infants. 1986

<sup>5</sup> National Diet and Nutrition Survey of children aged 1½-4½ years. 1992/3

<sup>6</sup> National Diet and Nutrition Survey of young people aged 4-18 years. 1997/8

<sup>7</sup> Dietary and nutritional survey of British adults. 1986/7

<sup>8</sup> National Diet and Nutrition Survey of people aged 65 years and over. 1994/5

were 8 times the median for the free-living group and 22 times for median for those in institutions.

Table 2 provides information on folate intakes from food and supplements adjusted for body weight and classified by age and sex. Body weight adjusted folate intakes are highest in infants and show a trend to decrease with age for children and young people.

### **Sources of folate in the diet**

Table 3 indicates the contribution made by different types of food to average intakes of folate by young people aged 15-18 years. This dataset was collected in 1997 and so most closely reflects current eating habits and fortification practices.

*The main food source of folate in this age group is cereals and cereal products (38%), of which half came from breakfast cereals and about a quarter came from bread. Many breakfast cereals and some breads are fortified with folate. Vegetables, potatoes and savoury snacks provided 27% of folate intake, of which about half came from potatoes. Drinks provided 10%, of which half came from beers and lagers.*

Infants obtained about a fifth of their folate intake from milk and milk products, a further fifth from commercial infant foods and 17% from infant formulas. Cereals and cereal products were the major sources of folate for other age groups, followed by vegetables, potatoes and savoury snacks.

Folate is often added voluntarily by manufacturers to foods such as breakfast cereals and bread. The universal fortification of flour with folic acid was discussed in a recent COMA report on folic acid and the prevention of disease<sup>9</sup>. The conclusions in the COMA report are subject to consultation.

### **Folate intakes from supplements**

Dietary supplements containing folate (including those prescribed) provided between 0.5% and 2.5 % of population average intakes of folate for pre-school children, young people and adults. However, for females aged 16 to 24 years supplements provided about 9% of population average intakes. For young people, the effect of supplements providing folate was only apparent for females aged between 7 and 18 years of age at the upper 2.5 percentile of the distribution. For example, supplements providing folate increased population average intakes from food sources alone by 13% at the upper 2.5 percentile for females aged 11 to 14 years.

For older people free-living in the community, dietary supplements containing folic acid (excluding those prescribed) provided 3% of population average intakes of folate for males and 6% for females. This increased to 9% and 18% respectively when prescribed supplements containing folic acid were included in the dataset. For the oldest group of males, prescribed and non-prescribed supplements together provided 40% of average folate intake.

<sup>9</sup> Department of Health. Folic acid and the prevention of disease. Report on Health and Social Subjects 50. London: The Stationery Office, 2000

For older people living in institutions, dietary supplements (excluding those prescribed) provided a negligible contribution to population average intakes of folate for men and 1% for women. However, when prescribed supplements containing folate were included, this increased to 29% for males and 31% for females overall, 38% for females aged 65-84 years, and 51% and 23% for males and females aged 85 years and over respectively.

Of course, the proportion of intake from supplements is much higher if supplement consumers are considered separately.

Table 4 shows the number of consumers of dietary supplements containing folate in each age group, together with the mean, median and range of intakes of folate from supplements for those who consumed them. No more than 5% of any group studied used supplements containing folate.

All women who could become pregnant have been advised, since the early 1990s, to take 400µg folic acid per day as a medicinal or food supplement prior to conception and until the twelfth week of pregnancy, to reduce the risk of neural tube defects. There were no intakes at this level from supplements containing folic acid for females in the 15 to 18 year age group in the most recent NDNS survey of young people (1997/8). This was also the case for older females of child-bearing age; however these data were collected in 1986/87, before the above guidance was issued, and the use of folate supplements has increased since then. The high intakes of folate from supplements in older people were due primarily to the use of prescribed supplements containing folic acid. One brand provided 5000 µg (5mg) per dose. Folic acid in doses of 500µg or greater requires medical prescription.<sup>10</sup>

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August 2000

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<sup>10</sup> Department of Health. Folic acid and the prevention of disease. Report on Health and Social Subjects 50. London: The Stationery Office, 2000.

Table 1: Total intakes of Folate

Age/sex	Absolute Folate intake ( $\mu\text{g/day}$ ) <sup>11</sup>							
	Food Only				Food and Supplements			
	2.5% ile	Mean	Median	97.5%ile	2.5% ile	Mean	Median	97.5%ile
<b>Infants (1986)</b> 6-12mths/M&F	58	106	102	172	*	*	*	*
<b>Pre-school children</b>								
1½-2½ yrs/M/F	59.6	120	114	222	59.6	120	114	225
2½-3½ yrs/M/F	60.7	133	126	252	60.7	134	127	254
3½-4½ yrs/M	58.8	143	138	244	58.8	145	140	256
3½-4½ yrs/F	62.7	138	134	248	62.7	140	135	251
<b>Young people (1997/8)</b>								
4-6 yrs/M	93	191	184	350	93	192	185	350
4-6 yrs/F	89	169	159	317	89	171	162	317
7-10 yrs/M	123	212	203	348	123	213	203	373
7-10 yrs/F	93	188	186	320	93	190	187	341
11-14 yrs/M	114	245	234	405	114	247	234	405
11-14 yrs/F	90	205	198	353	90	210	200	398
15-18 yrs/M	130	305	283	615	130	309	284	615
15-18 yrs/F	88	210	197	383	88	215	197	402
<b>Adults (1986/7)</b>								
16-24 yrs/M	140	302	285	600	140	302	285	600
16-24 yrs/F	85	198	194	330	91	217	198	343
25-34 yrs/M	164	317	303	549	164	319	303	563
25-34 yrs/F	84	206	198	348	84	208	198	349
35-49 yrs/M	138	321	308	555	138	322	310	555
35-49 yrs/F	105	220	212	383	106	224	213	410
50-64 yrs/M	156	300	289	545	156	301	289	569
50-64 yrs/F	101	218	214	354	102	222	214	402
<b>Older people free-living in the community (1994/5)</b>								
65-74yrs/M	128	282	275	458	130(130)	308(292)	276(276)	609(560)
65-74yrs/F	88	215	209	387	88(93)	248(228)	213(211)	481(480)
75-84 yrs/M	109	249	234	454	108(109)	256(256)	234(237)	487(488)
75-84 yrs/F	89	201	186	358	89(89)	265(217)	190(188)	509(436)
85 and over/M	76	234	219	375	77(76)	390(242)	239(232)	1972(461)
85 and over/F	65	184	170	347	65(65)	227(192)	174(172)	443(398)
<b>Older people living in institutions (1994/5)</b>								
65-84 yrs/M	88	234	223	452	86(88)	234(234)	222(223)	451(452)
65-84 yrs/F	100	210	199	346	109(108)	339(211)	199(200)	426(346)
85 and over/M	92	235	225	438	94(92)	479(236)	233(225)	5169(438)
85 and over/F	80	187	185	318	83(80)	242(191)	187(188)	479(332)

\* Data unavailable

<sup>11</sup> Data in brackets = intakes from food and supplements, excluding prescribed supplements

**Table 2: Bodyweight adjusted Folate intake**

Age/sex	Bodyweight adjusted Folate intake ( $\mu\text{g}/\text{kg}$ bwt /day) <sup>12</sup>		
	<i>intakes from food and supplements</i> <sup>13</sup>		
	Mean	Median	97.5%ile
<b>Infants (1986)<sup>14</sup></b> 6-12mths/M&F	11.100	10.640	18.030
<b>Pre-school children (1992/3)</b> 1½-2½ yrs/M&F	9.887	9.337	19.294
2½-3½ yrs/M&F	9.158	8.595	17.307
3½-4½ yrs/M	8.736	8.382	15.739
3½-4½ yrs/F	8.576	8.237	14.644
<b>Young people (1997/8)</b> 4-6 yrs/M	9.074	8.732	15.069
4-6 yrs/F	8.438	7.900	16.863
7-10 yrs/M	7.171	7.087	12.093
7-10 yrs/F	6.173	5.912	10.595
11-14 yrs/M	5.451	5.240	9.701
11-14 yrs/F	4.474	4.172	9.167
15-18 yrs/M	4.686	4.264	9.237
15-18 yrs/F	3.680	3.445	6.918
<b>Adults (1986/7)</b> 16-24 yrs/M	4.336	4.120	7.765
16-24 yrs/F	3.381	3.328	5.951
25-34 yrs/M	4.251	4.107	7.325
25-34 yrs/F	3.385	3.376	6.163
35-49 yrs/M	4.221	4.026	7.339
35-49 yrs/F	3.559	3.341	6.760
50-64 yrs/M	3.907	3.734	7.054
50-64 yrs/F	3.498	3.440	6.882
<b>Older people free-living in the community (1994/5)</b> 65-74 yrs/M	3.979	3.632	7.543
65-74 yrs/F	3.805	3.260	7.569
75-84 yrs/M	3.501	3.202	7.399
75-84 yrs/F	4.050	3.084	9.430
85 and over/M	5.899	3.567	27.988
85 and over/F	3.887	3.063	8.685
<b>Older people living in institutions (1994/5)</b> 65-84 yrs/M	3.447	3.212	6.045
65-84 yrs/F	5.709	3.189	8.347
85 and over/M	7.757	3.436	50.557
85 and over/F	4.324	3.019	7.810

<sup>12</sup> Body weights measured for each subject for all age groups except infants aged 6-12 months where reported body weights were used.

<sup>13</sup> Data includes intakes from prescribed folate supplements

<sup>14</sup> Intakes for infants aged 6-12 months are from food only.

Table 3<sup>15</sup>: Sources of Folate in the diet

Food Type	Contribution of food types to average daily intake of Folate	
	µg/day	% of total
Cereal and cereal products	100	38
- of which breakfast cereals	50	19
- of which bread	23	9
Milk and milk products	25	9
Egg and egg dishes	4	1
Fat spreads	0	0
Meat and meat products	17	7
Fish and fish dishes	2	1
Vegetables, potatoes and savoury snacks	70	27
- of which roast/fried potatoes and chips	22	8
- of which boiled, mashed, baked potatoes	12	5
Fruits and nuts	5	2
Sugar, confectionery and preserves	2	1
Beverages	27	10
- of which beers and lagers	12	5
Miscellaneous	7	3
<b>Total intake from food</b>	<b>259</b>	<b>100*</b>
<i>Intake from dietary supplements</i>	5	2
<b>Total intake from food and supplements</b>	<b>263</b>	<b>100</b>

\*Total allows for rounding

<sup>15</sup> NDNS: young people aged 4-18 years. 1997/8. 15-18 year group

**Table 4: Folate intake from supplements<sup>16</sup>**

<i>Age/sex</i>	<b>Consumers of Folate supplements</b>		<b>Folate intake from supplements (consumers only) (µg/day)</b>		
	<i>Number</i>	<i>%</i>	<i>Mean</i>	<i>Median</i>	<i>Range</i>
<b><i>Infants (1986)</i></b> 6-12 mths/M&F	*	*	*	*	*
<b><i>Pre-school children (1992/3)</i></b> 1½-4½ yrs/M&F	21	1	72.0	50.0	5.0 – 246.4
<b><i>Young people (1997/8)</i></b> 4-6 yrs/M&F	12	3	41.0	21.4	7.1 – 100.0
7-10 yrs/M&F	7	1	117.9	121.4	21.4 – 200.0
11-14 yrs/M	4	2	88.1	64.3	21.4 – 150.0
11-14 yrs/F	5	2	226.5	264.3	28.6 – 342.9
15-18 yrs/M	5	3	182.7	135.7	72.0 – 300.0
15-18 yrs/F	7	3	119.3	67.9	21.4 – 214.3
<b><i>Adults (1986/7)</i></b> 16-64 yrs/M	14	1	98.4	37.5	0.9 – 300.0
16-64 yrs/F	31	3	107.1	46.4	2.6 – 350.0
<b><i>Older people free-living in the community (1994/5)</i></b> 65 and over/M	26	4	667.7	300.0	12.0 – 5000.0
65 and over/F	32	5	854.8	300.0	0.5 – 10000.0
<b><i>Older people living in institutions (1994/5)</i></b> 65 and over/M	5	2	3328.9	2544.2	12.5 – 5000.0
65 and over/F	9	4	1909.5	67.8	15.0 – 5350.0

\* Data unavailable

<sup>16</sup> Data includes intakes from prescribed folate supplements

## ANNEX 3 TO EVM/00/18

**Folic Acid : Summary table of selected nutrition related information and existing guidance on regulations**

Unit of usage	$\mu\text{g/day}$		$\mu\text{g}/100 \text{ kcal}$
	Male	Female	
<i>UK DRV<sup>17</sup> for adults (19-50+)</i>			
LRNI	100	100	
EAR	150	150	
RNI	200	200	
<i>Mean adult UK dietary intake from food (all sources)</i>			
Adults (16-64) <sup>18</sup>	311 (312)	213 (219)	
65 years and over <sup>19</sup>			
free living	270 (279)	207 (220)	
institutionalised	235 (235)	200 (197)	
EU labelling RDA <sup>20</sup>	200		
Supplemental doses	400 - 800		
<b>Regulations</b>			
Infant formula <sup>21</sup>			minimum 4
Infant foods <sup>22</sup>			50
Weight reduction <sup>23</sup>			
whole daily diet replacement	200 per day		
meal replacement	60 per meal		
<i>Maximum total safe daily intake</i>			
EHPM 1997 <sup>24</sup>	1000		

<sup>17</sup> Committee on Medical Aspects of Food and Nutrition Policy (1991). Dietary Reference Values for Food Energy and Nutrients for the United Kingdom. Report on Health and Social Subjects 41. London: HMSO.

<sup>18</sup> Dietary and nutritional survey of British adults. 1986/7

<sup>19</sup> National Diet and Nutrition Survey of people aged 65 years and over. 1994/5

<sup>20</sup> The Food Labelling Regulations 1996

<sup>21</sup> The Infant Formula and Follow-on Formula Regulations 1995

<sup>22</sup> The Processed Cereal-based Foods and Baby Foods for Infants and Young Children Regulations 1999 (amended)

<sup>23</sup> The Foods Intended for Use in Energy Restricted Diets for Weight Reduction Regulations 1997.

<sup>24</sup> Vitamins and Minerals A Scientific Evaluation of the Range of Safe Intakes. European Federation of Health Product Manufacturers 1997.

