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

FOLIC ACID – STATEMENT ON THE TOLERABLE UPPER LEVEL (TUL)

Introduction.

1. It is well established that supplementation with folic acid can reduce the risk of having a neural tube defect (NTD) affected pregnancy. UK Government advice is that women should take a 400µg supplement of folic acid daily prior to conception up to the third month of pregnancy; women who have already had a NTD affected pregnancy are advised to take a 5 mg supplement (SACN, 2006; SACN, 2009). This remains the current advice (SACN, 2017).

2. However, as many women do not take supplements and many pregnancies are unplanned, the rate of affected pregnancies has not significantly changed since this advice was issued (Morris et al, 2016). Consequently, the Committee on Medical Aspects of Food and Nutrition Policy (COMA 2000), and later the Scientific Advisory Committee on Nutrition (SACN) have recommended that wheat flour should be fortified with folic acid to increase the folate status of the population. This recommendation came with the proviso that fortification should not increase the number of people who were currently exceeding the Guidance/Tolerable Upper Intake Level (GL/UL) for folic acid, meaning that levels in some supplements or other fortified products would need to be reduced (SACN 2017).

3. TULs (or equivalent) for folic acid have been established by a number of risk assessment bodies, including the US Institute of Medicine Food and Nutrition Board (IOM, 1998), the EU Scientific Committee on Food (SCF, 2000) and the UK Expert group on Vitamins and Minerals (EVM, 2003). All of these bodies set a maximum recommended intake of 1 mg/day folic acid (the EVM set a level for supplemental folic acid; see para 42) based on the observations of neurological effects in numerous case series and small studies of folic acid supplementation in patients with pernicious anaemia. However, there was some difference in the hazard identified. Whereas the IOM considered the main concern was the possible precipitation or exacerbation of neuropathy in vitamin B12-deficient individuals, the SCF and the EVM considered the hazard was the ability of folic acid to mask the diagnosis of pernicious anaemia. Whilst folic acid would improve haematological status, it would not prevent the neurological effects associated with the condition. The delay in diagnosis could allow the adverse neurological endpoint to progress until it was potentially irreversible. The SCF noted that they could not rule out the possibility that folic acid could increase the progression of neurological signs, and that this should be considered the most serious adverse effect. The EVM considered that the data

were not adequate to set a TUL and they subsequently set a guidance level (GL) for supplemental intake (in addition to folate in the diet) instead.

4. A recent paper by Wald *et al*., 2018 argues that the basis of the TUL is flawed (see TOX/2018/12 for details). The criticisms made in the paper apply to the IOM TUL but some will also be relevant to maximum intakes recommended by EVM and SCF since the same database was used to set the TUL. The Committee was asked to consider whether the analysis by Wald and colleagues had implications for the GL set by the UK EVM and cited by SACN (SACN, 2006). It should be noted that the EVM set their GL on supplemental intake of folic acid rather than total folates in the diet. Differences between levels set by the risk assessment bodies can be found in paragraph 27. SACN carried out modelling using a range of levels of folic acid fortification to determine that total exposure to folic acid (excluding dietary folates) should not be more than 1mg/day.

5. Following discussion of the Wald *et al*. paper and given that it was some years since the EVM GL was established, the Committee agreed that the TUL should be reconsidered, firstly by considering the basis on which it was set and then, if necessary, consideration of the rest of the database to determine whether a TUL was required based on other endpoints.

Background

6. Folates are a family of chemically related compounds based on the folic acid structure. In tissues, folates act as donors and acceptors of one carbon units in one carbon reactions (Shane, 2008). Most tissue folates are in a polyglutamate form in which the glutamate tail is extended via the gamma-carboxyl of glutamate. Metabolism of folates to polyglutamates by the enzyme folylpolyglutamate synthetase is required for biological activity as the polyglutamate forms are more effective substrates for enzyme activity than the monoglutamate forms of folic acid, which are the transport forms (Shane, 2008).

7. In food, folates typically occur in a reduced polyglutamyl form. Folic acid (pteroyl glutamic acid) is not found in nature. It is used in supplements because it is highly bioavailable, chemically stable and readily reduced to tetrahydrofolates, the active co-enzyme form.

8. Most folic acid and dietary folate is metabolised to 5-methyl-tetrahydrofolate during passage across the intestinal mucosa. When high doses of folic acid or other forms of folates are consumed, they may appear in the peripheral circulation unchanged (SACN, 2006; Shane, 2008; SACN 2017). The bioavailability of folic acid is close to 100% on an empty stomach and 85% if consumed with food. Bioavailability of food folates is estimated to be around 85% (IOM, 1998).

9. Although high plasma folate levels can be achieved with oral supplements, these decrease rapidly as the renal re-absorption threshold is exceeded and much of the dose is excreted within 24 hours. Plasma levels more than 100 times normal can be achieved, but tissue folate levels increase only marginally (often less than 2-fold) due to the limited ability of tissues to metabolise the large doses to the polyglutamate

form required for retention. At normal dietary intake, whole body folate turns over slowly with a half-life in excess of 100 days (Shane, 2008).

Function of folate.

10. Folate coenzymes are involved in three major inter-related metabolic cycles. These cycles are required for the synthesis of thymidylate and purines, precursors for DNA and RNA synthesis; the synthesis of the essential amino acid methionine from homocysteine, and; the interconversion of serine and glycine (Shane, 2008).

How vitamin B_{12} and folate are linked.

11. Vitamin B_{12} (cobalamin) consists of a central cobalt atom surrounded by a haem-like planar corrin ring structure. It is present in food bound to protein and is released in the stomach by the acid environment and by proteolysis of the binders by pepsin (Shane, 2008). The released B_{12} initially binds to R-binders (dietary proteins with an affinity for vitamin B12) but while passing through the small intestine, these are hydrolysed and the B_{12} binds to intrinsic factor, a glycoprotein, secreted by the parietal cells of the stomach, and is absorbed via receptors in the ileum.

12. The majority of vitamin B_{12} is stored in the liver. Mammals need B_{12} as a cofactor for two enzymes, cytosolic methionine synthase and mitochondrial methylmalonyl CoA mutase. These are the only two vitamin B_{12} dependent enzymes in mammals.

Methionine synthase

13. Methionine synthase is thus both folate- and vitamin B_{12}-dependent. It catalyses the methylation of homocysteine to regenerate methionine using 5-methyl-tetrahydrofolate as the methyl donor. Methionine is an essential amino acid and is often the most limiting amino acid in the human diet. Methylation reactions account for a large proportion of the methyl group intake in humans and the methionine synthase reaction allows salvage of its backbone after its use for methylation. The folate dependent methionine cycle is very sensitive to inadequate folate status and when folate status is poor, the failure to remethylate cellular homocysteine results in increased plasma homocysteine level which is an indirect indicator of folate insufficiency (Shane, 2008).

Methylmalonyl CoA mutase.

14. Mitochondrial β-oxidation of odd-chain dietary fatty acids produces propionyl CoA in addition to acetyl CoA. Propionyl CoA is converted to D-methylmalonyl CoA. These compounds may also be produced during the catabolism of certain amino acids including isoleucine, valine, methionine and threonine. D-methylmalonyl CoA is enzymatically racemised to the L-form and B_{12} dependent methylmalonyl CoA mutase then catalyses the conversion of this to succinyl CoA. This has several fates, including entrance to the citric acid cycle and involvement in the biosynthesis of haem. In the liver, the conversion of propionyl CoA to succinyl CoA allows the carbon skeleton of some amino acids to be used for gluconeogenesis.

B_{12} and folate deficiency - the metabolic basis for folate and B_{12} deficiency signs.

Megaloblastic anaemia

15. The classical sign of folate insufficiency is megaloblastic anaemia, a condition reflecting deranged DNA synthesis in the erythropoietic cells. Megaloblastic changes occur in all fast-growing tissues such as the bone marrow and the gut epithelia. Megaloblastic cells contain nearly twice the normal DNA content and the DNA is partially fragmented. Many cells are arrested in the G2 phase just prior to mitosis, and cells that do divide often undergo apoptosis. The defect in DNA synthesis has been ascribed to defective thymidylate synthesis, ultimately leading to an increase in double-stranded DNA breaks.

16. Megaloblastic anaemia is also a sign of pernicious anaemia, which is caused by an inability to absorb vitamin B_{12} due to the lack of intrinsic factor production. The anaemia that results is identical to that caused by folate deficiency. Since body stores of vit B_{12} are generally ample at the onset of the disease and turnover is slow, it can take some years before signs of deficiency become apparent.

17. In vitamin B_{12} deficiency, the vitamin B_{12} dependent methionine synthase enzyme is inactive and cytosolic folate is “trapped” as 5-methyl-tetrahydrofolate at the expense of other folate co-enzyme forms required for one-carbon metabolism such as thymidylate synthesis, leading to a functional folate deficiency or “methyl trap” in the cell (Shane, 2008). As 5-methyl-tetrahydrofolate is a poor substrate for folylpolyglutamate synthetase, the ability of tissues to accumulate folate is reduced and the folate deficiency is compounded by a drop in cellular folate levels.

18. As the defective DNA synthesis in pernicious anaemia is caused by an induced secondary folate deficiency, high levels of folate cause a haematological response in patients with megaloblastic anaemia due to vitamin B_{12} deficiency, but folate is ineffective in preventing the severe neurological pathologies associated with B_{12} deficiency

Pernicious anaemia

19. Pernicious anaemia is a disease of autoimmune origin in which atrophy of the gastric mucosae in the body and fundus of the stomach reduces the number of parietal cells that produce the intrinsic factor necessary for the intestinal absorption of vitamin B_{12}, which in turn is essential for erythropoiesis and myelin synthesis (Bizzaro and Antico, 2014). Parietal cells also produce chlorhydric acid, which helps release vit B_{12} from its protein-bound form in the stomach (Lahner and Annibale, 2009). Blocking autoantibodies bind to the parietal cells and to the vitamin B_{12} binding site of intrinsic factor (Toh *et al*., 1997). More details on pernicious anaemia can be found in TOX/2018/26.

Prevalence

20. Pernicious anaemia occurs worldwide and is probably underdiagnosed given that microcytic and macrocytic anaemia are usually treated with iron, folates and cobalamin without any more thorough investigation into the cause of anaemia

(Bizzaro and Antico, 2014). Even if a biopsy is performed, a generic histological pattern of chronic gastritis with intestinal metaplasia is often described.

21. Pernicious anaemia usually occurs in individuals of either sex aged over 30 years and is particularly frequent in Northern Europeans, especially Scandinavians (Bizzaro and Antico, 2014); it is present in other populations but is relatively infrequent in oriental populations. Banka *et al*., 2011 report a prevalence of 0.1-0.2% in the British population (citing Scott, 1960 as the original source of this value).

Diagnosis

22. B_{12} deficiency is best diagnosed using a combination of tests because none alone is completely reliable (Green, 2017). Serum B_{12} measurement used 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 a concentration within the reference range does not always indicate normalcy. This is partly due to the distribution of vit B_{12} 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.

23. The diagnosis of pernicious anaemia depends on the demonstration of megaloblastic anaemia, low serum vitamin B_{12} levels, gastric atrophia and the presence of antibodies to gastric parietal cells or intrinsic factor (Bizzaro and Antico, 2014).

24. The laboratory workup can be accomplished by systematic investigation starting with a concurrent assessment of vitamin B_{12}/folate status (Bizzaro and Antico, 2014). A deficit of intrinsic factor can be demonstrated using the Schilling test (this is a complex test involving radioisotopes and is not routinely performed, though is widely considered to be the gold standard for diagnosis (Andrès *et al*., 2012)). Other, less specific, laboratory indications include markedly elevated lactate dehydrogenase and mildly elevated bilirubin, total iron, and aspartate aminotransferase levels; the latter two can reflect intramedullary erythroblastosis. Fasting gastrin levels are elevated in many patients, while the levels of somatostatin are depressed (Bizzaro and Antico, 2014). Indications that antibodies should be investigated include, macrocytic anaemia not responding to oral therapy with vit B_{12}, the presence of other endocrine autoimmune disorders, dyspeptic symptoms not correlated with other gastrointestinal disease and siblings with pernicious anaemia. The antibodies can be detected by immunoblotting, ELISA and chemiluminescent immunoassay, and can be detected more frequently in gastric juice than in serum (Bizzaro and Antico, 2014).

25. In their guidance on anaemia - B_{12} and folate deficiency, NICE (2018) state that if vitamin B_{12} or folate deficiency is suspected, a full blood count to determine MCV, haematocrit and haemoglobin levels should be obtained as well as a blood film to help identify megaloblastic anaemia. Serum folate and cobalamin levels should be measured and investigations such as liver and/or thyroid function tests be conducted to establish the underlying cause.

Treatment

26. In the UK, vitamin B_{12}-deficiency anaemia is treated with i.m. injections of hydroxycobalamin every other day for 2 weeks or until signs improve (NHS, 2018). Subsequent treatment depends on whether the deficiency is dietary or due to pernicious anaemia. If the cause is pernicious anaemia, then an injection would be given every 3 months thereafter. Signs of deficiency improve rapidly, with increased reticulocyte count being the most useful indicator of a haematological response (Bizzaro and Antico, 2014). Gastric atrophy does not respond to vitamin treatment but does respond to steroids with partial regeneration and renewed secretion of intrinsic factor. The neurologic complications are serious as they may not be reversible after supplementation therapy (Toh *et al*., 1997).

How the TULs / GL for folic acid were set

27. The progression to neurological effects in subjects with pernicious anaemia treated with folic acid is well established and was used to set recommended levels of intake by several regulatory bodies. The US IOM established a TUL for supplemental intake of 1 mg/day intended to reflect a maximum safe intake over a lifetime, which would not precipitate or exacerbate neuropathy. The EU SCF also established a TUL of 1 mg/day, but this was intended to reflect a maximum safe intake over a lifetime that would avoid masking of the diagnosis of pernicious anaemia by folic acid. The UK EVM established a Guidance Level for supplemental intake, rather than a TUL, reflecting the limited nature of the available data, of 1 mg/day, which also reflected a lifetime's exposure that would avoid masking of the diagnosis of pernicious anaemia by folic acid. A GL was intended to represent a value which would not be expected to result in adverse effects but noting that this level may not be applicable to all life stages or for life-long intake. The database used consisted of case reports and case series along with a number of small studies. The historical background to the phenomena is given below along with an overview of the approaches taken by three risk assessment bodies who considered the topic.

28. It should be noted that although the majority of the papers are individual case reports or case report series, the studies by Spies *et al*, 1948; Ross *et al*., 1948; Will *et al* (1959) are small clinical trials. The majority of studies report the appearance of neurological signs and/or haematological relapse in individuals whose treatment was changed from liver extract or desiccated stomach preparation to folic acid. The relapses frequently followed an initial improvement in haematological parameters. However, in a few cases the folic acid treatment was considered to have exacerbated the neurological signs. These are Berk *et al*., 1948; Ross *et al*., 1948. Other studies considered this issue and concluded that there was no evidence for a direct neuropathic effect of folic acid; these include Chodos and Ross, 1951. Several later case reports are of delayed diagnosis resulting from the use of multivitamin preparations. These include Crosby, 1960; Ellison, 1960 and Baldwin & Dalessio, 1961.

29. Although the issue of delayed diagnosis has been discussed at various points, notably when TULs or their equivalents were being established, no additional data have been identified in the recent literature.

Historical background

30. It was known that pernicious anaemia could be treated effectively with liver extract, but the pure anti-(pernicious) anaemic factor, vitamin B_{12}, was not isolated until 1948 (Chanarin, 2000). Liver provides both folic acid and vitamin B_{12}.

31. Folic acid was identified and synthesised in 1945/6, leading to its "unexpected and remarkable" effects on restoring blood counts in the treatment of pernicious anaemia and nutritional macrocytic anaemia (discussed in Israëls and Wilkinson, 1949).

32. Although the results of folic acid treatment of patients with pernicious anaemia were initially striking, it soon became evident that even when blood counts were restored to normal levels, relapses were occurring and signs of involvement of the peripheral nerves such as paraesthesia and numbness in the limbs, and of the spinal cord tracts like ataxia and loss of vibration sense might become worse. Of more concern was the observation that patients who had not previously shown signs of nervous system disturbances developed such signs, often very acutely, after being treated with folic acid for variable periods (Israëls and Wilkinson, 1949). The occurrence and progression of subacute combined degeneration (SCD) in patients with pernicious anaemia under treatment with folic acid was first reported by Vilter *et al*., 1947, and Meyer, 1947 and was also noted by Welch *et al*., 1946 and by Hall and Watkins, 1947 (Bethell and Sturgis, 1948). Bethell and Sturgis (1948) noted that in some cases, the activity of the process was not arrested by increasing the dose of folic acid.

33. The recommendations on maximum levels of intake are discussed below in order of publication.

IOM (1998)

34. The TUL established by IOM applies to supplemental folate only since there was no evidence that dietary folate was of 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 Case reports considered by IOM.

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	2.5 y	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, 1947.	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

35. Secondly, the IOM noted that in addition to the human case reports, studies in rhesus monkeys (Agamanolis, 1976) and fruit bats (van der Westhuyzen *et al* 1982, 1983) show that vitamin B_{12} deficient animals receiving supplemental folate developed signs of neuropathology sooner than controls. Thirdly, there is a well-documented interaction between folate and B_{12} (Chanarin *et al*., 1989). 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 B12 deficiency. The delayed diagnosis could then result in an increased risk of progressive or unrecognised neurological damage.

36. 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 identified but a LOAEL of 5 mg was identified based on the cases in Table 1. IOM 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 the fact that a LOAEL rather than a NOAEL was used, resulting in a TUL of 1 mg/day folic acid. 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)

37. In the late 1990s it was proposed that, to improve market harmonisation, maximum levels for vitamins and minerals should be set for food supplements. The EU Scientific Committee on Food (SCF) and subsequently the EFSA Dietetic Products, Nutrition and Allergies (NDA) panel reviewed a range of vitamins and minerals to establish maximum upper levels. They noted that in the early days of research when B_{12} had not been identified as a separate vitamin, individuals with macrocytosis and other haematological abnormalities were treated with > 5 mg folic acid, with complete remission of signs occurring in > 60% of individuals with pernicious anaemia. Sub-optimal improvement was reported at dosages of 1-5 mg. At doses < 1 mg, any improvement was very rare.

38. In their review, the SCF noted the studies by Wagley (1948), Bethell and Sturgis (1948) and Schwartz (1950), which reported the treatment and subsequent neurological and haematological relapses of pernicious anaemia patients treated with folic acid. The SCF noted the claims in some studies that folic acid therapy in patients with pernicious anaemia might aggravate or even induce the neurological lesions but also cited the review by Dickinson (1995) which concluded that there was no convincing evidence for such an effect and pointed out that there were methodological flaws in the initial fruit bat study (van der Westhuyzen *et al*., 1983), in which vit B_{12} deficiency was induced by treatment with nitrous oxide, notably that the control animals were not given sham injections and the observations of flight being reduced to hops being very subjective. The B_{12} deficient bats receiving the oral folic acid reached the same stage of neurological impairment slightly, but not significantly, earlier as the untreated ones. With regard to the study in rhesus monkeys (Agamanolis *et al*., 1976), the SCF noted that the numbers of animals were small, ,

visual lesions observed in the monkeys were only rarely seen in humans and they occurred in some of the non-folic acid treated animals.

39. The SCF established a TUL of 1 mg/day for synthetic folic acid on the basis of masking of haematological signs in pernicious anemia patients at higher levels (SCF, 2000). The SCF considered that although there was no conclusive evidence in humans, the risk of progression of the neurological effects 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 in only a few cases were the doses between 1 and 5 mg/day. The LOAEL was estimated to be 5 mg and the TUL 1 mg for adults, with TULs for children being scaled on body weight. No data were available to suggest that other populations 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.

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

40. The 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 of flour with folic acid. Where it was possible to set a Safe Upper Level (SUL)[1] of intake for an individual nutrient, this was done, but where the data were not available or were less secure, guidance was given, if possible.

41. A full, detailed review of folic acid was conducted (EVM, 2002), considering the case reports, amongst other available data. Most, but not all, overlapped with those studies considered by the IOM, but also included Marshall *et al*, 1960, Hansen and Weinfeld, 1962, Vilter *et al.*, 1950, Vilter *et al.,* 1960. Two of the key papers cited in the assessment were Weissberg *et al*., (1950) and Harvey *et al*., 1950, studies 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.

42. For folic acid, a guidance level (GL) of 1 mg/day for supplemental intake (in addition to folates found in the diet) was established (EVM, 2003), since there were insufficient data to set a SUL. 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”.

Changes in B_{12} status since fortification

[1] Equivalent to a UL or TUL, representing a daily intake over a lifetime which would be unlikely to result in adverse health effects.

43. Mixed results have been found in population studies looking at the effect of folic acid fortification on vitamin B_{12} status and the masking of vitamin B_{12} deficiency. In cross-sectional studies looking at vitamin B_{12} status before and after fortification, one study found a higher prevalence of vitamin B_{12} deficiency following folic acid fortification [0.09% pre-fortification to 0.61% post-fortification] (Wyckoff and Ganji, 2007) whilst two studies found no such increase in vitamin B_{12} deficiency (Mills *et al*., 2003; Qi et al., 2014).

Consideration of the Wald *et al*. (2018) paper

44. Wald *et al* conclude that the IOM TUL is flawed. The basis for this conclusion is laid out below. They begin with two IOM observations:

- at doses of folate of 5 mg/day and above in patients with vit B_{12} deficient anaemia, there were more than 100 reported cases of neurological progression;

- at doses of less than 5 mg/day of folate (0.33 to 2.5 mg/day) in patients with vit B_{12} deficient anaemia, there are only eight well-documented cases of neurological progression;

45. Wald *et al.* went back to the original studies and from the "well-documented" cases calculated that 12 patients took less than 5 mg/day folic acid and 8 developed neuropathy (67%; 95% CI 35-90%), whereas 279 patients took more than 5 mg/day folic acid and 147 developed neuropathy (53%; 95% CI 47-59%). A further analysis using 3 dose categories (<5, 5-9.9, >10 mg/day) and using the Freeman-Tukey transformation (to allow for extreme estimates of variance in small studies and a random effects model to take account of the heterogeneity between the studies) gave percentages of 84, 66 and 54, suggesting a non-significant decrease in the proportion of patients showing signs of neuropathy following ingestion of folic acid. On this basis, Wald *et al.* concluded that the assertion that folic acid is directly neurotoxic is incorrect. Wald *et al.* further argue that detection methods for vitamin B_{12} status are sufficiently improved and the advent of vitamin B_{12} therapy mean that there is now no risk of "masking" the effects of vitamin B_{12} deficiency with folic acid. Wald *et al.* also criticise the IOM for including natural folate in the TUL, but this is not correct – the IOM applied the TUL to supplements and fortified foods, but not naturally present folate.

46. Wald *et al.* concluded that there was no dose-response relationship and that there should not be an TUL for folic acid, a water-soluble vitamin, any more than for other B vitamins (B1, B2, B5 or B12).

47. Members discussed the paper by Wald et al. (2018) on a number of occasions. They agreed that reviewing the basis of the TUL was useful and necessary but disagreed with some of the conclusions, namely regarding the ease of diagnosis of pernicious anaemia. Whilst improvements have been made to methods for diagnosis, these are not routinely available and pernicious anaemia still goes undetected in many. Limitations therefore still exist in this area and the masking of vitamin B_{12} deficiency by folic acid is still a risk in pernicious anaemia patients.

Summary and discussion

48. Pernicious anaemia results from an autoimmune atrophy of the gastric mucosa which reduces the number of parietal cells that produce intrinsic factor which allows the body to absorb vitamin B_{12}. The subsequent deficiency results in severe megaloblastic anaemia, as well as progressive damage to the nervous system due to demyelination and progressive damage to axons. Pernicious anaemia occurs throughout the world but is more common in Northern Europeans. The prevalence increases with age and it is slightly more common in women. The condition is under-diagnosed and thus many individuals with pernicious anaemia may not be aware they have the condition.

49. Patients may present with haematological or neurological signs, some of these may be non-specific, making diagnosis difficult. The condition is diagnosed first by assessing whether anaemia is due to vit B_{12} deficiency. This involves a combination of tests which include serum vitamin B_{12} and folate levels, standard haematology parameters such red cell and reticulocyte count and haemoglobin levels and other investigations, such as liver function tests, gamma-glutamyl transpeptidase, and/or thyroid function tests to exclude other possible causes. Confirmation of pernicious anaemia involves tests for serum anti-intrinsic factor and parietal cell antibody levels.

50. It is well established that folic acid can delay the diagnosis of pernicious anaemia, allowing progression of the neurological damage until it is severe and potentially irreversible. This occurs because folic acid can correct defective DNA synthesis due to B_{12} deficiency improving haematological status through an increase in red cell and reticulocyte numbers without also correcting the neurological damage which proceeds via a different mechanism.

51. This phenomenon has been used to establish recommended maximum upper intakes by a number of risk assessment bodies. The database used consists of a series of case reports, case series and small studies as well as some limited animal data. The maximum level set (usually as a Tolerable Upper Level or TUL) by the US IOM, EVM and the EU SCF was 1 mg/day of folic acid, as it was generally considered that there were no convincing data that this level of intake would permit or induce neurological signs, whereas at intakes of 5 mg/day the effect was clear.

52. It has been argued by Wald *et al*., 2018 that the TUL is flawed and is also no longer relevant given modern diagnostic techniques and that misplaced concern has prevented the use of folic acid to fortify flour and reduce the number of NTD affected pregnancies. The data are conflicting but studies from the US suggest that fortification does not appear to have increased the prevalence of B_{12} deficiency without anaemia.

53. It is well established that supplementation with folic acid can reduce the risk of having a NTD-affected pregnancy. UK Government advice is that women should take a folic acid supplement prior to conception and up to the third month of pregnancy. However, as many women do not take supplements and many

pregnancies are unplanned, the rate of NTD-affected pregnancies has not significantly changed.

54. Consequently, SACN have recommended that wheat flour should be fortified with folic acid to increase the folate status of the population. This recommendation came with the proviso that fortification should not increase the number of people who were currently exceeding the guidance level for folic acid, meaning that levels in some supplements or other fortified products would need to be reduced.

55. TULs or GL of 1 mg/day have been set by a number of risk assessment bodies, including the UK EVM, based on the observation that folic acid may mask the diagnosis of pernicious anaemia by improving haematological status while allowing neurological damage to progress. The US IOM considered that it was not possible to rule out the possibility that folic acid could exacerbate the neurotoxicity associated with pernicious anaemia and used this as the basis of its TUL. The EU SCF based their TUL on masking, but also noted that they could not rule out the possibility that folic acid could increase the progression of neurological signs, and that this should be considered the most serious adverse effect.

Conclusions

A risk benefit assessment by SACN and COT concluded that there were benefits in preventing NTD from fortification with folic acid as long as mean intakes did not increase substantially. The information available to the committee provides no basis for changing this conclusion. Modelling by SACN of a range of dietary exposures resulting from fortification of flour with folic acid at 300 µg/100g flour showed that folic acid intakes would not exceed 1mg/day.

56. The criticisms made in the Wald et al. paper apply to the IOM TUL but some are also relevant to maximum intakes recommended by the SCF since the same endpoints were taken into consideration when setting the TUL, and by the EVM, as largely the same database was used. Wald et al's main criticism of the IOM relates to the possibility of folic acid having a direct neurotoxic effect as the basis for the establishment of the TUL.

57. The original case reports have significant limitations. The adverse effects were first reported following the isolation of folic acid in the 1940s, when patients with pernicious anaemia were treated with folic acid rather than the meat or liver extract (which also contain B_{12}), which had been used as treatment previously and some of these patients had gone on to develop new neurological signs or to relapse. The way the data were presented and reported make it difficult to determine a dose-response relationship.

58. It was possible that any dose response was a flat one, i.e., once the folic acid was sufficient to correct the anaemia of B_{12} deficiency further increases did not have any additional effect.

59. There is no evidence that folic acid has a direct neurotoxic effect in humans, but it is able to mask diagnosis by treating the anaemia associated with B_{12} deficiency. Hence, the conclusion of the IOM is incorrect, on the basis of the human data that they evaluated.

60. The Committee considered a number of endpoints (carcinogenicity, dysplasia and metaplasia, cervical neoplasia, colorectal cancer, lung, oesophageal and stomach cancer, unmetabolised folic acid, potential effects on zinc status and absorption, decreased efficacy of folate antagonists and hypersensitivity to folate) on which to set an Upper Level or Guidance Level but the masking of B_{12} deficiency is considered the most relevant.

61. Wald et al. state that "The likelihood of masking …. [vitamin B_{12} deficiency…] disappeared during the latter half of the last century, with the introduction of specific assays for folate and B_{12} deficiency, and with the ready availability and common use of B_{12} therapy…… " and "…with the advent of reliable assays for B_{12} deficiency and the clinical necessity of measuring a person's B_{12} level if early neurological symptoms arise, concerns over the correct diagnosis were no longer an issue".

62. The COT does not fully agree with this view. Although serum B_{12} levels can be measured, these do not indicate whether the B_{12} is functional; the test for the latter is not widely available. In the event that reliable testing becomes routinely and consistently applied across the UK, then this would mitigate the residual risk posed by folic acid fortification/supplementation above the currently proposed levels, and would obviate the need for a GL/TUL.

63. When establishing their guidance level, the UK EVM took 5 mg/day folic acid as a LOAEL where there was some evidence of masking and 1 mg/day, where there was little evidence, as a NOAEL. The Committee agrees that, whilst there is appreciable uncertainty, this is a reasonable interpretation of this data set and that the EVM had taken an appropriate approach, setting a guidance level rather than a TUL as this indicated the supporting data were less secure.

64. The Committee reaffirmed the Guidance Level for supplemental folic acid of 1 mg/day on the basis of possible masking of pernicious anaemia in vit B_{12}-deficient subjects. This GL can continue to be used in risk-benefit assessment of folic acid fortification to reduce the number of neural tube defect-affected pregnancies.

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Abbreviations

AIG	Autoimmune Gastritis.
ATP	Adenosine Triphosphate
CoA	Coenzyme A
CI	Confidence Interval
CNS	Central Nervous System
DNA	Deoxyribonucleic acid
EFSA	European Food Safety Authority
ELISA	Enzyme Linked Immunosorbent Assay
EVM	Expert Group on Vitamins and Minerals
fL	Femtolitre
GL	Guidance Level
H	Hydrogen
HC	Haptocorrin
HLA-DR	Human Leukocyte Antigen-antigen D related
IDA	Iron Deficiency Anaemia
IFA	Intrinsic Factor Auto antibodies
i.m.	Intramuscular
IOM	Institute of Medicine Food and Nutrition Board.
i.v.	intravenous
K	Potassium
LOAEL	Lowest Observed Adverse Effect Level
mg	Milligram
MHC	Major Histocompatibility
MHTFR	Methylenetetrahydrofolate reductase
MCV	Mean Corpuscular Volume
NDA	Dietetic Products, Nutrition and Allergies panel
ng/L	Nanograms per Litre
NHANES	National Health and Nutrition Examination Surveys
NHS	National Health Service
NICE	National Institute for Clinical Excellence
NOAEL	No Observed Adverse Effect Level
OR	Odds Ratio
PCA	Parietal Cell Autoantibodies
pmol/L	Picomoles/Litre
RNA	Ribonucleic acid
SACN	Scientific Advisory Committee on Nutrition
SCD	Subacute combined degeneration
SCF	Scientific Committee on Food
SUL	Safe Upper Level
TC	Transcobalamin
TUL	Tolerable Upper Level

REFERENCES

Agamanolis, D.P., Chester, E.M., Victor, M., Kark, J.A., Hines, J.D., Harris, J.W. (1976). Neuropathology of experimental vitamin B12 deficiency in monkeys. Neurology, 26, 905-914.

Allen, R.H., Stabler, S.P., Savage, D.G., Lindenbaum, J. (1990). Diagnosis of cobalamin deficiency I: usefulness of serum methylmalonic acid and total homocysteine concentrations. Am. J. Hematol. 34, 90-8

Andrès, E., Serraj, K (2012). Optimal management of pernicious anaemia. Journal of Blood Medicine, 3, 97-103.

Baldwin, J.N., Dalessio, D.J. (1961). Folic acid therapy and spinal-cord degeneration in pernicious anemia. NEJM. 264, 1339-42.

Banka, S., Ryan, K., Thomson, W., Newman, W.G. (2011). Pernicious anaemia-genetic insights. Autoimmunity, Reviews, 10, 455-459.

Berk, L., Bauer, J.L., Castle, W.B. (1948). Folic Acid. A report of 12 patients treated with synthetic pteroylglutamic acid with comments on the pertinent literature. South African Medical Journal, 22, 604-611.

Best, C.N. (1959). Subacute combined degeneration of the spinal cord after extensive resection of ileum in Crohn's disease. British Medical Journal, 31 92) 862-864.

Bethell, F.H., Sturgis, C.C. (1948). The relation of therapy in pernicious anaemia to changes in the nervous system. Early and late result in a series of cases observed for periods of not less than ten years and, early results of treatment with folic acid. Blood, 3, 57-67.

Bizzaro, N., Antico, A. (2014). Diagnosis and classification of pernicious anaemia. Autoimmunity Reviews, 13, 565-568.

Chanarin, I. (2000). A history of pernicious anaemia. British Journal of Haematology, 111, 407-415.

Chanarin, I., Deacon, R., Lumb, M., Perry, J. (1989) Cobalamin-folate interrelations. Blood Review, 3, 211-215. Abstract only.

Chodos, R.B., Ross, J.F. (1951). The effects of combined folic acid and liver extract therapy. Blood, 6, 1213-1233.

COMA (2000) Folic Acid and the Prevention of Disease. Department of Health Report on Health and Social Subjects No 50. The Stationery Office, London 2000

Conley, C.L., Krevans, J.R. (1951). Development of neurologic manifestations of pernicious anaemia during multivitamin therapy. The New England Journal of Medicine, 245, 529-531.

Crosby, W.H. (1960). The danger of folic acid in multivitamin preparations. Military Medicine, 125, 233-235.

Dickinson, C.J. (1995). Does folic acid harm people with vitamin B_{12} deficiency? Quarterly Journal of Medicine, 88, 357-364.

Ellison, A.B. (1960). Pernicious anemia masked by multivitamins containing folic acid. JAMA, 173, 240-243.

EVM (2002) Expert Group on Vitamins and Minerals Review of Folic Acid. Available at: https://webarchive.nationalarchives.gov.uk/20101211075917/http:/www.food.gov.uk/multimedia/pdfs/evm0018p.pdf

EVM (2003) Expert Group on Vitamins and Minerals: Safe Upper Levels for Vitamins and Minerals, Food Standards Agency, London. Available at https://cot.food.gov.uk/sites/default/files/vitmin2003.pdf

Fowler, W.M., Hendricks, A.B. (1949). Folic acid and the neurologic manifestations of pernicious anaemia. American Practitioner and Digest of Treatment, 3 (10) 609-613.

Hall, B.E., Watkins, C.H. (1947) Experience with pteroylglutamic (synthetic folic) acid in the treatment of pernicious anaemia. The Journal of Laboratory and Clinical Medicine, 32, 622-634.

Hansen, H.A., Weinfeld, A. (1962) Metabolic effects and diagnostic value of small doses of folic acid and B12 in megaloblastic anemias. Acta Medica Scandinavica, 172, 427-443.

Harvey, E.A., Howard, I., Murphy, W.P. (1950). Absence of a toxic effect of folic acid on the nervous systems of persons without pernicious anaemia. New England Journal of Medicine, 242, 446-447.

Heinle, R.W., Welch, A.D. (1947). Folic acid in pernicious anaemia. Failure to prevent neurologic relapse. Journal of the American Medical Association, 133, 739-740.

Heinle, R.W., Dingle, J.T., Weisberger, A.S. (1947). Folic acid in the maintenance of pernicious anemia. The Journal of Laboratory and Clinical Medicine, 32, 970-981.

IOM (2000) Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin, and Choline available at: http://nationalacademies.org/hmd/Reports/2000/Dietary-Reference-Intakes-for-Thiamin-Riboflavin-Niacin-Vitamin-B6-Folate-Vitamin-B12-Pantothenic-Acid-Biotin-and-Choline.aspx

Israëls, M.C.G., Wilkinson, J.F. (1949). Risk of neurological complications in pernicious anaemia treated with folic acid. British Medical Journal, 2 (4636), 1072-1075.

Jacobson, S.D., Berman, L., Axelrod, A.R., Vonder Heide E.C. (1948). Folic acid therapy its effects observed in two patients with pernicious anaemia. Journal of the American Medical Association, 137 (10) 825-827.

Marshall, R.A., Jandl, J.H. (1960). Responses to “physiologic” doses of folic acid in the megaloblastic anaemias. Archives of Internal Medicine, 105, 352-360.

Meyer, L.M. (1947) Folic acid in the treatment of pernicious anaemia. Blood, 2, 50-62

Mills, J.L., Von Kohorn, I., Conley, M.R., Zeller, J.A., Cox, C., Williamson, R.E., Dufour, D.R. (2003). Low vitamin B-12 concentrations in patients without anaemia: the effect of folic acid fortification of grain. American Journal of Clinical Nutrition, 77, 1474-7

Morris, J.K., Rankin, J., Draper, E.S., Kurinczuk, J.J., Springett, A., Tucker, D., Wellesley, D., Wreyford, B., Wald, N.J. (2016). Prevention of neural tube defects in the UK; a missed opportunity. Arch. Dis. Child. 101, 604-607.

NHS (2018) https://www.nhs.uk/conditions/vitamin-b12-or-folate-deficiency-anaemia/treatment/

NICE (2018) Anaemia - B_{12} and folate deficiency https://cks.nice.org.uk/anaemia-b12-and-folate-deficiency#!diagnosissub:1

Qi, Y.P., Do, A.N., Hamner, H.C., Pfeiffer, C.M., Berry, R.J. (2014). The Prevalence of low serum vitamin B-12 status in the absence of anaemia or macrocytosis did not increase among older US adults after mandatory fortification. Journal of Nutrition, 144, 170-176.

Ross, J.F., Belding, H., Paegel, B.L. (1948). The development and progression of the spinal cord in patients with pernicious anemia treated with synthetic pteroylglutamic (folic) acid. Blood, 3:68-90.

SACN (2006). Scientific Advisory Committee on Nutrition. Folate and Disease Prevention, TSO, London.

SACN (2009) Report to CMO on folic acid and colorectal cancer risk. https://www.gov.uk/government/publications/sacn-report-to-cmo-on-folic-acid-and-colorectal-cancer-risk

SACN (2017) Update on folic acid. https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/637111/SACN_Update_on_folic_acid.pdf

SCF (2000). Opinion of the Scientific Committee on Food on the Tolerable Upper Intake Level of Folate. SCF/ CS/ NUT/UPPLEV/18 final. https://ec.europa.eu/food/sites/food/files/safety/docs/sci-com_scf_out80e_en.pdf

Schwartz, S.O., Kaplan, S.R., Armstrong, B.E. (1950). The long-term evaluation of folic acid in the treatment of pernicious anaemia. The Journal of Laboratory and Clinical Medicine, 35, 894-898.

Scott, E. Prevalence of pernicious anaemia in Great Britain. Journal of the College of General Practitioners, 3. 80-84.

Shane, B. (2008). Folate and vitamin B_{12} metabolism: Overview and interaction with riboflavin, vitamin B_6 and polymorphisms. Food and Nutrition Bulletin, 29 no 2 (supplement) S5-S16.

Spies, T.D., Vilter, C.F., Koch, M.B., Caldwell, M.H. (1945). Further Studies on folic acid in the treatment of macrocytic anaemia. Southern Medical Journal, 38, 781-5 [NB. original not obtained and checked]

Spies, T.D., Stone, R.E. (1947). Liver extract, folic acid, and thymin in pernicious anaemia and sub-acute combined degeneration. The Lancet, 1 (16440) 174-176.

Spies, T.D., Stone, R.E., Garcia Lopez, G., Milanes, F., Aramburu, T., Lopez Toca, R. (1948). The association between gastric achlorhydria and subacute combined degeneration of the spinal cord. Postgraduate Medicine, 4, 89-95.

Toh, B-H., van Driel, I.R., Gleeson, P.A. (1997). Pernicious Anemia. The New England Journal of Medicine, 337, 1441-1448.

van der Westhuyzen, J., Fernandes-Costa, F., Metz, J. (1982). Cobalamin inactivation by nitrous oxide produces severe neurological impairment in fruit bats; protection by methionine and aggravation by folates. Life Sciences, 31, 2001-2010.

van der Westhuyzen, J., Metz, J. (1983). Tissue-S-adenosylmethionine levels in fruit bats (*Rousettus aegyptiacus*) with nitrous-oxide induced neuropathy. British Journal of Nutrition, 50, 325-330.

Victor, M., Lear, A.A. (1956). Subacute combined degeneration of the spinal cord. The American Journal of Medicine, 20, 896-911.

Vilter, C.F., Vilter, R.W., Spies, T.D. (1947). The treatment of pernicious and related anemias with synthetic folic acid. I. Observations on the maintenance of a normal hematologic status and on the occurrence of combined system disease at the end of one year. The Journal of Laboratory and Clinical Medicine, 32 (3) 262-273.

Vilter, R.W., Horrigan, D., Mueller, J.F., Jarrold, T., Vilter, C.F., Hawkins, V., Seaman, A. (1950). Studies on the relationship between Vitamin B_{12}, folic acid, thymine, uracil, and methyl group donors in persons with pernicious anaemia and related megaloblastic anemias. Blood, 5, 695-717

Vilter, R.W., Will, J.J., Wright, T., Rullman, D. (1960). Interrelationships of vitamin B_{12}, folic acid and ascorbic acid in the megaloblastic anemias. American Journal of Clinical Nutrition. 12, 130-144.

Wagley, P.F. (1948) Neurologic disturbances with folic acid therapy.

Wald, N.J., Morris, J.K., Blakemore, C. (2018). Public health failure in the prevention of neural tube defects; time to abandon the tolerable upper intake level of folate. Public Health Reviews, 39:2.

Weissberg, J., McGavack, T.H., Vogel, M., Kenisberg, S. (1950). The effect of folic acid on the central nervous system of normal subjects and subjects with pernicious anaemia. Blood, 5, 148-154

Welch, A.D., Heinle, R.W., Nelson, E.M., Nelson, H.V. (1946). Infective utilization of conjugated pteroylglutamic (folic) acid in pernicious anaemia. Journal of Bilogical Chemistry, 164, 787-788. [Abstract only]

Will, J.J., Mueller, J.F., Brodine, C., Kieley, C.E., Friedman, B., Hawkins, V.R., Dutra, J., Vilter, R.W. (1959). Folic acid and vitamin B12 in pernicious anaemia. Studies on patients treated with these substances over a ten-year period. The Journal of Laboratory and Clinical Medicine, 53, 22-38.

Wyckoff, K., Ganji, V. (2007). Proportion of individuals with low serum B12 concentrations without macrocytosis is higher in the post-folic acid fortification period than in the pre-folic acid fortification period. American Journal of Clinical Nutrition, 86, 1187-92.