TOX/2018/12

COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

FOLIC ACID – SCOPING PAPER ON SETTING UPPER LEVELS OF INTAKE

Introduction

1. It is well established that folic acid supplementation prior to conception can reduce the risk of having a Neural Tube Defect (NTD) affected pregnancy. Consequently, UK Government advice recommends supplementation with 400 µg/day folic acid from the time contraception is ceased until the 12th week of pregnancy. Women who have already had a NTD-affected pregnancy are advised to take a supplement of 5 mg/day folic acid.

2. However, not all women use folic acid supplements and many pregnancies are unplanned. For example, it has been estimated that only 31% of women took folic acid supplements immediately prior to pregnancy, 62% started to take them once pregnancy was confirmed but too late to prevent NTDs and 8% of women did not take them at all (Bestwick *et al*., 2014). The proportion of women taking supplements prior to conception was lower in younger women and in non-white ethnic groups. The number of NTD-affected pregnancies did not change significantly between 1998 and 2012 (Morris *et al*., 2015).

3. The Committee on the Medical Aspects of Food Policy (COMA) concluded that universal fortification of flour would significantly reduce the number of conceptions and births affected by NTDs (DH, 2000); this was also endorsed by the Food Standards Agency (FSA) board. Low uptake of supplementation was discussed by the Scientific Advisory Committee on Nutrition (SACN) in 2006, in a report which recommended mandatory fortification of flour with folic acid. SACN's recommendations were re-iterated in 2009 and 2017; folic acid supplementation would still be recommended. However, the current position is that previous Government ministers have decided not to consider mandatory fortification but instead to promote the use of folic acid supplements as part of pre- and post-conception care advice, though the issue is currently being reconsidered.

4. Maximum intake levels of supplemental folate intake have been established by various expert bodies. These are either Guidance Levels (GLs) as set by the Expert Committee on Vitamins and Minerals (EVM) in 2003 or Tolerable Upper Levels (TULs) as set by US Institute of Medicine (IOM) and the EU Scientific Committee on Food (SCF) in 1998 and 2000 respectively but are all 1 mg/day and are based on either the masking of the neurological symptoms and/or the exacerbation of the neurological symptoms associated with deficiency of vitamin B_{12}, a related vitamin.

5. One of the recommendations made by SACN was that because of the uncertainties around high intakes of folic acid, the number of people exceeding the UL/GL should not increase; this would be achieved by reducing voluntary fortification in other foods and food supplements. Emerging evidence on the effects of long term high intakes should also be monitored.

6. At the last COT meeting in February, a recent paper by Wald *et al* (2018) was considered under Any Other Business. The paper argued that the basis of the TUL was flawed and that the concerns about the masking of vitamin B12 deficiency were no longer relevant. It was agreed that, given the age of the GL and related TULs, the evidence underpinning them should be examined.

7. It has also been suggested that folic acid could result in other adverse effects. Most notably it was proposed that it could promote colorectal cancer in individuals who had existing pre-neoplastic lesions. This was considered by a joint COC/SACN working group (SACN, 2009) who concluded that there was no clear explanation for the increase in colorectal cancer observed at the time of mandatory fortification in the USA and Canada but reiterated the advice that the number of individuals exceeding the GL/TUL should not increase (see paragraphs 30 and 31).

8. In general, this paper focusses on the neurological effects associated with B_{12} deficiency and the potential effect of folic acid supplementation.

Background

Function of folate.

9. Folate is required for cell division and cell maintenance. Folate is important as a co-factor in 1 carbon transfer reactions (the transfer of methyl, methylene and formyl groups) which maintains the methylation balance in, for example, the DNA repair and construction and amino acid synthesis as well as site-specific methylation of the cytosine base in DNA which regulates gene expression.

10. Rich sources of folate include green leafy vegetables, citrus fruit, peas, chick peas, asparagus and liver. It is not possible to reach the recommended supplementary level by dietary means.

Natural vs synthetic folate.

11. Folate is the generic name for a number of compounds sharing a similar activity as folic acid (pteroyl glutamic acid or PGA), that is, being involved in 1 C transfer reactions (SCF, 2000). Folic acid (PGA) is a synthetic folate compound used in food supplements and for food fortification as it is more stable than natural folates. Synthetic folic acid becomes active after reduction.

12. Natural (dietary) folates are largely reduced folates, that is, derivatives of tetrahydrofolate (THF) such as 5-methyl THF, 5-formyl THF and 5,10-methylene THF and exist mainly as pterylpolyglutamates with up to 9 additional glutamate molecules attached to the pteridine ring.

The link between B_{12} and folate

13. Folate and vitamin B_{12} (cobalamin) metabolism are linked because methionine synthase, a vitamin B_{12} dependent enzyme, is needed for the conversion of 5-methyl tetrahydrofolate to tetrahydrofolate (THF) which is the active substrate for folate polyglutamate synthesis within cells. In B_{12} deficiency, the conversion of 5-methyl THF to THF is suppressed, leading to a functional folate deficiency within the cells. Red cell folate levels and thus polyglutamate synthesis are reduced while serum folate levels are raised. Unreduced serum folic acid (PGA) is metabolised to polyglutamates within the cell by a B_{12} independent mechanism and thus may improve haematological status without correcting the vitamin B_{12} deficiency. Haematological abnormalities do not occur in all patients. Campbell (1996) (see SCF) estimated that 11-33% of patients with neurological problems had normal haematology. Lindenbaum *et al.* (see SCF) reported that Mean Corpuscular Volume (MCV) and haematocrit were normal in 28.4% of patients with neuropsychiatric disorders due to clinical cobalamin deficiency.

14. Some limited animal data are available indicate which that when B_{12} deficient fruit bats and monkeys are given folic acid, neurological symptoms develop more quickly (see paragraph 20).

Folate and B 12 deficiency

Folate

15. Inadequate folate intake leads to decreased serum folate concentration, followed by decreased red cell folate concentration, a rise in serum homocysteine concentration and megaloblastic changes in the bone marrow and other tissues with rapidly dividing cells (ultimately producing abnormally large, immature and dysfunctional cells) (IOM, 1998). Macrocytic anaemia then develops, first indicated by a decrease in red cell count. Eventually all 3 indicators of anaemia (haematocrit, haemoglobin concentration and erythrocyte concentration) are depressed. Since there are compensatory mechanisms, symptoms of anaemia do not tend to be apparent until the anaemia is severe. The symptoms include weakness, fatigue, difficulty concentrating, irritability, headache, palpitations, and shortness of breath; atrophic glossitis has also been reported. The symptoms may be apparent in vulnerable individuals such as the elderly when the anaemia is less severe.

Vitamin B_{12}

16. The major cause of clinically observable deficiency is pernicious anaemia; the haematological effects are indistinguishable from those of folate deficiency. Neurological complications are present in 75-90% of individuals with clinically observable B_{12} deficiency and may be the only manifestation in 25% of cases (IOM, 1998). The occurrence of neurological symptoms may be inversely proportional to degree of anaemia (Healton *et al*.,1991 and Savage *et al*., 1994 cited by IOM, 1998). Neurological symptoms include tingling and numbness, which is worse in the lower

limbs, disrupted vibratory and position sense and motor disturbance such as abnormalities of gait also occur. Cognitive changes may also occur, these include loss of concentration, memory loss, disorientation and frank dementia, with or without mood changes. In addition, visual disturbances, insomnia, impotency, and impaired bowel and bladder control may develop. The progression is variable, but usually gradual. Many of the neurological symptoms may be attributable to subacute combined degeneration of the cord (SCD); this is the degradation of the posterior and lateral columns of the spinal cord due to the loss of myelin. Whether the symptoms are reversible depends on their duration.

17. B_{12} deficiency is also frequently associated with gastrointestinal complaints including sore tongue, appetite loss, flatulence and constipation. These complaints may be related to the underlying gastric disorder in pernicious anaemia. In a prospective study of 50 patients admitted to hospital with vitamin B_{12} deficient megaloblastic anaemia, the most common finding was peripheral neuropathy; SCD was uncommon (Reynolds, 2007). About a quarter of the patients had either cognitive impairment or an affective disorder but in a third there was no detectable nervous system involvement. Pernicious anaemia was the cause of two thirds of the B_{12} deficiency. Vitamin B_{12} deficiency is most common in older adults.

Diagnosis of Vitamin B_{12} deficiency

18. Green (2017) considered that B_{12} deficiency was best diagnosed using a combination of tests because none alone were completely reliable. Serum B_{12} used measurement in isolation has a generally poor sensitivity and specificity for reliable detection of B_{12} deficiency. A low serum level does not always indicate deficiency and concentration within the reference range does not always indicate normalcy. This is partly due to the distribution within the serum where 70-90 % may be bound to the haptocorrin (HC) protein and is unavailable for immediate delivery to the cells; the remainder is bound to transcobalamin (TC), the functional B_{12} transport protein. Consequently, if the HC bound fraction is preserved, the serum B_{12} concentration may be within the normal range; this may be clarified by measuring B_{12} metabolites. The serum levels of holoTC, metabolites methyl malonic acid (MMA) and homocysteine (Hcy), and assays of B_{12} absorption and intrinsic factor[1] antibodies may also be measured.

19. NHS UK (2018) provide advice which states that the tests for possible B_{12} deficiency include measuring haemoglobin, the size of red blood cells and folate and B_{12} levels, but notes that the test for blood B_{12} only measures total B_{12} and not whether it is in an inactive form; tests for the latter are not widely available.

Expert Opinions

IOM (1998)

20. The Tolerable Upper Intake Level (TUL) established by IOM applies to supplemental folate only since there was no evidence that dietary folate was of

[1] Intrinsic factor is secreted by the stomach and enables B12 absorption.

concern. The IOM considered that there were three strands of evidence which suggested that excess supplemental folate might precipitate or exacerbate the neurological damage of vitamin B_{12} deficiency. Firstly, there were numerous case reports showing onset or progression of neurological complications in vitamin B_{12} deficient individuals receiving oral folate, these case reports are tabulated as below:

Table 1. Dose and duration of oral folate administration and the occurrence of neurological manifestations in patients with pernicious anaemia.

Study	Number of Subjects	Dose (mg/day)	Duration	Occurrence of Neurological Manifestations
Crosby, 1960	1	0.35	2y	1 of 1
Ellison, 1960	1	0.33-1	3 mo	1 of 1
Allen *et al*., 1990	3	0.4-1	3-18 mo	3 of 3
Baldwin and Dalessio, 1961	1	0.5	16 mo	1 of 1
Ross *et al*., 1948	4	1.25	9-23 mo	1 of 4
Chodos and Ross, 1951	4	1.25	3.5-26 mo	3 of 4
Victor and Lear, 1956	2	1.5-2.55	10-39 mo	2 of 2
Conley and Krevans, 1951	1	4.5	3 y	1 of 1
Schwartz *et al*., 1950	48	5	48 mo	32 of 48
Ross *et al*., 1948	2	5	48 mo	1 of 2
Conley and Krevans, 1951	2	5-8	2-2.5 y	2 of 2
Will *et al*., 1959	36	5-10	1-10 y	16 of 36
Bethell and Sturgis, 1948	15	5-20	12 mo	4 of 15
Chodos and Ross, 1951	11	5-30	3-25 mo	7 of 11
Israels and Wilkinson, 1949	20	5-40	35 mo	16 of 20
Wagley, 1948	10	5-600	12 mo	8 of 10
Ellison, 1960	1	5.4-6.5	2 y	1 of 1
Victor and Lear, 1956	1	6.68	2.5y	1 of 1
Berk *et al*., 1948	12	10	>17 mo	3 of 12
Best, 1959	1	10	26 mo	1 of 1
Spies and Stone, 1947	1	10	22 d	1 of 1
Ross *et al*., 1948	6	10-15	≤ 12mo	4 of 6
Hall and Watkins	14	10-15	2-5 mo	3 of 14

Heinle *et al*., 1947	16	10-40	≤ 12 mo	2 of 16
Jacobson *et al*., 1948	1	10-65	5 mo	1 of 1
Heinle and Welch 1947	1	10-100	4 mo	1 of 1
Spies *et al*., 1948	38	≥ 10	24 mo	28 of 38
Ross *et al*., 1948	7	15	28-43 mo	3 of 7
Chodos and Ross, 1951	1	15	10.5 mo	1 of 1
Fowler and Hendricks, 1949	2	15-20	4-5 mo	2 of 2
Vilter *et al*., 1947	21	50-500	10-40 d	4 of 4

Secondly, in addition to the human case reports studies in monkeys (Agamanolis *et al*., 1976) and fruit bats (van der Westhuyzen 1982, 1983) show that vitamin B 12 deficient animals receiving supplemental folate develop signs of neuropathology sooner than controls. Thirdly, there is a well-documented interaction between folate and B12 (Chanarin). IOM stated that although the association between folate treatment and neurological damage observed in human case reports does not prove causality, the hazard could not be ruled out and remained plausible given the results of the animal studies and the known interaction. The IOM further stated that it had been recognised for many years that excessive intake of folate supplements might obscure or mask the diagnosis of vitamin B_{12} deficiency. The delayed diagnosis could then result in an increased risk of progressive of unrecognised neurological damage.

21. To establish the TUL, case reports involving oral administration were used. Individuals with B_{12} deficiency may be apparently healthy and were considered to be part of the general population. The data did not allow a NOAEL to be established but a LOAEL of 5 mg was identified based on the cases in Table 1. It was noted that at doses of ≥ 5 mg/day there were more than 100 reported cases of neurological progression, whereas at doses < 5 mg/day there were only 8 well documented cases. In most cases throughout the dose range, folate supplementation maintained the patients in haematological remission over a considerable timespan. All but three of the cases were reported before the fortification of breakfast cereals in 1990. An uncertainty factor (UF) of 5 was used to account for the severity of the effects and fact that a LOAEL rather than a NOAEL was used. TULs for children were then established on the basis of scaling for body weight and no data were identified suggesting other vulnerable groups.

Scientific Committee on Food (2000)

22. In the late 1990s it was proposed that, to improve market harmonisation, maximum levels should be set for food supplements. The EU Scientific Committee on Food (SCF) and subsequently the EFSA NDA panel reviewed a range of vitamins and minerals to establish maximum upper levels.

23. The SCF established a TUL of 1 mg/day for synthetic folate. The SCF considered that although there was no conclusive evidence in humans, the risk of progression of the neurological symptoms in vitamin B_{12} deficient patients as a result of folic acid supplementation could not be excluded and should be considered the most serious adverse effect. In nearly all cases, the doses involved were greater than 5 mg/day, and only a few cases were the doses between 1 and 5 mg/day. The LOAEL was estimated to be 5 mg and the UL 1 mg for adults, with ULs for children being scaled on body weight. No data were available to suggest that other life groups would be more susceptible to folic acid. Further research on the effects of high folate intake on the symptomatology of B_{12} deficiency was recommended. SCF- in the early days when B_{12} was not identified as a separate vitamin, individuals with macrocytosis and other haematological abnormalities were treated with > 5 mg folic acid, with complete remission of symptoms occurring in > 60% of individuals. Sub-optimal improvement was reported at dosages of 1-5 mg.

24. In their review, the SCF noted studies by Wagley (1948) in which 10 patients with pernicious anaemia were given 5-25 mg folic acid/day, one patient had neurological symptoms after 8 days, and 2 had symptoms 2 months and 9 months respectively after treatment. Bethell and Sturgis (1948) reported that neurological symptoms deteriorated after 6-12 months treatment with 10 mg folic acid in 3/70 pernicious anaemia patients. Schwartz, reported that of 98 patients followed up for up to 3.5 y, 4 patients relapsed within 1 year and 19 the following year.

Expert Group on Vitamins and Minerals (2002 and 2003).

25. Expert Group on Vitamins and Minerals (EVM) reviewed a range of vitamins and minerals and recommended upper levels of intake where possible in order to inform UK Government policy on food supplements in response to the possible establishment of maximum levels for food supplements. The review was therefore unrelated to possible fortification with folic acid. Where it was possible to set a Safe Upper Level (SUL) of intake this was done, but where the data were not available or were less secure, guidance was given.

26. A full, detailed review of folic acid was conducted (EVM, 2002) considering the case reports, amongst other available data. Most but not all overlap with those studies considered by the IOM. For folic acid, a guidance level (GL) of 1 mg/day for supplemental folic acid intake was established. This was based on the potential masking of B_{12} deficiency. The EVM stated that “a general consistency of data indicated that supplementation with ≤ 1 mg/day folic acid does not mask vitamin B_{12} associated anaemia in the majority of subjects, whereas ≥ 5 mg/day does. The effects of doses of between 1 and 5 mg/day are unclear”.

27. Two of the key papers cited in the risk assessment were Weissberg *et al*., (1950) and Harvey *et al*., 1950 in which adverse neurological effects were not observed in healthy volunteers or patients with anaemias other than pernicious anaemia who were given 20 mg/day folic acid for up to 12 months.

Scientific Advisory Committee on Nutrition

Folate and disease prevention (2006)

28. SACN considered a number of health endpoints in this review, which examined both the risks and benefits of folate fortification. In particular, they considered the potential masking of vitamin B_{12} deficiency and the possibility that folate could increase the progression of pre-existing polyps to bowel cancer. It was noted that the evidence relating to the masking of deficiency consisted of poorly described case reports, while the data on bowel cancer suggested a potential dual effect of the nutrient depending on age, possibly genotype and intake of other nutrients. SACN considered the association between folic acid and increased risk of colorectal cancer to be unclear.

29. SACN recommended that fortification of flour was the most effective way of increasing folate intake in women most at risk of a NTD affected pregnancy as well as being beneficial to the population as a whole, by redistributing intakes and increasing them in low consumers. However, because of the uncertainties around adverse effects, SACN agreed that the number of people consuming more than the upper level of folic acid should not increase significantly. The upper level cited was the 1 mg/day established by the EVM.

Report to CMO on folic acid and colorectal cancer risk. (2009)

30. In 2007, the Chief Medical Officer (CMO) asked SACN to consider 2 papers by Mason *et al*., (2007) and Cole *et al*., (2007) which examined the relationship between folic acid and cancer risk. SACN worked jointly with COC and external experts to consider these papers along with a meta-analysis of other studies. The Working Group agreed that there was no certain explanation for the increase in colorectal cancer incidence observed in the USA and Canada at around the same time as the introduction of folic acid fortification and that increased rates of colorectal cancer screening, higher intakes of folic acid at the time of fortification, or other factors could be responsible.

31. The majority view of the Working Group was that, on balance, they supported SACN's original recommendation and the new evidence did not provide a substantial basis for changing it. In the event of fortification, concerns about cancer risk should be addressed by careful monitoring of emerging evidence.

Update on folic acid (2017).

32. In 2017, SACN re-considered their risk assessments for folic acid at the request of the Scottish Government. A number of endpoints were considered but the original SACN recommendations regarding the UL were reiterated.

33. SACN (2017) noted that the prevalence of vitamin B_{12} deficiency with or without anaemia did not increase after mandatory fortification in the USA. However, Selhub and Rosenberg (2016) using NHANES data, reported that high serum folate was associated with exacerbation of both clinical and biochemical anaemia in elderly individuals.

Other adverse effects of Folic Acid

34. Although this paper has largely focussed on the possible effects of folic acid on the neurological symptoms associated with B_{12} deficiency other possible adverse effects of folic acid on the nervous system have been suggested. Reynolds (2007) discussed the possible effects of folates on individuals with epilepsy. Vitamin treatment of anti-epileptic drug induced deficiency has been reported to exacerbate seizures and in laboratory animals, large doses of sodium folate have been reported to induce seizures but only where the animal is already vulnerable or where the blood-brain barrier is damaged. Folate induced models of epilepsy are used experimentally to study basic mechanisms and anti-epileptic drugs, while folic acid enhances the electrical kindling model of epilepsy and B_{12} can be used to kindle seizures directly. The IOM (1998) cited animal evidence that folate in the form of folic acid is neurotoxic and epileptogenic in animals but concluded there was no clear evidence for this in humans. The SCF (2000) also noted conflicting studies on epilepsy. Reynolds published a recent letter (Reynolds, 2017) which raised concerns that the move to fortification had overlooked the adverse effects on the nervous system, However, the Secretariat have not yet been able to acquire a copy of this paper.

35. A review by Selhub and Rosenberg (2016) discusses the various adverse effects potentially associated with high folic acid intake. In addition to effects on B_{12}, adverse effects on insulin resistance, natural killer cell number and cognitive ability in elderly women with a particular genetic polymorphism in the dihydrofolatereductase gene. A recent abstract by McGowan *et al*. (2018) reported that high serum level levels of unmetabolized folic acid in children at birth/early life was associated with the development of food allergies, either through increased exposure or an underlying genetic difference.

Wald *et al* (2018)

36. In the paper by Wald *et al*., (2018) (attached at Annex A) the background and history of folic acid is considered. The TUL established by the ION is discussed in detail. Wald argues that “masking” was a misleading term as it dated from a time when folate and B_{12} deficiency could not be distinguished so that the occurrence of neurological symptoms was interpreted as an adverse effect of folic acid rather than an inability to make the correct diagnosis and provide the necessary treatment. The likelihood of masking an incorrect diagnosis disappeared with the use of specific assays for B_{12} and folate and the availability of B_{12} therapy. A related concern was stated to be that folic acid fortification might reduce the occurrence of macrocytic anaemia in vitamin B_{12} deficient individuals and hence delay diagnosis of the deficiency. However, as macrocytic anaemia may be absent in 28% of B_{12} deficient individuals. Therefore, macrocytic anaemia should not be regarded as a requirement for the diagnosis of B_{12} deficiency. Again, with the advent of reliable assays for B_{12} deficiency and the clinical necessity of measuring B_{12} levels early if neurological symptoms occur; this would mean that concerns over the correct diagnosis were no longer an issue; this was acknowledged by the IOM report.

37. Wald and colleagues then discuss the TUL which they consider to be a misinterpretation of the data. IOM state that at doses of ≥ 5 mg/day there were over 100 reported cases of neurological progression but at doses < 5 mg/day there were only 8 reported cases. This comparison was used to set 5 mg as a LOAEL. The argument rested on patients receiving lower doses being taken to represent natural progression of the disease in the absence of treatment while the proportion of progression among patients was assumed to indicate exacerbation by folic acid. Wald *et al* (2018) argues that the analysis is incorrect. Of the studies considered by IOM, 3 included patients taking between 0.33 and 2.5 mg/day, 17 included patients taking 5 mg/day or greater and 3 included both. In the 23 studies 8/12 patients developed neuropathy (67% (95%CI 35-90%) while in the higher dose category 147/ 279 patients developed neuropathy (53% (95%CI 47-59%)). Therefore, the rate of disease progression was no greater in patients taking higher doses. The same data were included in a meta-analysis which had 3 dose categories <5, 5-9.9 and ≥ 10 mg/day using a Freeman-Tukey transformation to allow for extreme estimates of variances in small studies and a random effects model to take account of heterogeneity between the studies. This showed a non-significant decline in the proportion of patients developing neuropathy with increasing folic acid dose. The "illogicality" of attributing neurological toxicity to folic acid rather than the continuation of B_{12} deficiency together with the absence of a folic acid dose response indicates there is no evidence for a LOAEL and therefore no basis for an UL. It was noted that IOM did not set ULs for other B group vitamins such as B_1, B_2, B_5 and B_{12} which were water soluble and readily excreted. However, the UL for B_6 which results in peripheral neuropathy after high and/or sustained doses is not mentioned. The paper then went on to discuss the effect of fortification on exposure and consider the implications for policy.

Updated literature search

38. A limited literature search has not identified any new case reports or any new adverse effects in addition to those noted elsewhere in the paper.

Summary

39. The metabolism of vitamin B_{12} is linked to that of folate as a vitamin B_{12}-dependent enzyme is a co-factor in the metabolism of folate. This means that supplemental folic acid may improve the haematological symptoms while the neurological symptoms proceed undiagnosed.

40. Recommendations for the maximum intakes of folic acid intakes are based on a series of case reports describing the potential masking of the diagnosis of vitamin B_{12} deficiency and/or the exacerbation of its symptoms. A recent paper has argued that the analysis on which these upper levels are based is flawed and that developments in diagnostic techniques mean that the concern about masking is no longer relevant.

Questions for the committee

41. Does the committee wish to:

a) Proceed with the review of the evidence underpinning the GL/TUL for vitamin B_{12}?
b) If so, do the Committee wish to consider the original case reports?
c) Which other data would the Committee wish to review e.g. On epilepsy, cognitive impairment, other endpoints?
d) Do the Committee have any other comments?

Secretariat
March 2018

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