

RIVM report 340230001/2004

**Neurotoxicity of folic acid**

J.G.C. van Amsterdam, E.H.J.M. Jansen and  
A. Opperhuizen

This investigation has been performed by order and for the account of The Health Inspectorate of the Ministry of Health, Welfare and Sports within the framework of project 340230 Risk assessment folic acid supplementation.

# Contents

<b>SAMENVATTING.....</b>	<b>3</b>
<b>SUMMARY .....</b>	<b>4</b>
<b>1. INTRODUCTION .....</b>	<b>5</b>
<b>2. BACKGROUND INFORMATION .....</b>	<b>6</b>
2.1 SOURCES OF FOLATES.....	6
2.2 PHYSIOLOGICAL ROLE.....	7
<b>3. FOLATE DEFICIENCY .....</b>	<b>8</b>
3.1 PREVALENCE OF FOLATE DEFICIENCY .....	8
3.2 INDICATIONS FOR FOLIC ACID SUPPLEMENTATION .....	8
3.3 GUIDELINES OF FOLATE SUPPLEMENTATION .....	9
<b>4. ADVERSE EFFECTS .....</b>	<b>10</b>
4.1 MASKING OF VITAMIN B <sub>12</sub> DEFICIENCY .....	10
<b>5. DIRECT TOXICITY OF FOLATES .....</b>	<b>11</b>
5.1 ANIMAL STUDIES.....	11
5.2 HUMAN DATA.....	11
<b>6. TOXICITY OF FOLINIC ACID .....</b>	<b>14</b>
<b>7. NEUROTOXIC EFFECTS OF FOLATES.....</b>	<b>15</b>
7.1 ANIMAL STUDIES.....	15
7.2 HUMAN STUDIES .....	16
<b>8. EPILEPTOGENIC EFFECTS.....</b>	<b>18</b>
8.1 REDUCTION OF FOLATE BY ANTI-EPILEPTIC DRUGS .....	18
8.2 IMPAIRMENT OF SEIZURE CONTROL .....	18
<b>9. FOLATE DEFICIENCY AND NEUROTOXICITY.....</b>	<b>20</b>
<b>10. CONCLUSION.....</b>	<b>21</b>
<b>REFERENCES.....</b>	<b>22</b>
<b>ANNEX 1. OVERVIEW OF STUDIES DEPICTED IN TABLE 1.....</b>	<b>32</b>

## SAMENVATTING

Het huidige rapport is opgesteld naar aanleiding van de vraag om in een literatuuroverzicht de vermeende neurotoxische effecten van foliumzuur samen te vatten. Enkele dierexperimentele studies gaven aan dat foliumzuur, indien direct toegediend in de hersenen, neurotoxisch en epileptogeen is. In 1970 maakte een, overigens slecht gecontroleerde en niet gereproduceerde, studie melding van neurotoxische symptomen, zoals malaise, slaapverstoring en mentale stoornissen in veertien gezonde vrijwilligers, die een maand lang dagelijks 15 mg foliumzuur oraal kregen toegediend. Vijf jaar later werden convulsies gemeld na hoge intraveneuze toediening van foliumzuur aan een patiënt met een slecht gecontroleerde epilepsie. Overigens zijn er geen gegevens, die duiden op een direct neurotoxisch effect in de mens bij oraal foliumzuurgebruik. Op het risico voor maskering van vitamine B<sub>12</sub> deficiëntie door foliumzuur wordt kort ingegaan.

Klinische studies, waarbij patiënten langdurig (tot 3 jaar lang) 5 tot 15 mg per dag kregen toegediend gaven geen aanwijzing voor aan foliumzuur gerelateerde neurotoxiciteit. Tenslotte wordt uiteengezet, dat een deficiëntie in foliumzuur, die de synthese van S-adenosylhomocysteïne remt en stapeling van homocysteïne veroorzaakt, wél kan leiden tot neurotoxische schade.

## SUMMARY

The present review summarises the neurotoxicological effects of folic acid. Some studies in animals have shown that folic acid is neurotoxic and epileptogenic when applied directly to the brain. One poorly controlled and not further reproduced study from 1970 reported neurotoxic symptoms like malaise, sleep disturbances, and mental changes in 14 healthy volunteers who took daily 15 mg of folic acid for one month. Five years later seizures were reported in a patient with poorly controlled epilepsy after high intravenous doses of folic acid. There are no further data that indicate that oral folic acid is directly neurotoxic in humans. In addition, clinical studies, where daily doses of 5 to 15 for up to 3 years were applied, did not show any evidence of folate associated neurotoxicity. Moreover, a deficiency in folic acid, that induces a decrease in the synthesis of S-adenosyl-homocysteine and accumulation of homocysteine, may lead to neurotoxicological damage.

# 1. INTRODUCTION

Folates have been claimed to retain neurotoxic properties. The basis of this claim may be retrieved from results observed in older studies (before 1985) performed in vitamin B<sub>12</sub>-deficient patients. 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.

Following the identification and chemical synthesis of folic acid in 1946, but before the isolation of vitamin B<sub>12</sub>, folic acid was used, usually in doses of 5 mg or higher, to treat pernicious anaemia. However, contrary to the effect of folic acid supplementation on the haematological symptoms, the neurological abnormalities in vitamin B<sub>12</sub>-deficient patients are not cured by folic acid [1] (cf. section 4). Some early studies even claimed that folic acid therapy in patients with vitamin B<sub>12</sub> deficiency might aggravate, or even induce neurological lesions. More important in this respect is that folic acid supplementation may mask the diagnosis of pernicious anaemia.

Finally, one early study reported in 'The Lancet' in 1970 [2] scared everyone as this study reported neurotoxic symptoms like malaise, sleep disturbances, and mental changes in 14 healthy volunteers who took daily 15 mg of folic acid for one month. The results of this poor controlled study have, however, never been confirmed by others.

## 2. BACKGROUND INFORMATION

### 2.1 Sources of folates

Folate is the generic term for compounds that have a common vitamin activity (water-soluble B-vitamins). The folates include the synthetic form of the vitamin folic acid (PGA, pteroylglutamic acid, methylformate) that is not present in nature. Folic acid is used to indicate the parent compound, whilst folate is used in a generic sense to indicate one or a mixture of pteroylglutamates. Folinic acid (calcium folinate, methyl-THF) is a folic acid derivative, used as drugs in cancer therapy.

Most mammals, including man, cannot synthesise folic acid and hence must necessarily obtain it from food. Food supplements and food nutrition fortifications mostly contain pteroylmonoglutamate (PMG) which is a synthetic and more stable form of folic acid. Natural (dietary) folates are mostly reduced folates, i.e. derivatives of tetrahydrofolates (THF), such as 5-methyl-, 5-formyl- and 5,10-methylene-THF, and exist mainly as pteroylpolyglutamates (up to nine glutamate molecules attached to a pteridine ring). Green vegetables and citrus fruits contain high amounts of folates, mostly in its reduced form as polyglutamate. Before entering the circulation, single glutamine units (monoglutamates) are split off from the polyglutamate in the small intestine. In serum, folates are notably present as N<sup>5</sup>-methyl-THF and bound to specifically folate-binding proteins. In red blood cells various folates are present such as 5,10-methylene-THF, and most folates are stored in the liver. Daily food intake of folic acid in Europe is 250-300 µg; in the Netherlands the mean daily folate intake is 251 µg [3].

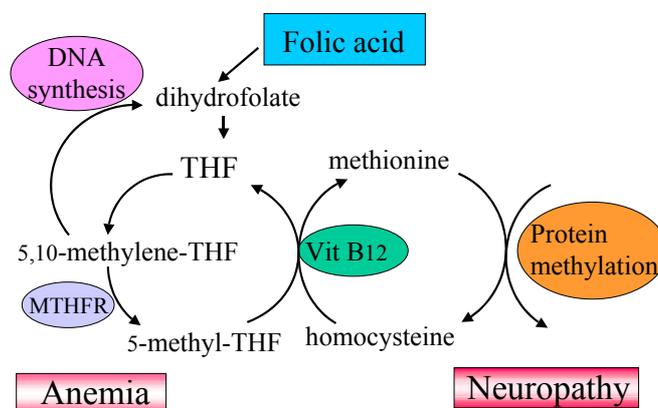


Figure 1. Metabolic pathways of folate.

## 2.2 Physiological role

The biological active THF-compounds serve as co-enzyme the single carbon transfer reactions, where for instance methyl-, formyl- or hydroxymethylgroups are transferred to substrates. The THF acts as a C1-acceptor and the substituted THF-compound as C1-donor. As such folic acid is important for protein synthesis, and DNA- and RNA-synthesis. Consequently, folic acid is required in rapid growing tissues, like the development and outgrow of the foetus, blood forming organs and the epithelium. In addition, folic acid is required for the synthesis of S-adenosylhomocysteine, which is extremely important for the further biosynthesis of brain neurotransmitters (serotonin and dopamine) and phospholipids, like phosphatidylcholine and phosphatidylserine (cf. Section 6). Finally, folic acid is involved in the metabolic pathway of homocysteine (cf. Fig. 1) so that folate supplementation effectively decreases homocysteine level.

## 3. FOLATE DEFICIENCY

### 3.1 Prevalence of folate deficiency

An increased prevalence of folate deficiency is present in elderly populations. Clarke et al. [4] recently reported among a sample of men and women aged 50-75 in Oxford, U.K., at least 10-20% was folate deficient. In a recent Dutch survey [5] 4% of men aged 50-79 -but not in other age-gender groups- were found to have low serum folate levels i.e. below the 2.5 percentile of Dutch blood donors (5 nM) which is not dramatically high. Secondly, some investigators have reported an association of oral contraceptive use with a slight reduction in folate level [5], although others have found no effect of these drugs on folate status (reviewed by Davis [6]). Finally, the chronic use of certain drugs increases the folic acid demand, and patients using such drugs should be supplemented, as well. Examples of such drugs are dihydrofolic acid reductase inhibitors like methotrexate, aminopterin, anti-epileptics, like hydantoin and barbiturates (cf. section 6.1), and anti-malaria drugs like pyrimethamine [7].

### 3.2 Indications for folic acid supplementation

Folic acid supplementation can be used to decrease the high levels of homocysteine found in hyperhomocyst(e)inemia. Maximal homocysteine decreasing effects (up to 25%) are observed at daily doses of 350 to 400 µg folic acid [8-10]. To decrease homocysteine level seems to be relevant, because associations have been observed between hyperhomocyst(e)inemia and various diseases, like cardiovascular disease [11-13], Alzheimer disease [14] and cancer [15]. There is, however, still no conclusive evidence on the importance of hyperhomocyst(e)inemia as risk factor for cardiovascular disease [11-13], so that supplementation of folic acid is only advised to patients with a strongly elevated homocysteine levels ( $> 15 \mu\text{M}$ ) [16].

The only actual indication of folate supplementation is the neural tube defect (NTD). Since 1993 the Dutch Health Council advocates women with child wish to use of folic acid (400 µg pteroylmonoglutaminic acid per day) starting four weeks before till at least eight weeks after conception to prevent neural tube effects [17]. Numerous studies have shown the efficacy of folate supplementation in NTD. One recent population-based result is the 19% reduction in NTD birth prevalence (from 37.8 per 100,000 live births before fortification to 30.5 per 100,000 live births) that followed folic acid fortification of food in the US. During the same period, NTD birth prevalence declined from 53.4 to 46.5 per 100,000 (PR 0.87; 95% C.I., 0.64-

1.18) for women who received only third-trimester or no prenatal care [18].

The supplementation of folic acid to women with child wish has been criticised by Davis [20]. In a part of the general population (5-15%) that has a variant of 5,10-methyl-THF reductase, folate deficiency may arise from a genetic defect. This enzyme is essential for catalysing the transfer of a methyl group to homocysteine to form methionine [19], and in principle the defect can be overcome by the mass action of large quantities of folate (food enrichment). It is, however, questionable to supply folate (at 5 mg/day) to the 85% of women who do not need it. According to Davis, a better way of dealing with this issue would be to find out who has an inherited abnormal reductase [20]. This criticism is only partly correct, as it is widely acknowledged that pregnant women simply have a higher demand of folate, indicating the need for folate supplementation.

### **3.3 Guidelines of folate supplementation**

The European Federation of Health Product Manufacturers Associations (EHPM) recommends a long-term upper safe level of 1000 µg/day [21]. The RDA (recommended daily allowance) in the Food Labelling Regulations (cf. EC Directive on nutritional labelling of foodstuffs 90/496/EEG) is 200 µg. The Council for Responsible Nutrition in the U.K. recommends an upper safe level of 400 µg folic acid per day for long term supplementation and 700 µg/day for short term supplementation [22].

According to the EVM-assessment group, an expert panel of the UK Food Standards Agency (FSA) on vitamins and minerals [23], a supplemental dose of 1 mg/day is not expected to cause adverse effects in the general population. Because of the consistency of the data, from a large number of studies in humans, no uncertainty factors were applied. Assuming a maximum intake from food of approximately 0.5 mg/day, a total dose of 1.5 mg/day is not expected to have any adverse effects.

The British National Formulary (BNF) notes that folic acid should not be prescribed alone in the presence of vitamin B<sub>12</sub> deficiency. Especially at the higher daily dose range of folic acid (1 to 15 mg) there is some evidence of a higher incidence of neuropathy in vitamin B<sub>12</sub> deficient patients. Data from randomised, controlled trials regarding this aspect are lacking. However, it has been generally concluded from anecdotal reports that oral folic acid supplementation at doses less than 5 mg/day is rarely associated with a direct adverse effect on vitamin B<sub>12</sub>-associated neurological damage. On this basis The Dutch Health Council [17] advised an upper daily intake level for adults of 1 mg (synthetic folic acid; PGA; pteroylmonoglutamic acid).

## 4. ADVERSE EFFECTS

Adverse effects that have been reported for folic acid are:

1. masking and exacerbation neurological symptoms due to vitamin B<sub>12</sub> deficiency;
2. epileptogenic and neurotoxic effects;
3. decreased efficacy of folate antagonists in cancer therapy;
4. impairment of zinc absorption and status.

Though the adverse effects of folate have been extensively reviewed [7, 24-26], there is no systematic toxicological evaluation of both natural and synthetic reduced folate compounds available. Both Campbell [24] and Dickinson [25] (the latter reviewed notably the neurological effects of folates) concluded that the information was inconclusive, and consists mainly of case reports or small groups of patients, and uncontrolled studies of questionable quality. The adverse effects that arose in those studies were not confirmed in larger trials. Monitoring side effects in thousands of patients receiving 0.4 to 4 mg folate and in smaller numbers of patients taking large doses for several years revealed no evidence for toxicity (cf. Table 1).

In general, folic acid is now considered as safe, excesses of the compound are mostly excreted in the urine. Gastrointestinal disturbances, and hypersensitivity reactions such as bronchospasm, skin rash have been reported. Adverse reactions to folic acid are rare at usual supplemental doses of up to 5 mg/day. The consequences of long-term excessive intakes are, however, not clearly established so that, in the opinion of the Dutch Health Council [28, 29], it is not possible to draw a definitive conclusion regarding the safety PMG. The available evidence with respect to the various safety issues is summarised below.

### 4.1 Masking of vitamin B<sub>12</sub> deficiency

Deficiency of vitamin B<sub>12</sub> induces virtually the same haematological symptoms as seen with folic acid deficiency. In subjects with a non-diagnosed low vitamin B<sub>12</sub> level, supplementation with folates normalises the haematological symptoms of the vitamin B<sub>12</sub> deficiency but not the neurological complications (neuropathy; nerve damage). As such, folic acid supplementation may mask vitamin B<sub>12</sub> deficiency. No data are available or will come available shortly (for ethical reasons it is hardly feasible to perform such studies) on the toxicity of folate in marginally vitamin B<sub>12</sub> deficiency. No controlled studies have been performed that show that folic acid treatment affects the course of untreated vitamin B<sub>12</sub> deficiency. This topic will be addressed more extensively in subsequent report that will appear in 2004.

## 5. DIRECT TOXICITY OF FOLATES

### 5.1 Animal studies

Folates are hardly toxic considering the high LD<sub>50</sub> values of 305 mg/kg (i.v. injection) and > 10 g/kg (p.o.) for folic acid in mice [30]. Parchure et al. [31] reported mean LD<sub>50</sub> values for intraperitoneal (i.p.) administered folic acid in the range of 85-330 mg/kg b.w. for different mouse strains tested. The symptoms observed in those studies, such as convulsions, ataxia and muscular weakness prior to death, occurred generally 3 to 4 days after treatment. Histopathological examination showed acute renal necrosis in many animals. Other studies have also shown that the parenteral administration of high doses of folic acid into rats (100 - 400 mg/kg b.w.) or mice (75 mg/kg b.w.) produces precipitation of the compound in the renal tubules and renal hyperplasia, hypertrophy and necrosis [32-36]. Hence, the renal effects appear to be non-specific.

In a study of the synergistic effects of folic acid and the anti-malaria drug pyrimethamine (an inhibitor of dihydrofolate reductase), female rats were supplemented from days 7-17 of gestation, by gavage. Folic acid treatment (50 mg/kg b.w. per day) showed no significant maternal or embryotoxicity, as compared with vehiculum [37].

### 5.2 Human data

*This paragraph originates from previous reports of Food Standard Agency [23, 38], which were further adapted and extended by the authors.*

In 1970 Hunter et al. [2] reported disturbing toxic effects after treatment of 14 (6 males and 8 females) healthy volunteers aged 22 to 57 years with a mean age of 36 years with 5 mg folic acid three times daily for 1 month. To exclude vitamin B<sub>12</sub> deficiency and possible neuropathy, serum vitamin B<sub>12</sub> was assessed at the start of the study and one month later (390 and 400 pg/ml, respectively). During treatment folate mean serum level rose from 4.5 µg/L at the start of the study to more than 120 µg/L (range 65 µg/L to >180 µg/L). In addition, the volunteers were interviewed weekly to trace neurological symptoms. During the treatment thirteen subjects showed a variety of toxic effects, whereas one volunteer remained unaffected throughout the study. Symptoms varied from vivid anxiety dreams, malaise and irritability, sleep disturbances, and over-activity. In four these developed within 72 hours, in the others in the last two weeks.

*Table 1. Absence of reported effects after folic acid supplementation to humans without apparent vitamin B<sub>12</sub> deficiency. This table is extracted from a report of the FSA-expert group on vitamins and minerals (reference 38) and further extended/up-dated.*

Subjects	Number subjects	Dose (mg)#	Treatment period	Reference
Healthy women	121	< 0.4	6 months	[40] Daly et al., 1997
Healthy women	144	0.5	4 weeks	[41] Brouwer et al., 1999
Hyperhomocysteine patients	100	0.65	6 weeks	[42] Ubbink et al., 1994
Healthy women	1871	0.8	>12 weeks	[43] Czeizel et al., 1992
Lactating women	42	1	3 months	[44] Mackey et al., 1999
Subjects incl. CAD-patients	242	1-2	3 weeks	[45] Malinow et al., 1997
Renal transplant recipients	60	2.4	12weeks	[46] Beaulieu et al., 1999
Healthy women	900	4	>12 weeks	[47] MRC 1991
Hypercholesterolaemia	20	5	4 weeks	[48] Verhaar et al., 1999
Healthy subjects	101	5	4 weeks	[123] Fokkema et al., 2001
Healthy subjects	16	5	5 weeks	[49] Chao et al., 1999
Healthy subjects	227	5	8 weeks	[50] Den Heijer et al., 1998
Patients with CAD	95	5	1-3	[51] Lobo et al., 1999 *
Healthy women	101	5	12-14	[52] Vergel et al., 1990
Women with CIN	331	5	6 months	[53] Childers et al., 1995
Haemodialysis patients	29	5	1 year	[124] van Gulderen et al, 1999
Healthy volunteers	20	10	2 weeks	[54] Wilmink et al., 2000
Myocard patients	16	10	6 weeks	[55] Landgren et al., 1995
Vitiligo subjects	52	10	≈3 months	[56] Juhlin et al., 1997
Male smokers	73	10	4 months	[57] Heimbürger et al., 1988
Cervical neoplasia patients	235	10	6 months	[58] Butterworth et al., 1992
renal dialysis patients	27	15	8 weeks	[59] Bostom et al., 1996
Folate-deficient epileptics	41	15	6 months	[115] Mattson et al., 1973
Psychiatric patients	41	15	6 months	[126] Godfrey et al., 1990
Folate-deficient epileptics	51	15	26 weeks	[128] Grant and Stores, 1970
Epileptics	30	15	< 1 year	[102] Gibberd et al., 1981
Folate-deficient epileptics	26	15	1-3 years	[99] Reynolds, 1967
Epileptics	24	20	11 weeks	[129] Jensen and Olesen, 1970
Psychiatric patients	22	15-30	8 weeks	[127] Alpert et al., 2002
Depressive patients	20	50	4-6 weeks	[125] Guaraldi et al., 1993
Patients with tropical sprue	50	100	2 weeks	[40] Suarez et al., 1947

# Daily dose (mg); \* this study reported adverse reactions: 1 in placebo and 1 folic acid group. CAD: coronary artery disease; CIN: cervical intraepithelial neoplasia.

*Table 2. Absence of reported effects after folic acid supplementation to humans without apparent vitamin B<sub>12</sub> deficiency (case studies).*

Number of subjects	Daily dose (mg)	Treatment period	Reference
1	30	10 weeks	[122] Richens, 1971
1	60	3 years	[27] Sheehy, 1973
4	1000	1 to 3 weeks	[119] Zettner et al., 1981
4	120-150	1 dose	[120] Czeizel and Tomcsik, 1999

This study was, however, not placebo controlled, and the results were not confirmed in a similar but double blind randomised study in 20 healthy subjects by Hellstrom et al. [39]. In contrast to the study of Hunter, serum folate levels increased in the study of Hellstrom et al. less (from 6.8 µg/L to 51.0 µg/L), and no differences between placebo and treatment groups were observed. Other more recent studies, summarised in Table 1 and 2 (further details of these studies have been described in Annex 1), showed no adverse effects, as well.

In some countries, cereals and other foods have been enriched for some years with PMG. As far as is known now, no adverse effects have been reported, though it is neither known whether such effect have been systematically registered. In the U.K. suspected adverse reactions to medicinal products are reported to the Committee on Safety of Medicines/Medicines Control Agency. The number of reports received depends on many factors, but is known to endure from considerable “under-reporting” of reactions. Most of the adverse reactions reported for products containing folic acid relate to multi-constituent products, and may, therefore, not be directly attributable to folic acid. For single constituent folic acid products a low number of adverse reactions have been reported with no trend or pattern to indicate a particular problem (no details could be retrieved). In this respect, Mills [60] pleaded for an adequate monitoring system to signal the adverse effects of folic acid supplementation.

**Comment by the authors:** *various clinical studies have shown that patients, receiving up to three years a daily dose of 5 to 15 mg folate, showed no adverse effects. It should, however, be noted that it was not the specific aim of most studies to determine such adverse effects. Studies using higher dosages and/or longer duration have not been performed.*

## 6. TOXICITY OF FOLINIC ACID

Folinic acid (the calcium salt is marketed as Leucovorin<sup>®</sup>) is formyl-tetrahydrofolic acid (N<sub>5</sub>-formyl-THF), an active metabolite of folic acid and thus naturally circulating in the body. In recent years, folinic acid has been introduced clinically in patients with advanced colorectal cancer, breast cancer, gastric cancer and head and neck cancer, to increase the therapeutic efficacy of 5-fluorouracil (5-FU) and to reduce methotrexate toxicity.

According to Martindale's pharmacopoeia, folinic acid is devoid of any serious toxicity but may rarely cause nausea. In mice, folinic acid is toxic at doses above 800 mg/m<sup>2</sup> body surface i.v. and a LD<sub>50</sub> i.v. value of 730 mg/kg.

Based on results from clinical studies folinic acid is recommended in very high dose, in combination with 5-fluorouracil (5-FU), for the treatment of cancer. The weekly dose of folinic acid is 500 mg/m<sup>2</sup> (900 mg/week; dose as a 2 hour infusion) given up to 48 weeks. Each cycle consists of five consecutive treatment weeks followed by a 3-week rest period. The cumulative dose during 48 weeks (6 cycles) is around 27000 mg per patient (6x5x900). This implicates an average daily dose of 80 mg per day. Folinic acid is also frequently administered in moderate to high doses at an appropriate interval after high dose methotrexate ("folinic acid rescue"). The recommendation for leucovorin rescues are: to administer 12 times a dose of 18 mg (approximately 10 mg/m<sup>2</sup>) every 6 hours (54 mg/day during 3 days) starting 24 hours after the beginning of the methotrexate infusion.

In combination with 5-FU, folinic acid may lead to more pronounced mucositis, precipitation of seizures, myelosuppression and diarrhea, but no neurotoxicological signs have been reported [61, 62].

**Comment by the authors:** *The natural folic acid derivative, folinic acid, shows no toxicological signs under clinical conditions when it is used in cycling regimes at a dose of 80 mg per day for 48 weeks (based on six treatment cycles).*

## 7. NEUROTOXIC EFFECTS OF FOLATES

### 7.1 Animal studies

Various animal experimental evidence is available, showing that in several species folates have powerful excitatory and convulsive properties, especially when applied directly into the brain [63-69]. For example, sodium folate is 100 times more epileptogenic than comparable nanomolar concentrations of sodium glutamate [68, 70]. This evidence is mainly based upon *in vitro* tissue and cell culture studies, and/or *in vivo* studies using very high dose levels (i.v. dosages 60-90 mg; note that LD<sub>50</sub> value is  $\approx$  300 mg/kg i.v.).

In one study with vervet monkeys a dose of 25.6 mg of folic acid per day was given to 3 male monkeys for 99 days without any obvious toxic side effect [71]. It was, however, not indicated what was examined in this study.

Direct injection of high doses of folic acid or methyl-tetrahydrofolate into the brain (i.c.v.) or spinal fluid causes seizures in rats [72-74]. At very high dose, intravenous of folic acid also caused convulsions in mice, but compared with i.c.v. injection, the intravenous EC<sub>50</sub>-value was 1000-fold higher, and the latency time to seizure largely prolonged [75]. If the animal is, however, already vulnerable to seizures or the blood-brain barrier is damaged locally, for example by a heat lesion, the dose of intravenous folate required to produce an epileptogenic effect is much reduced [68, 70].

The mechanism of the excitatory properties of folates is not really known, but there is some evidence that this property is related by blocking or reversing GABA-mediated inhibition [65, 76-79]. Epileptic phenomena induced by folates resemble those induced by dis-inhibitory compounds, like bicuculline, strychnine, and picrotoxin. In many respects they are also different from those induced by direct excitatory drugs such as kainic acid, carbachol, and neostigmine [80]. Based on *in vitro* results, Thomas et al. [81] recently suggested that the neurotoxic effects of folates were due to the release of glutamate residues from polyglutamates. Following oral administration, folic acid shows only minor effects. Hommes et al. [82] reported that changes in dietary folate content produced inverse effects on the pentylenetetrazol (PT) seizure thresholds of rats. Wistar rats were fed for 8-11 months diets containing folic acid at either 0.4 mg/kg ( $\approx$  0.02 mg/kg b.w./day; FA-deficient), 2.7 mg/kg ( $\approx$  0.14 mg/kg b.w./day; standard) or 50 g/kg ( $\approx$  2500 mg/kg b.w./day; FA-supplemented). Finally, 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, indicating a small pro-epileptogenic effect of folate.

Carl & Smith [83] reported that oral folic acid supplementation (20 mg/kg diet,  $\approx$  1 mg/kg b.w./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 [84] observed no effects of folic acid treatment (0.1 - 0.4 mg/kg b.w./day, for 19 days in drinking pregnant or non-pregnant rats.

## 7.2 Human studies

Weissberg et al. [85] reported an uncontrolled study on the neurological effects, following folic acid supplementation at 20 mg/day for 9-12 months, in 26 subject, 22 of whom were (non-pernicious) anaemia patients. Prior to therapy, 6 of the normal subjects and 7 of the anaemic subjects showed some abnormal neurological signs, 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. [86] reported that oral supplementation with folic acid (20 mg/day for 3-12 months) produced no signs of spinal cord or peripheral nerve damage in 40 healthy subjects without pernicious anaemia (13 subjects had mild hypochromic anaemia). Similarly, folic acid supplementation was not associated with 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 to 182 days [87].

Supplementation studies (15 mg/day for 45 days) with Parkinson disease patients did not show an effect on the incidence of neurological defects. Also, following i.v. treatment with dosages up to 150 mg, no adverse effects have been reported (for review see refs [7, 24]).

A case study on a 47-year-old woman with bilateral retrobulbar optic neuropathy showed that folic acid deficiency caused the disorder (serum vitamin B<sub>12</sub> level was normal) as her serum folic acid concentration was decreased. After treatment with oral folic acid and diet modification the patient's visual function returned to normal [88].

Folic acid levels were measured in the serum of 343 patients with various neurological diseases, and 36 patients (10.5%) showed decreased serum folate levels. Folate administration (15 mg/day) to folate-deficient patients improved neurological symptoms in 24 of 36 cases (67%). Neurological symptoms were more frequently improved by folate supplementation to

patients with neuropathy than exclusive encephalopathy [89].

In summary, there is no clear evidence for neurotoxicity induced by folic acid in humans. Some cases of neurological adverse effects have been reported following ingestion of folic acid tablets (3 mg), or folic acid containing multivitamin supplements, but it can not be excluded that these were due to an (undiagnosed) vitamin B<sub>12</sub> deficiency (see Dickinson [25]).

***Comment by the authors:*** *Studies in animals have shown that folic acid is neurotoxic and epileptogenic when applied directly to the brain. 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. The concerns about neurotoxicity are based on animal studies and one report of seizures in a patient with poorly controlled epilepsy after high intravenous dose [90].*

## **8. EPILEPTOGENIC EFFECTS**

### **8.1 Reduction of folate by anti-epileptic drugs**

Anticonvulsant pharmacotherapy may reduce blood levels of folic acid and dramatically increase homocysteine levels [91-93]. One preliminary study showed that pregnant women who use anticonvulsant drugs without folic acid supplementation have an increased risk of having a child with birth defects such as heart defects, cleft lip and palate, neural tube defects, and skeletal abnormalities. However, supplementation with folic acid greatly reduces the risk [94]. Consequently, some healthcare practitioners recommend that women taking (multiple) anticonvulsant drugs should be supplemented with 1 to 5 mg of folic acid daily, for three months prior to conception and during the first trimester, to prevent folic acid deficiency-induced birth defects [95-97].

### **8.2 Impairment of seizure control**

Folic acid should be given with caution to drug-treated epileptic patients, because seizure control by the drugs may be impaired. In one study with epileptic patients electroencephalographic changes were noted after administration of 7.2 mg of folic acid, and seizures after 14.4 and 19.2 mg seizures in a patient with poorly controlled epilepsy after high intravenous doses [90]. Other studies have indicated that as little as 0.8 mg of folic acid taken daily can increase the frequency and/or severity of seizures [98, 99]. This occurs, however, only in a small number of cases [100]. Other controlled studies in persons with uncontrolled, or drug controlled epilepsy at oral dosages between 15-20 mg/day or less showed no increased risk for seizures [24, 101]. These data are therefore inconclusive.

One well-controlled study, however, showed that the addition of folic acid to multiple anticonvulsant therapy reduced the seizure frequency, though the effect was not significantly better than with placebo [102]. In addition, three infants with seizures who were unresponsive to medication experienced immediate relief following supplementation with the active form of folic acid [103]. The reason of this inconsistency in results is not clear.

It has been further suggested that a folic acid deficiency induced by anti-epileptic drugs might form the basis for the neuropsychiatric toxicity associated with these drugs. This was recently addressed by Ali et al. who investigated the effect of the addition of folic acid to lamotrigine (LTG) therapy with respect to epilepsy, mood and memory in mice [104]. LTG exhibited a

dose-dependent increase in seizure threshold, whereas folate did not have any effect. LTG did not affect, whereas folic acid decreased, behavioural depression in the mouse Forced Swimming Test. The combination of LTG and folic acid significantly reduced depression while enhancing the effects on memory and seizure threshold. The authors concluded that folic acid shows a beneficial effect.

Because of the normally efficient blood-brain barrier mechanism that limits the entry of the vitamin into the nervous system, the risk to epileptic patients is small, especially in the short term. However, damage to the blood-brain barrier-for example, due to trauma-may lead to local accumulation of folate and patients with partial epilepsy may, therefore, be at some greater risk [64, 66].

## 9. FOLATE DEFICIENCY AND NEUROTOXICITY

Mechanistic reasons can be put forward that rather a deficiency in folate than a supra-maximal dose of folate triggers neurotoxicity. The mechanism of neurotoxicity due to folate deficiency is based on the increased levels of the endogenous methylating compound S-adenosyl-methionine (SAM) and homocysteine.

Folate (5-methyl-THF) provides the methyl group for the conversion of the amino acid methionine to SAM. SAM is further converted to S-adenosylhomocysteine (SAH) which is then metabolised to homocysteine (cf. Fig 1.). The amount of homocysteine present in a tissue or blood is usually low because it is further metabolised with the aid of dietary factors including vitamin B<sub>6</sub>, vitamin B<sub>12</sub>, folic acid, methionine, and choline. Note that SAM has shown to be much more effective in transferring methyl groups than other methyl donors, and is the major methyl donor for most methyltransferases.

Animal studies have shown that a deficiency of folic acid (or other dietary factors) results in decreased synthesis of SAM and permits accumulation of SAH or homocysteine [14, 105, 106]. Defects in methylation induces neurotoxicity [106-109]. Secondly, Poirier et al. [105] reviewed that diminished activity of methionine adenosyltransferase, the enzyme that actually synthesizes SAM, is associated both with demyelination of nervous tissue in man [110]. Finally, anti-convulsants like phenytoin and valproic acid might decrease the concentration SAM and increase the homocysteine level (cf. Poirier et al. [105]), decrease overall methylation of embryonic DNA, and induce reversible myelinolysis in the brain [111-114].

With respect to increased homocysteine levels due to inhibition of SAM-synthesis by folate deficiency, it has been shown that homocysteine acts as an agonist at the N-methyl-D-aspartate (NMDA) receptor, promoting excitotoxicity [115]. Under pathological conditions (stroke, head trauma) homocysteine may induce neurotoxicity through overstimulation of the NMDA-receptor [116, 117].

Ambrosch et al. [118] investigated the association between homocysteine and the prevalence of neuropathy in Type 2 diabetes mellitus in a total of 65 Type 2 diabetic subjects. Logistic regression analysis showed that homocysteine was significantly, though borderline, associated with the prevalence of neuropathy (odds ratio per 5 µM increase in homocysteine level: 2.60; 95% confidence interval 1.07-6.33).

## 10. CONCLUSION

The data that were available in 2003, and reviewed here, showed no evidence for a neurotoxic effect of folic acid. Since, no long-term studies or studies using high dose have been performed that would enhance our knowledge on this topic. However, intrathecal administration (direct administration in spinal / central compartment) of folate does induce neurotoxicity. The masking of vitamin B<sub>12</sub> deficiency by folic acid is potentially dangerous and this aspect will be described later in more detail.

Except for one poorly controlled study from 1970 performed in 14 volunteers receiving 15 mg folate daily for one month, and a case report from 1975 on seizures in one patient with poorly controlled epilepsy after high intravenous doses of folic acid, there are no data indicating that oral folic acid is directly neurotoxic in humans. A deficiency in folic acid, however, is more likely to induce neurotoxicological damage.

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## Annex 1. Overview of studies depicted in Table 1.

The descriptions below are based on abstracts retrieved from the Med-line database.

Vergel et al. [52] carried out an open trial, in which 101 women with a history of previous neural tube defect (NTD-) affected pregnancy. Subjects were given 5 mg/day folic acid, without any other vitamins for a period of around one menstrual period before conception until the 10<sup>th</sup> week of pregnancy (81 women fully-supplemented) or a shorter duration (20 women 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.

Bostom et al. [59] 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 15 mg folic acid, 100 mg vitamin B<sub>6</sub> + 1 mg vitamin B<sub>12</sub>, or placebo. All subjects also continued taking a pre-prescribed daily supplementation of 1 mg of 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 = 0.001, with baseline levels of 30 µM in both groups). To assess the potential side effects of the therapy, participants were asked to complete a symptom 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 trans-aminases (ALT, AST), creatinine or haematocrit associated with the therapy.

Wilmink et al. [54] 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.

Landgren et al. [55] 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 patients treated for 6 weeks either immediately following, or during weeks 6-12 after infarction. 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  $\mu\text{M}$ ,  $P < 0.001$  in treated subjects; mean increase of 1.7  $\mu\text{M}$ ,  $P < 0.001$ , in the untreated group). Adverse effects of the therapy were not reported.

Chao et al. [49] 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. Plasma homocysteine concentrations decreased from 22.7  $\mu\text{M}$  to 17.0  $\mu\text{M}$  implicating a reduction of 25% ( $P < 0.001$ ). Adverse effects of the therapy were not reported.

Verhaar. A randomised, double-blind, placebo-controlled, cross-over study by Verhaar et al. [48] 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.

Den Heijer et al. [50] 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  $> 16 \mu\text{M}$ ). 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). Nonhyperhomocysteinaemic 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). In all five treatment-type groups, median plasma homocysteine concentrations were  $\approx 12 \mu\text{M}$ . 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  $\approx 12 \mu\text{M}$  in all groups), as compared to placebo treatment (median reduction of 3%). Adverse effects of the treatment were not reported but for unknown reasons six subjects withdrew from the trial.

Lobo et al. [51] 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; placebo; 0.4 mg/day folic acid; 1 mg/day folic acid, and 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 µg/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. This effect was not seen in the placebo group (pre- and post-treatment level  $\approx 12$  µM). Seven subjects reported adverse reactions (no details were given) to the therapy (one in the placebo group; four in the 0.4 mg/day, one in the 1 mg/day, and one in the 5 mg/day folic acid group).

Beaulieu et al. [46] 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 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  $\approx 17$  µM in all groups to  $\approx 11$ , 13 and 14 µM 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.

Malinow et al. [45] 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.

Ubbink et al. [42] 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 hyper-homocysteinaemic men. Participants were randomised to daily supplementation, for 6 weeks, with either 0.65 mg folic acid; 0.4 mg vitamin B<sub>12</sub>; 10 mg vitamin B<sub>6</sub>; 0.65 mg folic acid + 0.4 mg vitamin B<sub>12</sub> + 10 mg vitamin B<sub>6</sub>, or 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.

Brouwer et al. [41] 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, or 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 in groups 1 and 2, respectively;  $P < 0.001$ ). Adverse effects of the therapy were not reported.

Heimbürger et al. [57] 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 hydroxycobalamin, 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.

Butterworth et al. [58] 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 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 papilloma virus type 16 infection. Adverse effects of the therapy were not reported.

Childers [53]. The effect of folic acid supplementation on the natural history of cervical intraepithelial neoplasia (CIN) was also evaluated in a multi-centre, randomised, double-blind, placebo-controlled trial. A total of 331 women with various forms of CIN were randomised to receive oral folic acid supplementation (5 mg/day) or placebo, for 6 months. Folic acid therapy did not affect serum retinol, retinyl palmitate,  $\alpha$ -tocopherol or  $\beta$ -carotene levels. Adverse effects of the therapy were not reported.

Juhlin and Olsson [56] 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. One hundred patients were treated with 10 mg/day folic acid + 2 mg/day vitamin B<sub>12</sub>, for  $\approx$  3 months. Treatment was associated with skin re-pigmentation, particularly if combined with exposure to the sun. Adverse effects of the therapy were not reported.

Mackey and Picciano [44] 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 control group, but not in the group treated with folic acid. Anthropometric indices of infant growth showed no significant differences between the groups. Adverse effects of the therapy were not reported.

Daly et al. [40] 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.

Creizel and Dudas [43]; MRC [47]. Two large randomised double blind placebo-controlled trials determined the protecting effect of PMG of neural tube defects [43, 47]. The two studies used daily doses of 4 mg PMG (900 women; starting at the day of randomisation till 12 weeks after conception [47], and 800 µg PMG 1817 women starting one month before conception till ninth week of pregnancy [43], respectively. 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 groups. No other adverse effects have been reported.

Zettner et al. [119] carried out a study of the absorption of folic acid in 4 hyperuricaemic men given mega-dose 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 and Tomcsik [120] 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. [121] reported that no toxic effects were observed in 50 patients with tropical sprue treated with oral folic acid at doses of 100-500 mg (single dose) or 5-100 mg/day (for periods of 10-14 days).

Richens [122]. Individual case reports also suggested that high-level folic acid therapy was without toxicity. Richens described one subject who took 30 mg/day folic acid for 10 weeks with no adverse effects.

Sheehy [27] 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.

Fokkema et al. [123] supplemented 101 healthy adults with folic acid (5 mg/day) and vitamin B<sub>12</sub> (1 mg/day) for 2 weeks and the same dosages of folic acid and vitamin B<sub>12</sub> plus vitamin B<sub>6</sub> (1 mg/kg/day) during the following 2 weeks. Mean fasting and 6-h postload homocysteine decreased after 4 weeks of vitamin supplementation by 3.5 µM (33.5%) and 8.5 µM (26.3%), respectively. No adverse effects were reported.

Van Guldener et al. measured plasma total homocysteine and methionine levels in chronic haemodialysis patients (N=29, 26 of whom completed the study) after an overnight fast, and 6 and 24 h after an oral methionine load (0.1 g/kg) [124]. The patients were randomised to treatment with folic acid 5 mg daily with or without betaine 4 g daily, and the loading test was repeated after 12 weeks. The patients were then re-randomised to treatment with 1 or 5 mg folic acid daily for 40 weeks, after which a third loading test was performed. At week 52, fasting and postload homocysteine levels did not differ significantly between patients on 1 or 5 mg folic acid daily. Plasma homocysteine half-life and plasma methionine levels after methionine loading were not altered by folic acid treatment. No adverse effects were reported.

Guaraldi et al. [125] treated 20 depressive patients, of which 16 completed at least 4 weeks of treatment, with a daily dose of 50 mg 5-methyltetrahydrofolate (MTHF) during 6 weeks. At endpoint a markedly significant improvement in their depressive symptoms were observed. There were no clinically relevant changes in routine laboratory tests, and no adverse events considered to be definitely drug-related were reported.

Godfrey et al. [126] treated in a double-blind, placebo-controlled trial 41 psychiatric patients with folate deficiency with folate 15 mg daily, for 6 months in addition to standard psychotropic treatment. Among both depressed and schizophrenic patients folate significantly improved clinical and social recovery. The differences in outcome scores between folate and placebo groups became greater with time. No adverse effects were reported.

Alpert et al. [127] studied folate supplementation in 22 patients with major depression as an adjunctive treatment among adults with inadequate response to an selective serotonin re-uptake inhibitor (SSRI). Patients (mean age 45.2), partial or non-responsive to an SSRI were enrolled in a 8-week prospective open trial. Leucovorin (folinic acid), which is metabolised to methylfolate, was added to SSRIs at 15-30 mg/day. Folate levels rose from 28 +/- 19 ng/mL to 301 +/- 203 ng/mL ( $p < 0.001$ ), but leucovorin appeared to be modestly effective as an adjunct among SSRI-refractory depressed individuals with normal folate levels. No adverse effects were reported.