TOX/2018/40

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

Discussion paper on the basis for the Upper Level for Folic Acid

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, 2015). Consequently, the Scientific Advisory Committee on Nutrition (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. 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 observation 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; this publication is attached as Annex 1 to this discussion paper). 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.

5. The Committee considered the paper by Wald *et al*., 2018 at their meeting in March 2018. They also discussed the Tolerable Upper Level for folic acid at their meeting in July 2018. During that discussion, members agreed that the end point used by the IOM, SCF and EVM was correct and appropriate and requested that the data be reanalysed to see if a dose response relationship could be determined

between folic acid intake and the masking of neuropathy in vitamin B12-deficient patients, and to see whether the analysis by Wald *et al.*, 2018 could be incorporated into the assessment. Members also asked for details of other end-points to see whether a TUL could be based on other endpoints.

6. This discussion paper provides information on the basis for the Upper Level (UL) or guidance level set by various risk assessment bodies and an analysis of the available data. Some of these data have been previously seen by the Committee but have been re-presented here in an expanded form. Also, in this paper are summaries of the other end points that may be considered on which to set an UL for folic acid if the committee consider the data on the effects of vitamin B12 deficient neuropathy are not considered adequate.

Upper Levels/Guidance Levels set by risk assessment bodies

The Institute of Medicine (IOM), 1998

7. The IOM set their UL on the results from 31 small studies and case reports given in Table 1 below.

Table 1: Case reports and studies 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

8. They concluded that “The weight of the limited but suggestive evidence that excessive folate intake may precipitate or exacerbate neuropathy in vitamin B12-deficient individuals justifies the selection of this endpoint as the critical endpoint for the development of a UL for folate.”

9. Using the data in Table 1 the IOM derived a LOAEL of 5 mg of folate:

• 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;

10. The LOAEL of 5 mg/day was divided by an uncertainty factor of 5 to give an UL of 1mg/day for all adults.

<u>The EC Scientific Committee on Food, 2006</u>

11. The Scientific Committee on Food (SCF) considered some of the original studies used by the IOM and some animal studies but primarily used reviews and the conclusions of the IOM to base their conclusions and set their UL.

12. 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 pointing 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.

13. The SCF established a TUL of 1 mg/day for synthetic folic acid. 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.

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

14. 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 (see above). 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.

15. A full, detailed review of folic acid was conducted (EVM, 2002) considering the case reports, amongst other available data. Most, but not all, overlap with those studies considered by the IOM, 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.

16. 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

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

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".

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

17. 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 outlined in paragraph 9:

• 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;

18. Wald *et al* went back to the original studies and from the "well-documented" cases calculated that 12 patients took less than 5mg/day folic acid and 8 developed neuropathy (67%; 95% CI 35-90%), whereas 279 patients took more than 5mg/day folic acid and 147 developed neuropathy (53%; 95% CI 47-59%). A further analysis using 3 dose categories (<5, 5-9.9, >10) 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 B12-deficiency related neuropathy following ingestion of folic acid. Wald *et al* argue that detection methods for vitamin B12 status are sufficiently improved and the advent of vitamin B12 therapy mean that there is now no risk of "masking" the effects of vitamin B12 deficiency with folic acid. Wald *et al* state that the assertion that folic acid is directly neurotoxic is incorrect. The views of other risk assessment bodies on the potential neurotoxicity of folic acid are given in paragraphs 24-35. 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.

19. 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).

Previous Committee discussion on the Wald paper

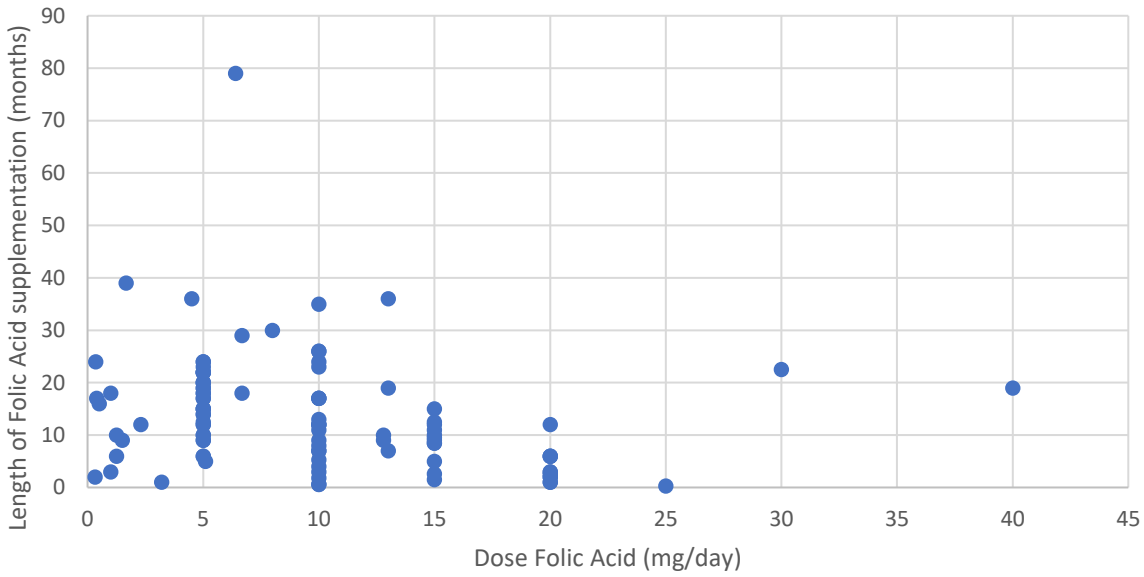
20. During their previous considerations, the Committee were provided with extensive background data on pernicious anaemia (TOX-2018-26). During their discussions, the Committee stated that, in the case of pernicious anaemia in particular, onset is slow, symptoms are non-specific and there is no routine test to reliably identify vitamin B12 deficiency in this population sub-set. Diagnosis can take 5 years or more. Therefore, the assertion by Wald et al, that there is no risk of masking the effects of vitamin B12 deficiency, does not apply in this group.

21. The Committee requested that the original data be reanalysed to see if any dose-response relationship could be determined. Some of the data from the previous discussion paper (TOX-2018-26) have been reproduced and /or expanded upon in this paper in order to provide sufficient detail for members to consider the data used to set the UL.

Additional analysis of the raw data used by the IOM and other bodies.

22. Data from the original studies used by the various risk assessment bodies, have been used to create Figure 1 below. Individual patients have been recorded where the dose and duration of folic acid consumption were given and there was evidence of neurological deterioration during the dosing period. Doses of folic acid often differed over time, so in some cases it has been necessary to use the minimum dose the patient received over the treatment period. The assessment of these studies is complicated by the limited reporting so that a total number of cases may be reported but with details provided for only some of them.

Figure 1: Studies and case reports showing onset or progression of neurological complications in Vitamin B12-deficient individuals receiving oral folic acid.



<u>Other end points that could be considered</u>.

23. Members requested details of other end-points that may be of interest in setting an upper level. Due to limited time, it has not been possible to undertake literature searches for all of these end points, but members are asked to consider if they would like more details on any of the endpoints outlined below.

24. Below are sections from a number of reviews looking at other biological end-points that could be considered if the Committee consider that the data on the "masking" or exacerbation of neuropathy in vitamin B12-deficient individuals is not adequate to set a guidance level or Safe Upper Level.

Direct neurotoxicity of folic acid

25. SACN have stated the following:

26. "Clinical symptoms associated with vitamin B12 deficiency include anaemia and neurological impairment. Treatment with high doses of folic acid can alleviate the anaemia ('masking' the vitamin B12 deficiency) which could delay diagnosis of vitamin B12 deficiency and may lead to irreversible neurological damage. It has also been suggested that folic acid might directly exacerbate neurological degeneration associated with marginal vitamin B12 status.

27. In 2006, SACN concluded that folic acid intakes up to 1 mg/d were not associated with neurological impairment in older people with low vitamin B12 status. Systematic reviews and meta-analyses published since then, of intervention studies of folic acid supplementation and observational studies, indicate either no relationship with cognitive decline or a lower risk associated with higher folate status.

28. No systematic reviews have evaluated the risk of folic acid masking or exacerbating vitamin B12 deficiency in adults. Evidence for this outcome is mainly from folic acid interventions over 1 mg/d. The prevalence of vitamin B12 deficiency with or without anaemia did not increase after mandatory fortification in the USA " (SACN, 2017).

29. The EVM stated the following:

30. "A number of animal studies have shown that folic acid is neurotoxic, but there are few data in humans. Supplementation studies in non-pernicious anaemia subjects have not shown evidence of neurotoxicity.

31. Weissberg et al (1950) reported an uncontrolled study of the neurological effects of 20 mg/day folic acid supplementation, for 6-12 months, in 26 normal volunteers and 22 (non-pernicious) anaemia patients. Prior to therapy, 6 of the normal subjects and 7 of the anaemic subjects showed some abnormal neurological signs (but not those of subacute combined spinal cord degeneration), which were not significantly altered during the therapy. Four subjects (1 normal, 3 anaemic) developed central nervous system (CNS) changes during the folic acid treatment, but these changes were not considered to be related to the therapy. Harvey et al. (1950) reported that oral folic acid supplementation (20 mg/day for 3-12 months) produced no indications of spinal cord or peripheral nerve damage in 40 healthy subjects without pernicious anaemia (13 subjects had mild hypochromic anaemia). Folic acid supplementation was not associated with signs or symptoms of neurotoxicity in a study of 18 patients with Parkinson disease who were treated with 15 mg/day folic acid therapy for periods of 14-182 days (McGeer et al 1972)" (EVM, 2006).

32. The SCF stated the following in 2000:

33. "Animal studies have shown that folic acid can be a neurotoxin, and can cause convulsions in laboratory animals (e.g. Hommes and Obbens, 1972; Spector, 1972). This evidence is in part based upon in vitro tissue and cell culture studies, and/or using very high dose levels (i.v. dosages 60-90 mg). There is however no clear evidence for a folic acid-induced neurotoxicity in humans. Some cases of neurological deterioration have been reported following ingestion of folic acid tablets (3 mg), or folic acid containing multivitamin supplements, but the presence of an (undiagnosed) vitamin B12 deficiency cannot be ruled out in these cases (see Dickinson, 1995). In one study with epileptic patients electroencelographic changes were noted after administration of 7.2 mg of folic acid, and seizures after 14.4 and 19.2 mg. However, in other studies no such changes or effects were observed after i.v. dosage of 75 mg (see Campbell, 1996). These studies are therefore inconclusive.

34. Hunter et al. (1970) reported disturbing toxic effects, i.e. sleep disturbances and mental changes, after treatment of healthy volunteers with 15 mg folic acid for 1 month. This study was however not placebo controlled, and the results were not confirmed in another double blind, randomised study (Hellstrom et al., 1971).

35. Concern has been expressed that folic acid might exacerbate seizures in persons with uncontrolled, or drug controlled epilepsy. However, no such effects were found in several controlled studies with dosages between 15-20 mg/day. Supplementation studies (15 mg/day for 45 days) with Parkinson disease patients did not show an effect on the incidence of neurological defects. Also after i.v. dosing with dosages up to 150 mg no adverse effects have been reported (for review see Butterworth & Tamura, 1989; Campbell, 1996). As already mentioned, some anticonvulsant drugs may reduce serum folate levels. However, as far as data are available, there seems apparently no increased risk for patients with epilepsy, or interference with anticonvulsant medication, at higher folate intakes" (SCF, 2000).

36. The IOM did not describe their considerations in detail but stated that there was no clear evidence to show that folic acid was neurotoxic in humans and stated:

37. *"The weight of the limited but suggestive evidence that excessive folate intake may precipitate or exacerbate neuropathy in vitamin B12-deficient individuals justifies the selection of this endpoint as the critical endpoint for the development of a UL for folate."*

Carcinogenicity

38. In 2009, a SACN subgroup with two invited members of the Committee on Carcinogenicity concluded that there were uncertainties regarding folic acid and cancer risk (SACN, 2017).:

39. "Prostate cancer risk: Findings from the different types of study are inconsistent and overall the evidence is inconclusive. Meta-analyses of RCTs of folic acid supplementation show no effect of folic acid on prostate cancer risk. Observational studies of dietary or total folate intake do not suggest an association. Observational studies of serum or plasma folate concentration suggest higher prostate cancer risk in men with relatively high serum or plasma folate concentration. The MTHFR genetic studies also suggest that higher blood folate concentrations are associated with an increased risk of prostate cancer.

40. Breast cancer risk: Findings from the different types of study are inconsistent but overall do not suggest an adverse association. Meta-analyses of RCTs report no effect of folic acid supplementation on breast cancer risk. Observational studies of dietary and total folate intake do not show an association with breast cancer risk. Observational studies of serum or plasma folate concentration also report no association. The MTHFR genetic studies suggest higher blood folate concentrations are associated with lower breast cancer risk.

41. Colorectal cancer risk: Findings from the different types of study are inconsistent and overall the evidence is inconclusive. Meta-analyses of RCTs report no effect of folic acid supplementation on colorectal cancer risk. Meta-analyses of observational studies are heterogeneous but suggest a protective association of folate intakes above about 400 µg/d. Observational studies of serum or plasma folate concentration provide no clear evidence of an association with colorectal cancer risk. The MTHFR genetic studies suggest that higher blood folate concentrations are associated with an increased colorectal cancer risk.

42. Overall cancer risk: Findings from the different study types are inconsistent but overall do not suggest an adverse association. RCTs show no effect of folic acid supplementation on overall cancer risk. The MTHFR genetic studies suggest higher folate concentrations reduce overall cancer risk.

43. *It is difficult to draw firm conclusions on folate and cancer risk on the basis of evidence from RCTs and observational data. The data are heterogeneous and, overall, do not suggest a detrimental effect of folic acid/folate on cancer risk. However statistical power is generally low, particularly for the RCTs. There are also uncertainties about the inference of nutritional effects from genetic evidence."*
(SACN, 2017)

44. The EVM has stated the following:

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

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

47. The UK Committee on Medical Aspects of Food and Nutrition Policy (COMA), however, recently concluded that there was insufficient evidence for linking folate intake to cancer prevention (Department of Health 1998)" (EVM, 2002).

48. The SCF stated the following:

49. "Folic acid has been associated with an increased incidence of oropharynx, hypopharynx and all cancers (Selby et al., 1989), but, as indicated by the authors of this epidemiological study, these cancers are largely smoking- and/or alcohol-related and this finding likely related to these confounding factors. In other (observational) studies an inverse relation was found between folate intake and/or status and colorectal cancer (e.g. Giovanucci et al., 1993; White et al., 1997), and with cervical cancer (Butterworth, 1993). Treatment of smokers with 10 mg folic acid plus 500 µg hydroxocobalamin for 4 months resulted in a reduction in atypical bronchial squamous metaplasia (Heimburger et al., 1988)" (SCF, 2000).

50. The IOM stated the following:

51. "In a large epidemiological study, positive associations were found between supplemental folate intake and the incidence of cancer of the oropharynx and hypopharynx and of total cancer (Selby et al., 1989). However, the authors of this study suggest that these associations might have been related to unmeasured confounding variables such as alcohol and smoking. Additionally, other studies suggest that folate might be anticarcinogenic (Campbell, 1996).

52. Experimental data indicate that changes in folate status may influence the process of neoplastic changes in certain epithelial tissue: a negative change in folate status may stimulate carcinogenesis. It is unclear if supraphysiological doses obtained from supplements afford any protection.

Dysplasia and Metaplasia

53. Dysplastic and metaplastic changes have been reported to reverse in response to high-dose supplemental folate. Butterworth and coworkers (1982) conducted a prospective, controlled clinical intervention trial giving supplements of 10 mg/day of folate to 47 women with dysplastic changes in the epithelium of the uterine cervix. They observed a significant attenuation of dysplasia, but the alteration in cytology may have been an attenuation of dysplasia or simply a reduction in megaloblastic cellular changes. A subsequent intervention trial by the same research group (Butterworth et al., 1992b) was unable to reproduce the results. However, the subjects in this second intervention study initially had the lowest grade of dysplasia, which has a greater than 60 percent spontaneous rate of reversion to normal. Heimburger and colleagues (1988) observed a significant reduction in metaplastic change in bronchial epithelial tissue in response to 10 mg of folate with 500 µg of

vitamin B12 given daily for 4 months to 36 subjects compared with changes in 37 subjects given a placebo. These findings may be questioned because of spontaneous variation in bronchial cytology, small sample size, short duration of trial, and the very high doses of folate and vitamin B12 used.

54. It has been hypothesized that poor folate status by itself is not carcinogenic but may enhance an underlying predisposition to cancer (Heimburger et al., 1987; Mason and Levesque, 1996). Support for this theory includes data from a case-control intervention trial of patients with cervical dysplasia who also were at significantly higher risk for cervical cancer because of cervical infection with human papilloma virus-16 (HPV-16) (Butterworth et al., 1992a). Subjects with the HPV-16 infection had a fivefold greater risk of having dysplasia if they also had diminished erythrocyte folate values (660 nmol/L [303 ng/mL]) (Butterworth et al., 1992a). On the basis of these data and other data from study of the colorectum (Lashner, 1993), Mason and Levesque (1996) suggested that even a minor decrease in folate status may promote carcinogenesis. Potential mechanisms for folate-related enhancement of carcinogenesis include the induction of DNA hypomethylation (Kim et al., 1997), increased chromosomal fragility or diminished DNA repair (Kim et al., 1997), secondary choline deficiency, diminution in natural killer cell surveillance, misincorporation of uridylate for thymidylate in DNA synthesis, and facilitation of tumorigenic virus metabolism (Mason and Levesque, 1996).

Cervical Neoplasia

55. Although several studies suggest that increased consumption of folate reduces the relative risk of cervical neoplasia (Brock et al., 1988; Potischman et al., 1991; Verreault et al., 1989; Ziegler et al., 1990, 1991), statistical significance was not attained in these studies after adjustments were made for confounding variables. These studies had several limitations: folate intake was assessed with a food frequency instrument that had not been validated for folate intake (Mason and Levesque, 1996); because subjects were not stratified for HPV infections as was done by Butterworth and colleagues (1992a), the inverse association between folate intake and cervical neoplasia in high-risk subjects was not examined; and the subjects had either carcinoma in situ or invasive cancer—advanced stages of neoplasia that may be unresponsive to folate (Heimburger et al., 1987; Mason and Levesque, 1996). Therefore, the effect of folate status on carcinogenesis in the cervix remains uncertain.

Colorectal Cancer

56. Data supporting the modulation of carcinogenesis by folate status are the strongest for the colorectum. Patients with chronic ulcerative colitis are at increased risk for colonic cancer and also coexisting folate deficiency. Sulfasalazine, a drug taken chronically by these patients, inhibits folate absorption (Halsted et al., 1981) and metabolism (Selhub et al., 1978). Lashner and coworkers (1989) observed that the rate of colonic neoplasia was 62 percent lower in folate supplemented patients with chronic ulcerative colitis than in unsupplemented patients and that sulfasalazine therapy was associated with an increase in the risk of dysplasia. These observations were not statistically significant but pointed to an important area of investigation. Lashner (1993) subsequently compared prospectively the erythrocyte folate

concentrations in patients with neoplastic changes in the colorectum with those for disease-matched control patients without neoplasia. The mean erythrocyte concentration was significantly lower in the individuals with neoplasia (988 nmol/L [454 ng/mL]) than in the control patients (1,132 nmol/L [520 ng/ mL]) but was still well within the normal range, which is in line with observations of erythrocyte folate concentrations and dysplasia in the uterine cervix (Butterworth et al., 1992a). Meenan and colleagues (1996) described the lack of association between erythrocyte folate levels and colonic biopsy specimens in healthy individuals, indicating the potential difficulty in predicting localized folate deficiency. In a subsequent report (Meenan et al., 1997), epithelial cell folate depletion occurred in neoplastic but not adjacent normal colonic mucosa.

57. In general, epidemiological studies support an inverse relationship between folate status and the rate of colorectal neoplasia (Mason and Levesque, 1996). Two large, well-controlled prospective studies support the inverse association between folate intake and incidence of colorectal adenomatous polyps (Giovannucci et al., 1993) and colorectal cancers (Giovannucci et al., 1995). In these two studies, moderate-to-high alcohol intake greatly increased the neoplastic risk of a low-folate diet. There was a significant 35 percent lower risk of adenoma in those in the highest quintile of folate intake (approximately 800 µg/day) relative to those in the lowest quintile (approximately 200 µg/day, relative risk approximately 0.65). The adverse effect of high alcohol intake coupled with a low folate diet was confirmed by Glynn and colleagues (1996), who observed a significant fourfold increase in risk of colorectal cancer.

58. Physicians' Health Study participants with the MTHFR polymorphism had reduced risk of colon cancer, but low folate intake or high alcohol consumption appeared to negate some of the protective effect (Ma et al., 1997) (see Appendix L for further discussion of MTHFR polymorphism).

59. More evidence for or against a causal relationship between folate status and colorectal cancer will be provided by data from prospective controlled intervention trials that are currently under way.

Lung, Esophageal, and Stomach Cancer

60. *As reviewed by Mason and Levesque (1996), data are not sufficient for making conclusions regarding the possible role of folate in reducing the risk of cancer of the lung, esophagus, or stomach."* (IOM 1998).

Unmetabolised folic acid

61. *"In 2006, SACN concluded that there were insufficient data to assess the long-term effects of exposure to unmetabolised folic acid (UMFA) in the systemic circulation. Evidence published since then shows no clear dose-response relationship between folic acid intake and the appearance of UMFA in the systemic circulation. UMFA comprises 1-3% of total folate regardless of age, level of intake, and whether blood is sampled in the fasted or non-fasted state. These observations question the interpretation that levels of UMFA are indicative of intakes above the short-term capacity of the body to metabolise folic acid"* (SACN, 2017).

62. The EVM did not consider unmetabolised folic acid in the blood stream.

63. The SCF did not consider the direct effects of unmetabolised folic acid in the blood stream in detail but did note that it's presence was an additional uncertainty in the risk assessment.

64. The IOM did not consider unmetabolised folic acid in the blood stream.

65. Recent literature has noted an association between unmetabolized folic acid and a decrease in the cytotoxicity of Natural Killer Cells in two human studies (Paniz et al, 2017; Troen et al, 2006) and one animal study (Sawaengsri et al, 2016), but limited information is available on other effects.

Potential effects on zinc status and absorption:

66. The SACN did not consider this end point.

67. The EVM stated the following:

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

69. Folic acid supplementation has been reported to have a negative effect on zinc status, although many studies have not observed this effect. Milne et al (1984) noted increased faecal zinc loss in 4 men given supplemental folic acid (0.4 mg every other day for 6 months) compared with 4 men not given supplements, but reported that reduced urinary losses maintained overall zinc balance. In an uncontrolled study of healthy women, 0.35 mg/day oral "folate" (presumably folic acid) supplementation for 2 weeks, either with (10 pregnant women) or without (10 non-pregnant volunteers) concurrent iron supplementation, significantly reduced the subsequent bioavailability of a single dose of 200 mg ZnSO4, but did not affect fasting serum zinc levels (Simmer et al 1987). A negative correlation between serum folate and zinc levels was reported in a retrospective study of 60 preterm infants supplemented with mg/day folic acid for various periods during the first 16 weeks of life (Fuller et al 1992). Some authors have suggested that correlations between low plasma zinc concentrations, high plasma folate concentrations and pregnancy complications or foetal distress may be due to impairment of zinc absorption by folic acid supplementation during pregnancy (Mukherjee et al 1984).

70. *Other studies have shown no adverse effects of folic acid supplementation, at doses up to 10 mg/day for several weeks or months, on serum or red cell zinc status in adults (Butterworth et al 1988, Tamura et al 1992, Hambidge et al 1993, Kauwell et al 1995) (data from these studies are summarised in Table 5). Keating et al (1987) reported that ingestion, in water, of 25 mg zinc (as ZnSO4) with or without 10 mg folic acid, produced similar changes in serum zinc concentrations in 6 men over a 4 hour period, with peak levels 2 hours post-ingestion, whilst Arnaud et al (1992) found*

no effect of 200 mg folic acid on serum or urinary zinc levels in response to a concurrent dose of zinc gluconate (30 mg elemental zinc) in 10 subjects. It has been suggested that, as zinc levels naturally decline during pregnancy, the association of folic acid supplementation with reduced zinc levels in the later stages of pregnancy is not necessarily causal (Tamura & Goldenberg 1996)" (EVM, 2002).

71. The SCF stated the following on this endpoint:

72. "Dietary zinc deficiency and a relative shortage of maternal zinc has been associated with NTD in human (Milunsky et al., 1992). It has been suggested (Quinn et al., 1990) that in the presence of a zinc deficiency the administration of high-dose folate increases the teratogenicity of such a deficiency. The enzyme gamma-glutamyl hydrolase is zinc-dependent and converts polyglutamates to monoglutamates, which is an important step in the absorption of folate. Therefore, the availability of folate is dependent on the glutamyl hydrolase activity, which is regulated by the concentration of zinc (Canton et al., 1990).

73. *Some earlier studies indicated competitive interactions between folic acid and zinc, however, results are conflicting. In reviews on this item from Butterworth and Tamura (1989) and from Zimmerman and Shane (1993) it is concluded that there is as yet no convincing evidence for negative effects of folate supplements on serum or red cell zinc contents (in a study in which women were dosed with 10 mg/day for 6 months), nor for negative effects of folic acid supplementation on zinc status in pregnant women. Contradictory results most likely result from methodological problems in assessment of zinc status/bioavailability"* (SCF, 2000).

74. The IOM stated the following:

75. "*Intestinal Zinc Absorption. Although there has been some controversy regarding whether supplemental folate intake adversely affects intestinal zinc absorption (Butterworth and Tamura, 1989), a comprehensive review of the literature reveals that folate supplementation has either no effect on zinc nutriture or an extremely subtle one (Arnaud et al., 1992; Butterworth et al., 1988; Hambidge et al., 1993; Keating et al., 1987; Milne et al., 1984; Tamura, 1995; Tamura et al., 1992). In a study of prenatal folate supplementation, Mukherjee et al. (1984) noted a significant association between the occurrence of fetomaternal complications and the combination of low maternal plasma zinc and high maternal plasma folate concentrations. However, this study may have failed to control for potential confounding factors. Furthermore, these findings are not supported by Tamura and colleagues (1992), who found high serum folate concentrations to be associated with favorable pregnancy outcomes including higher birth weight and Apgar scores of newborns, reduced prevalence of fetal growth retardation, and lower incidence of maternal infection close to the time of delivery"* (IOM 1998).

Decreased efficacy of folate antagonists:

76. The SACN did not consider this end point.

77. The EVM stated the following:

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

79. Limited data are available regarding the effects of folic acid supplementation on the efficacy of anti-folate chemotherapy in cancer patients. One (retrospective) study in children receiving high-dose MTX therapy for acute lymphoblastic leukaemia showed an association of folic acid-containing multivitamin use with a reduction in MTX toxicity, but did not address the issue of potentially reduced effectiveness of the chemotherapy in those children taking supplements (Schroder et al 1986). A phase I clinical study of the anti-folate agent, lometrexol (which selectively inhibits GARFT –a folate-dependent enzyme involved in purine synthesis) showed that treatment of patients with folic acid (5 mg/day for 7 days prior to, and 7 days following, lometrexol treatment, at 4 week intervals) increased the maximum tolerated dose of lometrexol 10-fold. The authors reported that the mechanism responsible for this reduction in lomotrexol toxicity had not been defined, but thatpharmacokinetic studies carried out on the treated patients suggested that folic acid did not enhance lomotrexol plasma clearance (Wedge et al 1995, Laohavinij et al 1996)" (EVM 2002).

80. The SCF stated the following on this end point:

81. *"Folate antagonists such as methotrexate are used in the treatment of various cancers, e.g. leukemia, and also in rheumatoid arthritis, bronchial asthma and psoriasis. There are also a number of other drugs that interfere with folate metabolism, such as pyrimethamine, phenytoin, colchicine, etc. The FDA discussed*

the issue of potential effects of increased folate intake on the efficacy of antifolate therapy and concluded that there are relatively little data (Food Labelling: Health Claims and Label Statements; Folate and Neural Tube Defects, 1993). The American College of Rheumatology has stated that a dose of 1 mg/day of folic acid does not appear to inhibit the efficacy of low-dose methotrexate therapy in rheumatoid arthritis. High dose folic acid is also used to reduce methotrexate toxicity (see Campbell, 1996). So, there is currently little scientific data on potential adverse effects of high folate intakes on antifolate medication" (SCF, 2000).

82. The IOM stated the following:

83. "Methotrexate is a folate antagonist that has been used frequently and successfully in the treatment of nonneoplastic diseases such as rheumatoid arthritis, psoriasis, asthma, primary biliary cirrhosis, and inflammatory bowel disease (Morgan and Baggott, 1995). Methotrexate has been especially effective in the treatment of rheumatoid arthritis (Felson et al., 1990), with efficacy established in numerous trials (Morgan et al., 1994). Patients with rheumatoid arthritis are frequently reported to be folate deficient, and folate stores are decreased in patients with rheumatoid arthritis who take methotrexate (Morgan et al., 1987, 1994; Omer and Mowat, 1968). Some of the side effects of methotrexate administration, such as gastrointestinal intolerance, mimic severe folate deficiency (Jackson, 1984). When patients are also given high-folate diets or supplemental folate, there is a significant reduction in toxic side effects with no reduction in drug efficacy. It has been recommended that patients undergoing chronic methotrexate therapy for rheumatoid arthritis increase folate consumption (Morgan et al., 1994) or consider folate supplements (1 mg/day) (Morgan et al., 1997)" (IOM, 1998).

Hypersensitivity to folate:

84. The SACN did not consider this end point.

85. The EVM stated the following:

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

Symptoms disappeared on discontinuation of the therapy, but returned when supplementation was given again 3 months later, with a subsequent positive reaction to intradermal folic acid challenge. Woodliff & Davis (1966) described allergic reactions to i.v. folic acid in 2 patients.

87. *It has, however, been noted that in some cases hypersensitivity reactions attributed to folic acid may have been caused by other components of the therapy (e.g. tartrazine) (Gotz & Lauper 1980, Butterworth & Tamura 1989)"* (EVM, 2000).

88. The SCF stated the following:

89. *"A limited number of case reports have been published on hypersensitivity reactions to oral and parenteral folic acid, but it cannot be excluded that these reactions were due to other components in the formulations. So, hypersensitivity may occur, but is most likely very rare (see Campbell, 1996)"* (SCF, 2000).

90. The IOM stated the following:

91. *"Hypersensitivity. Individual cases of hypersensitivity reactions to oral and parenteral folate administration were reported (Gotz and Lauper, 1980; Mathur, 1966; Mitchell et al., 1949; Sesin and Kirschenbaum, 1979; Sparling and Abela, 1985). Such hypersensitivity is rare, but reactions have occurred at supplemental folate doses as low as 1 mg/day (Sesin and Kirschenbaum, 1979)"* (IOM, 1998).

Questions for the Committee

I) Are the Committee satisfied that neuropathy in Vitamin B12-deficient patients is the most relevant end-point on which to set the Upper Level or Guidance Level?

II) Do Members have any comments on the way the current UL has been set by the IOM or the guidance level by EVM?

III) Do Members agree with the criticisms by Wald *et al* of the IOM upper level, and are they relevant to the guidance level set by the EVM?

IV) Is there a dose-response relationship between folic acid and neuropathy in vitamin B12-deficient individuals?

V) Can Members suggest any additional ways in which the available data could be treated to help identify a dose-response relationship.

VI) If not, do members wish to recommend another end point on which to set an UL or which should be investigated further?

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TOX/2018/40 Annex 1

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

Discussion paper on the basis for the Upper Level for Folic Acid

In this annex is a copy of the paper by Wald et al, Public Health Reviews, 39: 2
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https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5809909/pdf/40985_2018_Article_79.pdf