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

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

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 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.

2. Consequently, SACN have recommended that wheat flour should be fortified with folic acid to increase the population. This recommendation came with the proviso that fortification should not increase the number of people who were currently exceeding the Tolerable Upper Level (TUL) for folic acid, meaning that levels in supplements or other fortified products would need to be reduced.

3. The Department of Health and Social Care (DHSC) have recently announced a consultation on the fortification of flour with folic acid.

4. The Committee discussed the basis of the TUL at their meetings in July and October 2018 and agreed that the data on which the TUL is based should be reanalysed to see if any dose-response relationship could be determined. They also agreed to produce a statement which could be used to feed into the consultation being carried out by DHSC.

5. At the previous meeting, the Committee asked if it was possible to identify the dose of folic acid which would reverse the anaemia associated with vitamin B_{12} deficiency. It has not been possible to identify a figure. Current opinion is based on historical studies carried out before the roles of folate and vitamin B12 were known. Since the relationship between folic acid supplementation and masking of vitamin B12 deficiency has been identified, further studies to elucidate the relationship further would be unethical.

6. The Committee are invited to comment on the draft statement attached as Annex 1 to this paper.

Secretariat

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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 400 µg supplement of folic acid 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 (Morris et al, 2016). Consequently, 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 Tolerable Upper Level (TUL) for folic acid, meaning that levels in supplements or other fortified products would need to be reduced.

3. TULs (or equivalent) for folic acid have been established by a number of regulatory authorities, including the UK Expert group on Vitamins and Minerals (EVM), the EU Scientific Committee on Food (SCF) and the US Institute of Medicine Food and Nutrition Board (IOM). All of these bodies set a maximum recommended intake of 1 mg/day folic acid based on the observations from numerous case series and small studies that folic acid was able to mask the diagnosis of pernicious anaemia, by improving haematological status but without correcting the neurological effects associated with the condition. The delay in diagnosis could allow the adverse neurological endpoint to progress until they were potentially irreversible. The IOM further noted that some evidence suggested that folic acid could increase the progression of neurological symptoms, while the SCF noted this could not be ruled out.

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 and one of the same endpoints was used to set the TUL. The Committee was asked to consider whether the analysis by Wald and colleagues had implications for the Guidance Level (GL) set by the UK EVM and cited by SACN.

5. Following discussion of the Wald 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 see 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 form (Shane, 2008).

7. 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 dietary folate and folic acid is metabolised to 5-methyl-tetrahydrofolate during its 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 when the, 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 may occur, 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. Under 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 2 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 catalyses the methylation of homocysteine to produce methionine using 5-methyl-tetrahydrofolate as the methyl donor. Methionine is an essential amino acid because mammals are unable to synthesise homocysteine and it is not normally present in the diet. It 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 dietary odd chain 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 amino acids including isoleucine, valine, methionine and threonine. The B_{12} dependent methylmalonyl CoA mutase catalyses the conversion of L-methylmalonyl CoA 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 symptoms.

Megaloblastic anaemia

15. The classical symptom 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. Since body stores are generally ample at the onset of the disease and turnover is slow, it can take some years before deficiency symptoms become apparent.

16. Megaloblastic anaemia is also a symptom of impaired vitamin B_{12} status. Classical pernicious anaemia is caused by an inability to absorb vitamin B_{12} due to the lack of intrinsic factor production.

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 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 (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 is found worldwide and is probably underdiagnosed given that microcytic and macrocytic anaemia are treated with iron folates and cobalamin without any more thorough investigation (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 both sexes aged over 30 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 are 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 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.

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 cell 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 laboratory results include markedly elevated lactate dehydrogenase and mildly elevated bilirubin, total iron, and aspartate aminotransferase levels, both markers of intramedullary erythroblastolosis. Fasting gastrin levels are elevated in many patients, while the levels of somatostatin are depressed (Bizzaro and Antico, 2014). Indications to search for antibodies include, macrocytic anaemia not responding to oral therapy, the presences 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 iron (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, pernicious anaemia is treated with i.m. injections of hydroxycobalamin every other day for 2 weeks or until symptoms 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. Symptoms 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 replacement therapy (Toh *et al*., 1997).

How the TULs for folic acid were set

27. The masking of the diagnosis of pernicious anaemia by folic acid is well established and was used to set recommended levels of intake by several regulatory bodies. The US IOM and the EU SCF established a TUL of 1 mg/day intended to

reflect a maximum safe intake over a lifetime. The UK established a Guidance Level of 1 mg/day also reflecting a lifetimes' exposure but reflecting the limited nature of the available data. A GL was intended to represent a value which would not be expected to cause 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 phenomenon is given below along with an overview of the approaches taken by three regulatory 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 symptoms 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 of them the folic acid treatment was considered to have exacerbated the neurological symptoms. 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 effect; 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 have been established, no additional data have been identified in the recent literature.

Historical background

30. It was known that liver extract could treat pernicious anaemia, but the pure anti-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 in the treatment of pernicious anaemia and nutritional anaemia (discussed Israëls and Wilkinson, 1949). The first report on the effect of folic acid in the treatment of macrocytic anaemia was published in 1945 by Spies *et al*. (1945).

32. The results of folic acid treatment were initially striking but it 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 sign, often very acutely, after being treated with folic acid for variable periods (Israëls and Wilkinson, 1949). The occurrence and progression of Sub acute combined degeneration (SCD) in patients with pernicious anaemia under treatment with folic acid was first reported by Vilter *et al*., 1947, 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) note 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 Tolerable Upper Intake Level (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 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 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} 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.

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) 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 monkeys (Agamanolis *et al*., 1976), it was noted that the visual lesions observed in the monkeys were only rarely seen in humans.

39. The SCF established a TUL of 1 mg/day for synthetic folic acid (SCF, 2000). 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 TUL 1 mg for adults, with TULs 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.

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 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.

41. 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

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

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 risk 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 folic acid intake 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

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 UL is incorrect. 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, 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), there are only eight well-documented cases;

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 B_{12}-deficiency related neuropathy following ingestion of folic acid. Wald *et al* 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 state that the assertion that folic acid is directly neurotoxic is incorrect. Wald *et al* also criticise the IOM for including natural folate in the UL which is not correct – the IOM applied the UL 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 UL for folic acid, as is the case 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 made, namely regarding the ease of diagnosis of pernicious anaemia. Whilst improvements have been made to diagnosis methods, 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 B12 deficiency by folic acid is still a risk in pernicious anaemia patients.

Summary and discussion

48. Pernicious anaemia is an autoimmune condition which results in atrophy of the stomach 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 anaemia as well as progressive damage to the nervous system due to demyelination and progressive damage to axons. It is found 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 symptoms or neurological symptoms, some of these may be non-specific, making diagnosis difficult. The condition is diagnosed with a combination of tests which include serum vitamin B_{12} and folate levels, intrinsic factor and parietal cell antibody levels as well as standard haematology parameters such red cell and reticulocyte count and haemoglobin levels. Other tests may also be used to identify underlying causes.

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. There are also limited data which suggest that folic acid can also exacerbate the progression of the neurological symptoms.

51. This phenomenon has been used to establish recommended maximum upper intakes by a number of regulatory authorities. 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 delay diagnosis, 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 TUL for folic acid, meaning that levels in supplements or other fortified products would need to be reduced.

55. TULs of 1 mg/day have been set by a number of regulatory authorities, 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. Both the US IOM, and the EU SCF also considered that it was not possible to rule out the possibility that folic acid could exacerbate the neurotoxicity associated with pernicious anaemia.

Conclusions

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 EVM and SCF since some of the same endpoints were used to set the TUL. Wald's main criticism of the IOM relates to them using the possibility of folic acid having a direct neurotoxic effect in the establishment of the TUL.

57. The original case reports have significant limitations. The adverse effects were first reported following the first isolation of folic acid in the 1940s when patients were treated with folic acid rather that the meat or liver extract which had been used as treatment previously and some of these patients had gone on to have new neurological symptoms 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 treat B_{12} deficiency further increases did not have any additional effect.

59. There is no evidence that folic acid has a direct neurotoxic effect but it is able to mask diagnosis by treating the anaemia also associated with B_{12} deficiency.

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 B12 deficiency is the most relevant.

61. The COT does not agree with the comment in the Wald paper that diagnosis is sufficiently improved that the possibility of masking is no longer relevant. Although serum B_{12} levels can be measured, these do not indicate whether the B_{12} is functional; the test for the latter was not widely available.

62. 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 there is little else that could be done with 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.

Statement 201X/XX
XXXX 201X

Abbreviations

AIG	Autoimmune Gastritis.
ATP	Adenosine Triphosphate
CoA	Coenzyme A
CI	Confidence Intervals
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	Femtolitres
H	Hydrogen
HC	Haptocorrin
HLA-DR	Human Leukocyte Antigen-antigen D related
IDA	Iron Deficiency Anaemia
IFA	Intrinsic Factor Auto antibodies
i.m	Intra muscular
IOM	US Institute of Medicine Food and Nutrition Board (IOM).
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	The EU Scientific Committee on Food
SUL	safe Upper Level
TC	Transcobalamin
TUL	Tolerable Upper Level

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