TOX/2018/26

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

FOLIC ACID – ESTABLISHMENT OF THE ORIGINAL TOLERABLE UPPER LEVEL (TUL) BY IOM, EVM and SCF

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). 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 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.

6. This discussion paper provides information on the case studies as well as background information on pernicious anaemia including its symptoms and prevalence and on the linked metabolism of folic acid and vitamin B_{12}.

7. The Secretariat have been in contact with the Pernicious Anaemia Society and they have kindly provided some information on pernicious anaemia itself as well as their thought on the topic of folic acid fortification. This is attached at Annex A

Background

8. 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).

9. 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.

10. 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).

11. Although high plasma folate levels can be achieved, these decrease rapidly as the renal threshold is exceeded and much of 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.

12. 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 methionine from homocysteine, and; the interconversion of serine and glycine (Shane, 2008).

How vitamin B_{12} and folate are linked.

13. 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 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.

14. 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

15. 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.

16. 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; it can enter the citric acid cycle, and be involved 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

17. 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.

18. 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.

19. 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.

20. 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- see also Annex A

21. 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).

22. The autoimmune nature of the process that brings on gastric atrophy is demonstrated by the presence of autoantibodies secreted against intrinsic factor and by the frequent coexistence in the patients of other autoimmune disorders. Autoimmune gastritis[1] is a risk factor for stomach cancer (de Leon *et al*., 2012).

23. Patients with pernicious anaemia have two types of antibodies. One of these is to parietal cells, recognising the gastric enzyme H^+/K^+- ATPase (which secrets H^+ ions in exchange for K^+ ions), the antibodies are present at high levels initially but decline due to the progression of autoimmune gastritis and the loss of parietal cell mass. The other antibody is to intrinsic factor or its binding site in the small intestine, these are considered to be specific markers for diagnosing pernicious anaemia. The antibodies interfere with the absorption of the vitamin B_{12} intrinsic factor complex in the terminal ileum. Antibodies to the binding site (type I) occur in 70% of patients,

[1] Autoimmune gastritis is also known as atrophic body gastritis or type 2 gastritis.

while antibodies to a remote site away from the binding site but which impedes binding of intrinsic factor to the intestinal mucosae occur in 35-40% of patients but rarely occur alone (Toh *et al*., 1997; Bizzaro and Antico, 2014). Positivity to intrinsic factor antibodies has been reported in the serum of 40-60% of patients increasing to 60-80% with increasing duration of disease. In the gastric juices, the antibodies are present in 80% of patients, secreted by plasma cells that infiltrate the mucosae. These antibodies bind to residual intrinsic factor and impede the absorption of ingested B_{12}. Achlorhydria favours the formation of the antibody-antigen complex. The presence of the antibodies is a characteristic of autoimmune gastritis (AIG). This leads to atrophy of the fundus and the body of the stomach but not the antrum. In patients with AIG, parietal cells are lost from the gastric mucosae and a chronic humoral and cellular infiltrate appears. The detection of the parietal cell antibodies identifies individuals with asymptomatic AIG, the most common form of the disease before mucosal atrophy leads to iron malabsorption, ferropenic anaemia, impaired vitamin B_{12} absorption and pernicious anaemia (de Leon *et al*., 2012).

24. It is possible that *Helicobacter pylori* infection may be involved in pernicious anaemia, in whom the active infection has been gradually replaced by an autoimmune disease and a burnt-out infection and the irreversible destruction of the gastric body mucosae (Lahner and Annibale, 2009).

25. The heritability of pernicious anaemia is unknown, but familial clustering has been recognised. In a study of 142, patients, 19% had a relative who also had the condition. In relatives of pernicious anaemia patients, the levels of parietal cell and intrinsic factor antibodies was also increased, in a few families a pattern consistent with dominant Mendelian inheritance has also been demonstrated (discussed Banka *et al*., 2011).

26. A genetic susceptibility for pernicious anaemia is suggested by a specific HLA-DR (Human Leukocyte Antigen-antigen D related)[2] pattern. Blocking experiments with anti-DR and anti-DQ[3] antibodies indicate that the DR antigen represents the HLA restriction element in atrophic body gastritis (discussed Bizzaro and Antico, 2014). The genotypes HLA-DRB*03 and HLA-DRB1*04 which are known to be associated with other autoimmune conditions such as type 1 diabetes and autoimmune thyroid disease were significantly associated with pernicious suggesting that autoimmunity may play a role (Lahner and Annibale, 2009).

Signs and symptoms

27. Pernicious anaemia is progressive over a span of years from a mild chronic inflammation of the stomach body to an advanced state associated with a lack of vitamin B_{12} (Bizzaro and Antico, 2014). The symptomology is dominated by a profound megaloblastic type anaemia, and in the most serious cases by neurological alterations which can precede the diagnosis of gastric atrophy by several decades.

[2] HLA-DR is a MHC class II cell surface receptor encoded by the human leukocyte antigen complex on chromosome 6 and its ligand, a 9 amino acid or longer peptide constitutes a ligand for the T cell receptor.

[3] DQ is a cell surface receptor protein with close genetic linkage to HLA-DR.

Patients tend to present with symptoms related to anaemia and less frequently with neurological symptoms (Lahner and Annibale, 2009).

28. Patients usually exhibit symptoms of anaemia with pallor, fatigue, light-headedness or tachycardia; onset may be sufficiently insidious that anaemia is not initially suspected. Inhibition of DNA synthesis due to vitamin B_{12} deficiency causes megaloblastic changes in bone marrow and other rapidly dividing cells such as the gastrointestinal epithelium. Involvement of the small bowel epithelium may result in malabsorption and diarrhoea with weight loss: anorexia is also commonly found. Glossitis is a frequent sign of megaloblastic anaemia with the patient displaying a painful, smooth, red tongue. The elevation in bilirubin levels caused by ineffective erythropoiesis manifests as jaundice.

29. Iron deficiency is a known feature of pernicious anaemia as the reduction in the production of gastric acid prevent the reduction and absorption of iron from food (Hershko *et al*., 2006).

30. Neurologic abnormalities are seen in pernicious anaemia due to B_{12} deficiency. This starts with myelin loss, but can progress to axonal death and neuronal death if left untreated. Peripheral numbness and paraesthesia (a burning or tickling sensation) are the initial symptoms, with subsequent development of weakness and ataxia. The appearance of motor symptoms is indicative of Subacute Combined Degeneration (SCD)[4] involving the dorsal and lateral spinal columns. Mental disturbance may also be present ranging from forgetfulness to psychosis/

Subacute combined degeneration (SCD)

31. Victor and Leaf (1956) note that symptoms of nervous system disease are present in 75-89% of patients with pernicious anaemia, with a generally uniform mode of onset and progression. Initially, the patients notice general weakness and paraesthesias, followed by a variety of other sensations including numbness, stiffness, feelings of hot or cold and shooting pains. The paraesthesias progress steadily and are usually present in the distal parts of all four limbs. Less frequently, cramping, bladder and bowel problems and disturbances of vision, hearing and taste may occur. As the condition progresses, stiffness of the limbs may occur affecting gait. If untreated, the symptoms may progress to an ataxic paraplegia. Neurological examination may indicate disturbance of all parts of the nervous system but mainly of the posterior and lateral columns of the spinal cord. Loss of vibration sense is the most frequent sign, being more pronounced in the legs compared to the arms, position and cutaneous sense may also be affected. Examination of the motor system indicates loss of power, spasticity, changes in the tendon reflexes and extensor plantar responses, usually limited to the legs. Psychological signs may also occur, ranging from irritability, apathy, somnolence, suspiciousness and emotional instability to marked confusion or depressive psychosis.

32. The pathological lesions observed are a diffuse but uneven degeneration of the white matter, with multiple foci of spongy degeneration often in relationship to small blood vessels; the myelin sheaths and axis cylinders are both affected. There

[4] Also known as combined system disease.

is relatively little fibrous gliosis[5]. The lesions appear to spread from the posterior column of the spinal cord and are scattered through the lateral funiculi. In advanced cases, comparable lesions may be found in the brain.

33. The mechanism underlying the demyelination disease that occurs in some B_{12} deficient individuals is uncertain (Shane, 2008) and the available animal models are limited.

34. It has been suggested that reductions in methylmalonyl CoA mutase activity could be involved. Vitamin B_{12} deficiency causes accumulation of methylmalonyl CoA in the mitochondria, an elevation in circulating methyl malonate, acidosis, and elevated methylmalonate excretion. The accumulation of methylmalonyl CoA depletes the Co A pool available for other mitochondrial enzymes and metabolites, and the increased propionyl CoA can be incorporated into long chain fatty acids in place of acetyl CoA. A role for the accumulation of unusual fatty acids in myelin as an explanation for the demyelination has been proposed. However, the evidence (Shane, 2008 citing Metz, 1992) is unconvincing. Since, in particular, individuals with severe genetic impairment of the *mut* locus which encodes the mutase enzyme suffer from a variety of severe clinical conditions and metabolic abnormalities, but do not develop SCD (Shane, 2008).

35. Monkeys treated with nitrous oxide, which inactivates methionine synthase, develop neurological disease similar to that seen in humans, symptom development is retarded by methionine supplementation suggesting that the neurological disease may result from a defect in methionine synthesis (Agamanolis *et al*, 1976- see Annex B for further details). In the few known cases of genetic defects in methionine synthase or in the enzymes responsible for methyl cobalamin synthesis, the patients develop the expected megaloblastic anaemia but some also exhibit demyelination. Similarly, some patients with defects in methylenetetrahydrofolate reductase exhibit the same neurological symptoms as are observed in vitamin B_{12} deficiency but do not have megaloblastic anaemia. Studies with gastrectomized rats (a model for extreme vitamin B_{12} malabsorption, result in rodents with demyelination and other symptoms of SCD (Buccellato *et al*., 1999; Scalabrino *et al*., 2000)

36. This suggests that the demyelination is most likely to be related to defective methionine synthesis (Shane, 2008).

Prevalence

37. 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.

38. Pernicious anaemia usually occurs in individuals of both sexes ages over 30 and is particularly frequent in Northern Europeans, especially Scandinavians (Bizzaro and Antico, 2014); it is present in other populations but is relatively

[5] Gliosis is a non-specific reaction to central nervous system damage, consisting of proliferation or hypertrophy of several different types of glial cells.

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).

39. Lehner and Annibale, 2009 cited an early study (Wintrobe *et al*, 1981) which noted a female preponderance of 1.7-2.0 to 1 for pernicious anaemia.

40. In the general population the prevalence of pernicious anaemia is 0.1%; in subjects > 60 y it is 1.9% (Andrès and Serraj, 2012). Carmel gives a frequency of 0.1% in the population, rising to 1 % in the North European elderly[6]. Bizzaro and Antico, 2014 give a higher prevalence, stating that in the general population, the prevalence increases with age from 2.5% to 12%; this is based on the study by De Leon *et al.,* 2012 (see below) a survey of the general population rather than an estimate of a diagnosed population. Although commonly considered to be a disease of older people, this is not exclusively the case. In a series of 177 pernicious anaemia patients described by Lahner and Annibale, 2009 around half of them were < 60 y. In particular, 4% were < 30 y and 10% 30-40 y. Younger people represent about 15% of pernicious anaemia patients and may seek medical advice due to the symptoms related to anaemia such as weakness and asthenia (abnormal physical weakness and lack of energy) (Lahner and Annibale, 2009). Andrès *et al*, 2007 reported that in their study of 172 French patients (median age 70 y) with cobalamin deficiency, 33 % of them had pernicious anaemia, but stated that in their experience, pernicious anaemia accounted for 15-25% of cases of cobalamin deficiency in elderly patients.

41. Maktouf *et al*., 2005 studied 478 Tunisian patients with megaloblastic anaemia; blood folate and cobalamin were measured in 439 of them with low serum cobalamin only being found in 242 and low folate in only 6. The Schilling test, which specifically diagnoses pernicious anaemia) was performed on 120 randomly selected patients and was positive in 103 of them. Serum antibodies were measured in 94 patients with diagnosed pernicious anaemia, 60.6% had antibodies to parietal cells, 42.6% to intrinsic factor and 25.5% to both. The authors noted that the picture of pernicious anaemia in Tunisia was consistent with that elsewhere but the patients appeared to be younger with 21.5% being less than 30 y. A high degree of consanguinity was noted.

42. Pernicious anaemia is more frequent in carries of other autoimmune conditions. 41% of a population with pernicious anaemia also had autoimmune thyroid disease, with 10% presenting with vitiligo or alopecia, indicating that a sub-group of the patients could be considered as having a type II autoimmune polyendocrine syndrome (Lahner and Annibale, 2009). A study by Hershko *et al*., (2006) investigated the overlap between iron deficiency anaemia (IDA) and autoimmune gastritis (AIG). 160 patients with AIG identified by hypergastrinemia and strongly positive parietal cell antibodies were investigated. 83 of the patients had IDA (microcytic), 48 with normocytic indices and 29 with macrocytic anaemia. Compared to the macrocytic anaemia patients, those with IDA were 21 years younger (41 ± 15 y compared to 62 ± 15 y) and were mostly women. All groups had a high prevalence of thyroid disease and diabetes suggesting autoimmune polyendocrine system.

[6] The reference given is “Chanarin I. The Megaloblastic Anaemias 2nd Ed Boston, Mass: Blackwell Scientific Publications Inc: 1979. It has not been possible to obtain this, so the original source of the estimate is unclear.

Stratification by age showed a regular increase in mean corpuscular volume (MCV), serum ferritin and gastrin and a decrease in cobalamin level. The prevalence of *helicobacter pylori* infection decreased from 87.5% at < 20 y to 47.5% at 20-40, 37.5% at 41-60 and 12.5% at > 60 y. The authors concluded that rather than being a disease of the elderly, pernicious anaemia was a disease starting many years before the establishment of clinic cobalamin deficiency by an autoimmune process which was likely triggered by *H. pylori* infection. In an earlier study (Hershko *et al*., 2005) of 150 ambulatory patients with IDA but without apparent gastrointestinal disease, AIG was found in 27% of them, with serum cobalamin being below normal in about half. The majority of the subjects were young women, many with coexistent *H. pylori* infection.

Specific population studies- undiagnosed pernicious anaemia

43. de León *et al*., (2012) conducted a cross-sectional study of 429 individuals (262 women and 167 men) from the Canary Islands. The individuals were recruited from the 6729 participants of CDC (Cardiovascular Diabetes and Cancer) de Canarias study. This cohort is a random sample of the adult population of the Canary Islands aged 18-75 y. Parietal cell autoantibodies (PCA) were found in 7.8% of the population (95%CI 5.3-10.35%) being more common in women (10.7% compared to 4.8% in men, OR; 95%CI = 2.2; 1.0-4.8, p = 0.032). Prevalence was higher in post-menopausal women than in pre-menopausal women (13.9% compared to 5.2%, 2.9; 1.1-8.0, p= 0.029). Of the 36 individuals who were positive for PCA, 2 were also positive for intrinsic factor autoantibodies (IFA) giving a prevalence of 0.5% in the population. The authors noted that detection of PCA identified individuals with asymptomatic AIG the most common form of the disease before mucosal atrophy leads to iron malabsorption, ferropenic anaemia, impaired vitamin B_{12} absorption and pernicious anaemia.

44. Carmel (1996) reported a prospective study of 729 free living US adults (430 men and 299 women) aged > 60 y recruited from a range of sites including adult education centres, social clubs, an apartment complex and a Veteran Affairs outpatient clinic. To minimise self-selection by the subjects, mention of the symptoms and manifestations of B_{12} deficiency was avoided. The 729 subjects consisted of 299 women and 430 men with the ethnic backgrounds being 298 white, 179 black, 153 Latin American and 99 Asian American. Where ages were known, 257 individuals were aged 60-69, 335 were 70-79 y and 123 were 80 y or older. B_{12} was measured in blood by RIA using pure intrinsic factor with the remaining serum, where available, being tested for blocking (type I) anti-intrinsic factor antibody, regardless of the reported B_{12} level due to the specificity of the test and its sensitivity for the diagnosis of pernicious anaemia. All subjects found to have low B_{12} or a positive antibody test were invited for follow up examination, where further blood tests were conducted, including complete blood cell count and fasting serum gastrin level as well as absorption testing including Schilling tests. Eight subjects were shown to have pernicious anaemia from abnormal Schilling test results while a further nine declined follow up testing but were diagnose based on the presence of anti-intrinsic factor antibodies in their blood. Three of the 17 subjects had previously been suspected of having pernicious anaemia. Four of the 8 individuals who attended follow up were found to have mild anaemia and/or neurologic abnormalities. Fourteen (1.9%) of 729 elderly people were found to have previously

unsuspected pernicious anaemia, with mild B_{12} deficiency in most of the subjects. The prevalence was reported to be 2.7% in women and 1.4% in men, when considered by ethnicity this was 4.3% and 4.0% in black and white women respectively and 1.8 and 2.3 % in black and white men. No cases were identified in Latin American or Asian American individuals. Three additional patients were identified whose pernicious anaemia was previously suspected but inadequately diagnosed, if these individuals had been included in the analysis, the prevalence of pernicious anaemia in this population would have increased to 2.3%. The authors considered that the study was unlikely to have significantly overestimated prevalence due to selection bias due to the methods used but noting that even if there had been no pernicious anaemia in the non-recruited individuals, the prevalence would still have been 1%. They go on to argue that the figure was likely to be an under estimate since in the deficient subjects who declined follow up testing, pernicious anaemia could only be diagnosed if anti-intrinsic factor had been detected. Since this was present in just 70% of patients with pernicious anaemia and only 95 of the 729 subjects could be tested it was possible that another 3-4 subjects may not have been detected. In addition, they note that pernicious anaemia is most common in women aged > 60y who were 60.6% of the US population but only 41.0% of the study population. The author estimates that if extrapolated to the whole population, 800,000 individuals in the US have undiagnosed and untreated pernicious anaemia and were thus as risk from the presence of large amounts of folate, and this would not include younger people with unrecognised PA or those with B_{12} deficiency from other causes.

Diagnosis

45. 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. Consequently, if the HC bound fraction is preserved, the serum B_{12} concentration may be within the normal range; this may be clarified by measuring B_{12} metabolites. The serum levels of holoTC, metabolites methyl malonic acid and homocysteine, and assays of B_{12} absorption and intrinsic factor antibodies may also be measured.

46. 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). The anaemia is macrocytic and normochromic with reduction in the absolute number of reticulocytes. The haemoglobin level may be severely reduced. The patient's red blood cells exhibit marked anisopokilocytosis (variance in size and shape) and numerous oval macrocytes (megalocytes). Hypersegmented neutrophils are considered a hallmark of megaloblastic anaemia and typically precede the macrocytosis and anaemia. However, in advanced cases the neutrophils may be rare or absent probably due to ineffective granulopoiesis. Hypersegmented neutrophils support the diagnosis of megaloblastic anaemia, but are not unique as

they can occur in other types of anaemia. A bone marrow examination shows a hypercellular bone marrow with a shift towards immaturity and abnormal maturation of erythroid and myeloid cell lines.

47. 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).

48. 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. The implications of the results are discussed noting that neurological symptoms occurred when MCV was normal in 25% of cases. The clinically normal level of cobalamin is unclear but < 200 ng/L (148 pmol/L) is sensitive enough to diagnose deficiency in 97% of people. In elderly individuals, low serum cobalamin (100-160 ng/L) may be present in the absence of anaemia or macrocytosis and clinically significant deficiency may be present with cobalamin levels in the normal range. If cobalamin deficiency is confirmed, NICE (2018) recommend checking for anti-intrinsic factor antibodies. Individuals with normal cobalamin levels bur other symptoms such as SCD or megaloblastic anaemia should also be tested for antibodies. It is noted the presence of the antibodies is strongly predictive for pernicious anaemia but not very sensitive so that the absence of the antibodies did not rule out the diagnosis of pernicious anaemia. Similarly, NHS UK (2018a) provide advice which states that the tests for possible B_{12} deficiency include measuring haemoglobin, the size of red blood cells and folate and B_{12} levels, but notes that the test for blood B_{12} only measures total B_{12} and not whether it is in an inactive form or not; tests for the latter are not widely available.

Treatment

49. In the UK, pernicious anaemia is treated with i.m. injections of hydroxycobalamin every other day for 2 weeks or until symptoms improve (NHS, 2018b). 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).

50. In Asian countries, the preferred treatment is injections of methylcobalamin as this is thought to repair the myelin sheath. The different forms of cobalamin have different metabolic fates and functions, with some authors arguing that a combination approach may be needed. (Thakkar and Billa, 2015. See also Annex A).

MTHFR gene

51. Methylenetetrahydrofolate (MHTFR) reductase is an enzyme which converts 5,10-methylene tetrahydrofolate to 5-methyltetrahydrofolate. Deficiency in MHTFR is the most common genetic cause of elevated homocysteine, a condition which increases the risk of cardiovascular disease (SACN, 2006).

52. In their update on folic acid, SACN (2017) stated that a relatively common genetic polymorphism of MHTFR involves a C to T substitution at base 677 resulting in an enzyme with lower folate processing capacity. Individuals with that polymorphism have a significantly lower blood folate concentration for the same dietary intake of folate. Mothers with the TT genotype have a significantly increased risk of having a NTD affected pregnancy compared to those with a CC genotype.

How the TULs were set

53. 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 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. The individual case reports are described in Annex A to this paper where they have been presented in chronological order rather than by increasing dose as below. Some limited data from animal studies are also available and are also included in the annex.

54. 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.

55. 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

56. 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}.

57. 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).

58. 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 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 (Bethel and Sturgis, 1948). Bethel and Sturgis (1948) note that in some cases, the activity of the process was not arrested by increasing the dose of folic acid.

IOM (1998)

59. 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

These cases are described in detail in Annex B, where they have been presented in

60. Secondly, the IOM noted that in addition to the human case reports studies in rhesus monkeys (Agamanolis, 1976) and fruit bats (van der Westhuyzen 1982, 1983) show that vitamin B_{12} deficient animals receiving supplemental folate develop signs of neuropathology sooner than controls. Thirdly, there is a well-documented interaction between folate and 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 of unrecognised neurological damage.

61. 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)

62. 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 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.

63. 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.

64. 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.

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

65. 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)[7] of intake for an individual nutrient, this was done, but where the data were not available or were less secure, guidance was given.

66. 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.

67. 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

68. Mills *et al*. (2003) reviewed the laboratory results for whom a vitamin B12 concentration was measured at the Veterans Affairs Medical Centre in Washington between 1992 and 2000 to assess whether fortification had increased the number of patients with low vitamin B_{12} concentrations but no anaemia (as determined by MCV

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

and haematocrit). The level of fortification was intended to limit exposure to below 1 mg but, the authors consider that it was likely to be rather higher than that. There were 1573 subjects with low B_{12}. Data on race and sex were available for a subset of individuals only. The participants were 69, 24 and 7% African-American, White or other races respectively and 96.1% male). The proportion without anaemia did not increase significantly from the pre-fortification period (39.2%) to the period of optional fortification in March 1996 (45.5%) and the post-fortification period (37.6%) from January 1998. The proportion did not change significantly over the three time periods (age adjusted OR;95%CI 1.00;0.88-1.13, p = 0.096). These findings did not change when the analysis was restricted to individuals aged > 60y (anaemia was found to be more common in the younger subjects) or if a more conservative definition of low B_{12} was used (<150 pmol/L rather than < 258 pmol/L) or if the analysis was done as pre- vs post fortification. It was concluded that if confirmed, the results would indicate that food fortification had caused a major increase in masking of vitamin B_{12} deficiency. The folate status and intake of the participants was unknown.

69. Wyckoff and Ganji (2007) reviewed the medical records of individuals ≥ 19 y from a US hospital who had B_{12} and MCV measured. Of these, there were 633 individuals (261 men and 372 women) with low serum vitamin B_{12} levels (<258 pmol/L). The race of the participants was 337 White, 143 Black and 153 unknown and 263 and 370 individuals were < 65 y and ≥ 65 y respectively. MCV was significantly lower (88.6fL) in the post-fortification period than in the pre (94.4fL; p<0.001) or peri (90.6 fL; p<0.007) fortification period. The proportion of subjects with low serum B_{12} levels without macroytosis was significantly higher (≈87%) in the post-fortification and peri fortification (≈85%) periods than in the pre (≈70 %; p<0.001) fortification period. In a sex, race and ager adjusted analysis, the OR for having a low serum vitamin B_{12} without macrocytosis was 3.0 (95%CI: 1.7-5.2) in the post fortification period relative to the pre-fortification period. In general, serum B12 levels were slightly higher in the post-fortification period but when a multiple comparison test was applied, there were no significant differences in the median concentrations in the three time periods; the folate status and intake of the participants was unknown. The authors concluded that subjects with low serum B_{12} were likely to be without macrocytosis in the post fortification period. MCV should not be used as a marker for vitamin B_{12} insufficiency it was considered possible that folic acid fortification may have led to a correction of macrocytosis associated with vitamin B_{12} deficiency. In support of the view that there might be a risk of masking deficiency, the authors cited a study by Ray *et al*., (2003) which demonstrated that in a population of 25,000 elderly Canadian women, the prevalence of high folate and low vitamin B_{12} increased from 0.09% in the pre-fortification period to 0.61% post fortification (prevalence ratio 7.0: 95%CI: 2.6-19.2).

70. Qi et al. (2014) examined NHANES data from 1991-1994 (pre-fortification) and 2001-2005 (post-fortification) of adults aged > 50 y to establish whether the prevalence of serum B_{12} deficiency (<148 pmol/L) and marginal deficiency (148-258 pmol/L) with and without anaemia Haemoglobin < 130 g/L for men and < 120 g/L for women) and with and without macrocytosis (MCV >100 fL) had changed. There were 2922 individuals in the pre-fortification period and 4946 in the post-fortification period. The authors used multinomial logistic regression adjusted for age, sex, ethnicity, BMI, C-reactive protein and vitamin B_{12} supplement use. Pre-fortification

and post-fortification serum B_{12} deficiency without anaemia [4.0 vs 3.9%; adjusted prevalence ratio (aPR) (95%CI: 0.98; 0.67-1.44)] or without macrocytosis [4.2 vs 4.1%; aPR (95%CI: 0.96; 0.65-1.43)] remained unchanged. Marginal deficiency without anaemia [25.1 vs 20.7%; aPR (95%CI: 0.82; 0.72-0.95)] or without macrocytosis [25.9 vs 21.3%; aPR (95%CI: 0.82; 0.72-0.94)] were both significantly lower after fortification. After fortification, higher folic acid intake was associated with a lower prevalence of low serum B_{12} status in the absence of anaemia or macrocytosis. The results suggested that the prevalence of low vitamin B_{12} status among older US adults did not increase after fortification and did not support concerns that folic acid fortification adversely affected the clinical presentation of vitamin B12 deficiency among older adults.

Summary and discussion

71. 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.

72. 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.

73. 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 defect 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.

74. 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.

75. It has been argued 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.

76. At the last COT meeting it was agreed that the data on which the TUL was set should be examined to see whether it is appropriate. This paper has also provided information on pernicious anaemia, its features, prevalence and diagnosis to establish whether it is still relevant, given current diagnostic methods.

Questions for the Committee

77. The Committee are asked to consider:

a) Is the TUL appropriate given the available data on which it was set?

b) If the TUL is appropriate, is it still relevant given current methods/approaches to the diagnosis of pernicious anaemia?

c) If the TUL is no longer appropriate or relevant does it need to be modified or discarded?

d) If the TUL is no longer appropriate or relevant, should the rest of the database be reviewed to consider whether a TUL is required based on a different endpoint?

e) If the TUL is both appropriate and still relevant, is further action needed?

f) Would a formal risk-benefit analysis be of value for this topic?

Secretariat
June 2018

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

FOLIC ACID – ESTABLISHMENT OF THE TOLERABLE UPPER LEVEL (TUL)

Contribution from the Pernicious Anaemia Society.

This information has not been made available as it was provided in confidence.

Data on many aspects of the diagnosis and treatment of pernicious anaemia can be obtained from the Pernicious Society website

https://pernicious-anaemia-society.org/

Secretariat

June 2018

Annex B to TOX/2018/26

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

FOLIC ACID – ESTABLISHMENT OF THE TOLERABLE UPPER LEVEL (TUL)

ORIGINAL CASE REPORTS

Secretariat

June 2018

Case reports used by IOM

1. The papers described below are those which were listed in Table 1 of the main paper. They have been presented in chronological order, although it should be noted than IOM ordered them by increasing dose.

Hall and Watkins, 1947.

2. This paper presents a series of 14 case reports of individuals with pernicious anaemia, 9 of whom were diagnosed just prior to the study. In the others, a sensitivity to liver extract had developed and the patients were in severe relapse at the time of admission, two others had neglected treatment and the final individual had received a single liver extract injection. The patients were treated with both i.m and oral doses, with the oral doses ranging from 5- 30 mg/day for periods of 5 weeks to 1 year, with the majority being treated for 6-112 months.

3. Increased numbers of reticulocytes were measured, peaking at days 5-14, with the increase being stated as less than reported elsewhere (no specific reference given) and also being less than would be expected following liver extract therapy. Erythrocyte numbers and haemoglobin levels also increased; this was sometimes equivalent to the effect observed with liver extract treatment, but for other patients was markedly delayed. In three patients, erythrocyte number did not increase into the normal range despite increased folic acid doses (although it did increase following liver extract treatment. The degree of macrocytosis decreased in all cases, but returned to normal in only 3 individuals. In the six patients with low leukocyte levels, these increased, stayed below normal levels or decreased in 2 cases each. Platelet levels increased in 4 of 7 patients who had thrombocytopaenia prior to treatment. In twelve patients who had undergone sternal puncture, megaloblastic regeneration in the bone marrow prior to folic acid treatment was observed, but treatment resulted in a rapid change to normoblastic marrow in 7-10 days.

4. Folic acid improved glossitis in the 7 patients who had it, but in some patients, this was slower that would be expected from liver therapy treatment. The condition of 2 patients that had peripheral neuropathy and early signs of SCD did not improve during 5 or 6 weeks of treatment, even though one of the patients was receiving a folic acid dose of 100 mg/day. Early but definite signs of SCD were observed in 3 patients who had not had involvement of the spinal cord prior to folic acid treatment. Two patients experienced paraesthesia in the extremities, which improved slightly after treatment but relapsed 2-4 months later. Signs of spinal cord involvement were the loss of the sense of vibration and position in the lower limbs slight incoordination, presence of the Romberg sign (a standing individual is asked to close their eyes, an increased loss of balance is interpreted as a positive test), slight ataxia and in 1 patient, the bilateral presence of the Babinski great toe sign (this is a reflex whereby the big toe is extended when the sole of the foot is stimulated). The neurological manifestations began within 2-5 months of treatment, in case developing when erythrocyte levels were normal although there was a slight degree of macrocytosis. In other patients with neurological involvement, the effect on haematological

parameters varied – either with remission not occurring or erythrocyte numbers increasing and subsequently declining (this patient was receiving a maintenance dose of 15 mg/day folic acid at this time). The authors concluded that folic acid and the active agent in liver extract might be different.

Heinle et al, 1947; Heinle and Welch, 1947.

5. This study provided data on 41 patients with macrocytic anaemia, of these 16 had been treated with oral doses of 10-40 mg/day folic acid (usually 10-120 mg), 11 for 6-12 months and 5 for less than 6 months. Of the 16 patients, 8 showed no increase in erythrocyte levels, 3 an increase but none a moderate or marked increase. The other 5 patients were treated from haematological relapse and all showed an improvement in erythrocyte number and haemoglobin concentration.

6. Neurologic relapse occurred in 2 patients receiving oral dose of folic acid (a third occurred in a patient receiving i.m. injections). In the first of these (also reported in Heinle & Welch, 1947 in more detail) a 68 year old male in relapse was treated with 10 mg/day folic acid. He had experiences progressive weakness, slight numbness in the hands and burning of the mouth and gums for around a year prior to admission. Examination showed that some reflexes were absent, and vibratory sense was reduced in one leg but was normal elsewhere, the Romberg sign was absent and position sense was intact. Following treatment, there were significant increases in erythrocyte levels and haemoglobin and a complete disappearance of macrocytosis. Eighty three days after treatment started, there was a sudden and rapidly progressive onset of paraesthesia with rapid loss of deep reflexes and vibratory sensation. As reported elsewhere, there was a spike in the number of reticulocytes as well as slower increase in red and white cell count and haemoglobin levels which plateaued after 8 weeks. At the time of neurological relapse, erythrocyte and haemoglobin levels were normal. “Vigorous” liver extract therapy was required to improve the neurological lesions, which had not completely resolved at the time of reporting. In the second case, a 51 year old woman developed severe sensitivity to the liver extract treatment and the liver-stomach preparation tried did not maintain the blood at “satisfactory levels”. She was then given oral folic acid at 40 mg/day which was gradually reduced to 10 mg/day. A marked improvement in the blood and the patient’s wellbeing was reported. After 1 year of treatment, the patient reported soreness in the tongue and mouth and increasing numbness in the feet and hands. The liver-stomach treatment was reinstated which improved the numbness but the symptoms of sensitivity also returned. The authors concluded that folic acid was not a complete substitute for liver extract therapy because of the lack of efficacy on neurologic relapses but it did have a place in the treatment of macrocytic anaemia, particularly for patients who had becomes sensitive to liver extract.

Spies and Stone, 1947

7. It was reported that 21 patients who had been previously maintained on liver extract therapy, were selected for study and given a weekly dose of 70-105 mg folic instead for 10-12 months. After 5-8 months of therapy, paraesthesia in the extremities and an unsteady gait developed in 4 cases. Neurological signs gradually appeared and multiplied until there was clear evidence of SCD. The dosage of folic acid was increased to 50-500 mg/day for 10-40 days but this was not followed by

any improvement. The folic acid was discontinued and liver extract treatment resumed; the symptoms began to resolve 10-12 days later.

8. In a separate study, folic acid was given to 9 patients with SCD. Two patients appeared to show some improvement, though this was not completely clear. The other 7 patients deteriorated whilst treated with folic acid and did not improve until liver extract was given. Details were provided on a representative case report. In this, a 76 year old woman was admitted with an inability to walk unassisted and numbness in areas of the legs, arms and trunk; the symptoms had been progressing for some years until they had rapidly worsened prior to admission; the patient had never had severe anaemia (haemoglobin and cell counts were lower than normal but not severely so). The patient was treated with oral doses of 10 mg/day folic acid for 22 days. Cell count and haemoglobin levels improved but urinary incontinence persisted and the patient began to complain of failing appetite, abdominal discomfort, vertigo and depression. Neurological examination showed that there had been no improvement in nerve involvement and additional symptoms had appeared for the first time. Folic acid was discontinued and i.m. injections of liver extract started; although her symptoms improved, they did not completely resolve.

Vilter et al, 1947

9. Data reported on a group of 28 patients who had become sensitive to liver extract and were treated with folic acid for 1 year. Twenty one of these patients had pernicious anaemia, which had been satisfactorily controlled, 3 had pernicious anaemia which had haematologically relapsed, 2 had sprue (coeliac disease) I had nutritional macrocytic anaemia and 1 had macrocytic anaemia secondary to ileosigmoidostomy. Of the 21 pernicious anaemia patients in remission, doses of 10 or 20 mg per day or 30 mg 3 times per week maintained the haematological remission for 1 year. In the 3 patients in relapse, there was an initial response to the folic acid but increased dosage was needed to maintain it. SCD developed in 4 of the patients at 5-8 months and this was not resolved by increasing the folic acid dose; although the symptoms rapidly resolved when liver extract was given. In these cases, the symptoms were initially tingling, numbness, stiffness in the extremities and unsteady gait. The symptoms occurred when the haematology was normal. An additional patient had symptoms of nervous system degeneration prior to treatment, with symptoms including tingling, numbness, hypesthesia to touch and diminished reflexes; this was not improved by folic acid treatment.

Jacobson et al, 1948

10. In the first of two reported cases, a 78 year old woman was admitted to hospital following an eight month period where she had noticed gradually increasing malaise, anorexia, weakness and exertional dyspnoea as well as numbness and tingling of the toes and fingers. Examination revealed absent reflexes and loss of vibratory sensation; sense of motion and position were unaffected. The levels of haemoglobin and the red cell count were very low and the patients was diagnosed as having pernicious anaemia in relapse. The patient was given i.m. injections of 25 mg/day folic acid for 11 days followed by an oral dose of 65 mg/day for a further 30 days. The patient's appetite and clinical appearance improved, red cell count and haemoglobin levels increased and bone marrow returned to normal. The folic acid

dose was then changed to 195 mg twice per week for 3 and a half weeks. The numbness and tingling also disappeared. The patient was discharged to outpatients taking a daily dose of 10 mg/day. Two months later the numbness and tingling resumed and these became worse despite an increase in folic acid dose, eventually she became irritable and confused although the objective findings were unchanged. At this time, her red cell count and haemoglobin levels had declined slightly.

11. In the second case, a male aged 62 was admitted to hospital after complaining of progressive weakness for 1 year as well as anorexia, exertional dyspnoea and tingling and numbness in his toes. On examination, the patient was confused and irrational and weakness and loss of vibrational sense was observed in the lower extremities. Red cell and reticulocyte count, and haemoglobin levels were very low and the bone marrow indicated megaloblastic erythropoiesis; pernicious anaemia was diagnosed. Following a blood transfusion, the patient was initially treated for 4 days with an unspecified dose of folic acid, decreasing to 20 mg/day i.m. folic acid for 9 days, decreasing to 20 mg 3 times/weak and finally changing to an oral folic acid dose of 10 mg/day. Two weeks after treatment, the patient was alert and rational. Neurological examination indicated that vibratory sense was reduced or absent in the legs, but the sense of motion and position were intact. Haematological parameters had significantly improved but were still low. The patient was discharged but continued to take the oral dose of folic acid. Two weeks later, the patient was symptomatically well and his red cell count was stable, however, the numbness and tingling had recurred and he had difficulty walking; this progressed such that he was readmitted five weeks after discharge. Neurological examination indicated that vibratory sense was absent in the lower limbs, there was ataxia in the arms and reflexes were absent or hyperactive. There was no haematological relapse, although the presence of pathological neutrophils indicated an incomplete remission. The patient was treated with liver extract and the symptoms improved although they did not completely resolve.

12. Serial measurements for the haematology and neurology were reported for both patients. These show an initial spike in reticulocyte count followed by a decline to the initial levels along with a steadier increase and levelling off in red cell count and haemoglobin levels (in both patients these peak at around 60 days of treatment). The authors concluded that a clinical trial of 50 mg/day folic acid would establish whether folic acid would be useful in the treatment or maintenance of patients with pernicious anaemia.

Spies et al, 1948.

In this study the susceptibility of individuals with macrocytic anaemia (with and without achlorhydria) to SCD was investigated. It included 160 patients of whom, 38 had a histamine refractory achlorhydria on repeated gastric analyses who had been tentatively diagnosed as having Addisonian pernicious anaemia (the other participants had conditions including sprue or macrocytic anaemia related to pregnancy). The subjects were treated with 10-50 mg folic acid, but most usually 10 mg/day. The study participants also had to follow a restricted diet which excluded, meat, meat products, fish, poultry, milk and eggs. Haematological examinations were conducted daily, and regular gastric analyses were performed, bone marrow samples were taken at baseline, at the peak of reticulosis and when reticulocytes

returned to normal. Within 2 years, but usually within 2 months, 28 of the 38 pernicious anaemia cases had developed SCD regardless of the folic acid dose used. The neurological disturbances were rapidly relived with liver extract treatment. There was no evidence of SCD in the other macrocytic anaemia patients. Few details of the overall study are provided but some cases are reported in more detail, including one of pernicious anaemia. In this, a 68 year old male had been treated for pernicious anaemia with liver extract for over 10 years prior to the study. He ate liver occasionally and took liver extract intermittently and thus relapsed prior to the study. The blood responded well to the folic acid treatment. The neurological symptoms developed a year later with numbness of the feet which spread to the knees, leading to increased difficulty in walking. Vibratory sensation was reduced and reflexes abnormal and there was a marked ataxia in the lower extremities; symptoms largely resolved with liver extract treatment. The paper also briefly reports three other cases where the SCD was successfully treated with B_{12}.

Ross et al. 1948.

13. This study investigated the effects of using synthetic folic acid rather than liver extract to treat pernicious anaemia in 22 patients. The diagnosis was established by the demonstration of a macrocytic hyperchromic anaemia with associated leukopenia and thrombocytopenia, a histamine refractory gastric achlorhydria and a response to folic acid or liver extract with reticulocytosis and restoration of normal blood values. In many cases, a megaloblastic bone marrow typical of pernicious anaemia was apparent before treatment was started. The blood tests conducted were haemoglobin concentration, haematocrit and leukocyte, erythrocyte, and reticulocyte counts; bone marrow samples were also taken. Liver extract was given to some patients in addition to folic acid (when they did not respond to folic acid treatment alone). A number of patients had been previously treated with liver extract. The results of the study were summarised as follows. 21 patients with pernicious anaemia were treated with folic acid alone for 8 to 17 months. Satisfactory blood levels (not specifically defined but the term appears to indicate haematocrit and haemoglobin levels and blood cell count) were maintained in all cases receiving oral doses of 1.25 to 15 mg/day folic acid. Severe hematologic relapse occurred within 6 months in one case treated with monthly injections of 30 mg folic acid. Doses of 15 mg/day folic acid induced satisfactory haematopoietic responses in 3 cases with pernicious anaemia in severe relapse but only a slight response in a 4^{th} patient with mild pernicious anaemia but severe SCD. Ten patients showed a significant improvement in blood values for a few months after substitution of folic acid for liver extract. With one exception, these subsided to pre-folic acid levels comparable with those previously maintained with liver extract alone. This suggested that a combination of folic acid and liver extract may maintain a better hematologic status than either substance alone.

14. One patient with severe SCD did not improve during folic acid therapy, eleven patients developed or showed progression of SCD during treatment. Neurologic disease developed in most of these patients when the peripheral blood was normal. Progression could be rapid. The institution of liver extract therapy in addition to folic acid in patients who developed SCD whilst being treated with folic acid, failed to

prevent further progression in four cases and only partially arrested it in one case, though improvement occurred more rapidly when folic acid was discontinued. SCD occurred with greater frequency in patients on large daily doses of folic acid than it did in patients on low or intermittent doses, suggesting that large doses of folic acid could aggravate or precipitate neurologic disease. The authors suggested this could be because the folic acid could be interfering with glutamic acid metabolism.

15. The patients treated with the highest dose of folic acid (15 mg/day) were discussed in more detail; some of the key points being given as below:

Case no. 1- Patient given 15 mg/day for 12 months. The regeneration of erythrocytes, leukocytes and platelets was rapid, blood remained normal for eight months, but subsequently fell to anaemic levels, “definite subjective and objective signs of SCD appeared during the 12 month of therapy”.

Case no. 2 Patient given 15 mg/day folic acid for 28 days- slight hematologic improvement in response to folic acid, but no improvement in SCD, no further follow up.

Case no 3 Patient given 15 mg/day folic acid for 16 months, when mild anaemia and sub-acute SCD developed. SCD continued to progress when liver extract therapy was introduced in addition to the folic acid.

Case no 22. Patient given an oral dose of 15 mg/day folic acid along with a monthly dose of 30 mg/day folic acid by injection. The hematologic response was described as “excellent” but there was an “explosive” development of SCD following 9 months of folic acid treatment when the peripheral blood and bone marrow were apparently normal.

16. Fewer details are given for other cases, but of interest are:

Case no. 4. Patient given 15 mg/day folic acid for 12 months. Blood levels high and macrocytosis better than when previously treated with liver. SCD began in 11th month and progressed rapidly despite subsequent additional treatment with liver.

Case no. 8. Patient given 10 mg/day folic acid for 12 months, blood levels the same as with liver extract therapy. Probable development of early SCD around 7 months.

Case no 10. Patient given 5 mg/day folic acid for 12 months, blood levels initially higher than with liver extract therapy. Definite development of SCD.

Case no 12. Patient given 1.25 mg/day folic acid for 12 months, blood levels better than when maintained with liver extract, SCD progressed.

Case 13. Patient given 1.25 mg/day folic acid for 12 months, blood levels improved initially, but decreased at 10 months, definite progression of SCD.

Case 14. Patient given 1.25 mg/day folic acid initially followed by 15 mg from 9 1/2 months. No effect on blood levels. SCD developed at 91/2 months which progressed rapidly when the dose of folic acid was increased and continued when liver extract was given.

Case 15. Patient given 1.25 mg/day folic acid initially followed by 15 mg from 9 1/2 months. No effect on blood levels. SCD developed at 7 months and progressed despite an increase in folic acid.

Case 17. 100 mg/day folic acid (i.m) per month followed by 1.25 and then 15 mg/day oral folic acid. Evidence of SCD developed at 2 months and progressed despite an increase in folic acid.

Bethel and Sturgis, 1948

17. This paper describes a case series of 70 patients with pernicious anaemia who had been observed for at least 10 years to allow the evaluation of different types of therapy on the progression of the disease. As part of this, early results for 15 patients treated with folic acid were presented. The patients were generally treated with 5 to 10 mg/day folic acid, with one receiving 20 mg/day for periods of time ranging from 2-14 months as reported (some patients were also given folic acid injections at various points during treatment) - not all of the 15 cases noted are reported in detail. Four patients in the series were stated to have had “unsatisfactory results with respect to their neurological status”. Two of the cases were considered to be equivocal with the changes being slight or the folic acid dose being too low. In the first of these, the patient had paresthesia, mild ataxia, impaired vibratory sense and swaying in the Romberg position. The symptoms were not improved by 15 mg/day parenteral folic acid followed by 5 mg/day oral folic acid for 2 months. The neurological symptoms were moderately improved by treatment with i.m. liver extract. In the second equivocal case, the patient had moderately severe neurological disease with paraesthesia, ataxia and deep sensory disturbances, she was treated with 10 mg/day folic acid for two months, reporting that the numbness and tingling had become worse, although there was no demonstrable improvement in the neurologic signs. The symptoms were moderately relieved by treatment with liver extract.

18. Two cases were reported where SCD progressed actively during folic acid treatment. In the first of these a patient who was diagnosed with pernicious anaemia had experienced fatigue, paraesthesia and ataxia, developing severe depression shortly before diagnosis. He was treated with two injections of liver extract, then exclusively by 20 mg/day folic acid for 2 months. The neurological symptoms progressed such that walking became more difficult until he was unable to leave his bed developing symptoms of severe spastic ataxic paraplegia and loss of sphincter control. His haematological status had also declined. Following treatment with liver extract the neurological symptoms partially resolved after several months treatment, with the haematological status improving more rapidly. In the second case, the patient reported impaired sense of taste and anorexia progressing to numbness and tingling in the extremities and difficulty in walking. He was diagnosed with pernicious anaemia with moderately advanced postero-lateral column degeneration. He was initially treated with 10 mg/day i.m. folic acid for 10 days followed by a 10 mg/day oral dose. Although he was discharged walking unaided he returned some weeks

later unable to stand or walk without assistance; his haematological status had improved. Following treatment with liver extract, the neurological symptoms partly resolved.

19. The paper contains a note stating that one of the 6 patients receiving folic acid who had been described as not developing neurological manifestations, subsequently developed severe paraplegia while being treated with folic acid. The "early results" may therefore be too early to identify all those who might have a neurological relapse. The authors considered whether the evidence suggested that folic acid only improved the haematological parameters but they reported that the neurological symptoms of some patients also improved.

Wagley, 1948.

20. This paper reported 14 cases of macrocytic anaemia (10 of pernicious anaemia, 1 of tropical sprue and 2 of non-tropical sprue and 1 of macrocytic anaemia associated with total gastrectomy). Eight of the 10 patients with pernicious anaemia showed neurologic disturbances occurring from 8 days to 12 months after treatment. The oral doses of folic acid used by these individuals ranged from 5- 45 mg/day over periods of 8 days to 18 months. Reduced vibratory sense and tingling of the hands and feet were the most frequent developments with one patient also experiencing numbness along with a feeling of weakness. Two patients had difficulty in voiding urine. The onset of signs and symptoms was abrupt and severe in 2 cases. The neurological changes were described as being more marked in the sensory rather than the motor modality. Two patients treated with 5 or 10 mg folic acid for 9 and 4 months respectively did not experience neurological relapses.

Berk et al, 1948.

21. Twelve patients were treated with i.m. folic acid for up to 17 months. The planned dosage was 10 mg/day initially along with a diet which excluded meat, fish, eggs or other recognised haematopoietically active substances. This was followed by a maintenance phase of a 75 mg/week i.m. dose with no dietary restrictions. There were some exceptions to this schedule and some patients were given oral doses of 50 mg/day folic acid after 10 to 32 months treatment. The reticulocyte responses and initial rises in red cell count and haemoglobin were comparable to those seen with liver extract therapy and remission was maintained for periods up to 16 months. The blood values did not return to normal in four patients, this was attributed to insufficient therapy (2 cases) or iron deficiency (1 case) but remained unexplained in the fourth case. Two patients showed a decline in their blood levels after they had been initially satisfactorily maintained for 4-5 months on folic acid; normal blood values were restored following treatment with liver extract.

22. Neurological manifestations developed for the first time one of five patients who had received 75 mg. week i.m. for 8-17 months and in both patients treated with 50 mg/day oral doses of folic acid for 6 or 11 months. The way that data are reported in this study and the variation in treatment regimens used means that only limited conclusions can be drawn. The variations in treatments arose from efforts to restore declining blood levels. The authors note the reports of Ross *et al*. (1948) and Wagley (1948) and discuss the observation that the acute onset and rapid spread of the

neurological lesions seen in some cases was greater than in untreated cases or where liver extract treatment was used, suggesting that folic acid could be the “toxic agent” possibly by interfering with glutamic acid metabolism in the nervous system.

Fowler & Hendricks, 1949.

23. It has not been possible to obtain this paper. However, according to the table included in the IOM opinion, neurological manifestations occurred in 2 /2 cases treated with 15-20 mg folic acid for 4-5 months.

Israëls and Wilkinson, 1949.

24. This paper reported on 20 cases of patients developing neurological changes on treatment with folic acid, but also summarised the earlier literature which they stated had reported the same patients in different journals and did not always provide details, noting also where the folic acid treatment was believed to have made the neurological symptoms worse.

25. The 20 patients were divided into 4 groups – (1) Four patients with signs of nervous system disease developing acutely during treatment, (2) nine patients with signs of nervous system disease developing gradually during treatment, (3) three patients who had nervous system disease initially and (4) Four patients who had not developed nervous system disease at the time of publication. The patients were treated with doses of folic acid ranging from 2.5 to 40 mg/day, though 10-20 mg/day was most usual for periods of 1-35 months, the majority for over 1-2 years. Illustrative case reports are given for cases (1 each for groups 1, 2 and 3).

26. From group one, a 63 year old woman was treated for pernicious anaemia with 20 mg/day folic acid. After 4 weeks treatment, her haematological status was partially restored and the folic acid dose was decreased to 10 mg/day, after a further 5 months where her measurements were back in the normal range it was decreased again to 2.5 mg/day. However, by 9 months her red cell count and haemoglobin levels had started too decline and she reported a loss of sensation in her legs, her symptoms were stable until 15 months when, within 7 days she lost the use of her legs and became unable to walk. The patient was then treated with liver extract and desiccated stomach, her symptoms resolved completely over an eight month period.

27. For group 2, a 58 year old man had been diagnosed with pernicious anaemia some years previously but had neglected treatment. He was admitted with symptoms including severe anaemia; there was no indication of neurological disease. He was initially treated with i.v and i.m. folic acid for 10 days and then an oral dose of 20 mg/day, reduced to 10 mg/day after 2 months and reduced twice more as haemoglobin levels and blood count improved; however, the folic acid doses were subsequently increased as these values began to decline. After 14 months, his haemoglobin levels and blood count were normal but he was experiencing aching legs, though no objective abnormalities of the CNS were identified. Some months later, the patient developed numbness and parasthesiae in the arms and legs. Vibration and joint sense were impaired and Romberg’s sign was present. His haematological status had also declined. Following treatment with desiccated stomach, the symptoms largely resolved.

28. As an example of Group 3, a 46 year old man presented with numbness in the hands and fingers and stiffness in the legs. His haemoglobin levels and red cell count were slightly lower than normal, but the bone marrow was megaloblastic and there was achlorhydria. Reflexes were largely normal and vibration and joint position sense were normal. The patient was treated with 20 mg/day folic acid. After 4 months, red cell count and haemoglobin had not improved and the numbness and paraesthesia in the hands had worsened. His reflexes had not changed but vibration sense had disappeared. Treatment with folic acid was replaced by desiccated stomach which rapidly improved the haematological status, with the neurological symptoms resolving over a 16 month period.

29. Reviewing all the cases, the authors concluded that folic acid was given over a range of durations (3-35 months) before neurological symptoms occurred, there was no time of particular danger, nor any time after which a patient could be considered “safe.” Similarly, it was not possible to predict the likely onset of neurological complications either from this information or the data available on haematology (red cell count and haemoglobin). Overall, 80% of the patients who were being treated with folic acid had developed or increased signs and symptoms of nervous system that could be treated rapidly with desiccated stomach or liver extract, therefore there was a serious risk that patients with pernicious anaemia given folic acid alone may develop nervous system disease or that it could get worse. However, 4 patients were well maintained on folic acid, suggesting that there was some dissociation between the haematological and neurological features of the condition. The action of folic acid on the neurological system was not the same as that of the liver extract but the initial effect on bone marrow and blood appeared to be similar. The acute neurological syndrome they had observed was rarely seen in the natural course of posterolateral sclerosis with pernicious anaemia but there was insufficient evidence to show that folic acid could cause lesions directly in the cord.

Schwartz et al, 1950

30. Ninety-eight patients with pernicious anaemia were treated with 5 mg/day folic acid for 48 months. The condition of the patients and their liver extract treatment needs were well characterised and the patients were in complete neurological and haematological remission for periods of years or months. The patients were then seen at 4 weekly intervals. Of the patients observed, fifty-eight relapsed, twenty-six interrupted treatment and only twelve remained in satisfactory remission. In the relapsed patients, 23 had a haematological relapse (4 within the first year, 8 within the second year and 11 after 2 years. This was completely reversed by treatment with liver extract. Of the patients who had a neurologic relapse, 4 relapsed within a year and 19 within one and two years. The abnormal neurological findings (hyperactive deep reflexes, positive Romberg sign and impairment of vibratory sensitisation) were not reversible in 8 of the 23 cases despite intensive treatment with liver extract for six months or more. There were 9 cases of combined relapse which were largely hematologic in nature and showed a similar pattern to the hematologic relapses. These cases were all reversible. Three further cases reported sore tongue and weight loss. Of the 26 patients where therapy was interrupted, 12 died or moved away but the others voluntarily interrupted their treatment as they did not feel as well taking the folic acid as they had with liver extract treatment.

The study had started before the concerns about folic acid were published in the literature. By which time, the present study had already obtained similar findings. The authors noted that the patterns of neurologic and hematologic relapse were different (the neurologic relapses occurred more quickly) suggesting that different mechanisms might be involved. When compared to spontaneous hematologic relapse, the folic acid treated patients generally relapsed later. The authors concluded that the folic acid had an initial beneficial effect but that this progressively diminished.

Chodos and Ross, 1951

31. This paper followed on from Ross *et al* (1948) and was based on 18/22 of the original patients and attempted to establish whether folic acid had a direct deleterious effect. Four additional case of patients with hypochromic, microcytic anaemia were also considered as were 2 cases of post-gastrectomy macrocytic anaemia and 1 case of non-tropical sprue. In the earlier study it was suggested that there was apparent progression of SCD even after the addition of liver extract to the treatment. Further studies indicated that SCD which developed in some instances when patients with pernicious anaemia were treated with folic acid did not usually progress when parenteral liver extract was administered even if folic acid therapy was continued. Two patients did show progression of the neurological disease but both had sub-optimal nutrition and “organic abnormalities” which might have interfered with storage or utilisation of the liver extract. Of the new cases of hypochromic microcytic anaemia, the patients were treated with oral doses of 20-30 mg/day folic acid for periods of 3-25 months; no evidence of neurological disease was observed. Of the 2 cases of post-gastrectomy microcytic anaemia, 5 months treatment with folic acid at doses of 15 mg/day (3 weeks), 5 mg/day (2 weeks) and 10 mg/day (2 months) following a diagnosis of anaemia resulted in a moderate improvement of the anaemia but also signs and symptoms of SCD. These improved dramatically when folic acid was withdrawn and liver extract therapy introduced. In the second case, macrocytic anaemia developed which was treated with liver extract, when this was withdrawn a mild relapse was successfully treated with folic acid. No evidence of neurological disease was apparent after 21 months treatment with 30 mg/day folic acid. The patient with non-tropical sprue was given 15 mg/day folic acid for 2 months and whilst paraesthesia of the fingers was exacerbated, this was not thought to be related to folic acid treatment.

32. It was considered that prolonged administration of folic acid and liver extract to patients who had no pre-existing neurological disease did not result in any neurological relapses. The authors concluded that folic acid did not have a direct effect, provided there was no complicating disease or sub-optimal nutrition.

Conley and Krevans, 1951.

33. This paper reported 5 cases where the patients had previously unrecognised pernicious anaemia prior to presenting with neurologic symptoms but little or no anaemia. None of the patients had been aware of taking folic acid, but each had been taking multivitamin supplements; it was possible to identify the supplements in 4 of the 5 cases.

Case 1 had been taking a supplement containing 5 mg for 2 years “whenever he felt tired” before presenting with numbness in the hands and a stumbling gait. There was no evidence of anaemia.

In case 2, a 75 year old woman had been treated for anaemia with liver injections. This was discontinued and for the 21/2 years before admission, she took a supplement containing 1 mg/day folic acid. There was no evidence of anaemia.

In case 3, the patient had taken capsules giving a total daily dose of 4.5 mg folic acid. Haemoglobin and red cell counts were low.

In case 4, the patient consumed a dose of 2.25 mg/day for 5 months before physical symptoms (weakness, tiredness and numbness) were apparent although depression had been suspected before the supplements were given. The patient developed slight anaemia while under observation

In the final case the patient had taken a multivitamin supplement for 1 year but it was not possible to identify it. The patient had presented with numbness and pins and needles and there was no evidence of anaemia.

Victor and Lear, 1956

34. Victor and Lear (1956) presented 9 case reports. Three were considered by IOM. However, there appear to be four cases reported where folic acid treatment was given.

A 57 year old woman presented with numbness and aching pain in the legs and feet, problems with balance and irritability and forgetfulness. She had been treated for pernicious anaemia for 29 months with supplements containing a daily dose of 3.34-6.68 mgs of folic acid and 50-100 µg of vitamin B_{12} having been previously treated with liver extract. Blood counts and haemoglobin were normal throughout with only a slight variation in red blood cell size being apparent. The symptoms were largely resolved following treatment with i.m. injections of vitamin B_{12}.

A 65 year old male patient presented with difficulty in walking, which had progressed from weakness, giddiness and tingling which had begun over 4 years previously. He had initially been treated for anaemia with liver injections and transfusions before the treatment was changed to oral vitamin supplements which were taken for the 27 months prior to presentation. The dose of folic acid was 1.67 mg/day with the exception of a 3 month break where supplements which did not contain folic acid were used and 6 weeks prior to presentation when a third type of supplement with a daily dose of 2.55 mg folic acid was consumed. Blood morphology was normal throughout as was a bone marrow smear. Six months after B_{12} treatment had started, the symptoms had improved but not completely resolved.

A patient with early signs of SCD (numbness, tingling, cramp and difficulty in walking) but no anaemia was treated with multiple vitamins including folic acid at a daily dose of 3 mg/day for 18 months. The neurological symptoms progressed for nearly two years until anaemia developed and a neurologic diagnosis was made, by which time the patient, now aged 65, had no feeling in his hands and feet and was unable to use stairs. The patient was treated with B_{12} for 5 months when his red blood cell count was restored to normal and the neurological symptoms considerably improved. This case was not included by IOM.

A 53 year old woman had been treated for anaemia with capsules containing liver, iron and multiple vitamins plus occasional liver injections for 8 years along with eating liver every day. Her red cells and returned to normal and stayed stable throughout this period. She discontinued the medication and the anaemia returned, she was then treated with a supplement containing 6.68 mg/day folic acid After some weeks she noticed feelings of stiffness and cold in her hand and feet, but over the months her blood count returned to normal but the neurological symptoms remained progressing until the patient presented unable to walk. The neurological symptoms improved but did not fully resolve following treatment with parenteral B_{12}.

35. A number of cases were also described to illustrate the difficulties in SCD diagnosis, for example, cases where the patients presented with severe SCD but only mild anaemia and extensive delays in diagnosis had occurred or where other conditions were suspected, again delaying diagnosis.

Will et al, 1959

36. Between 1945 and 1948, 46 patients with pernicious anaemia were given an oral dose of 30 mg folic acid 3 times per week (approximately 12.9 mg/day); 36 of the patients were followed for 1-10 years until relapse occurred or the patients were lost to the study for other reasons. Twenty-nine of the patients had been previously maintained on liver extract while 7 had not been treated prior to the folic acid administration. Some of the patients were selected for additional studies in which, interactions between folic acid and B_{12} were examined; these were the effects of large doses of folic acid on the efficacy of B_{12} as well as the effect of folic acid on serum B_{12} and of B12 on urinary folic acid and folinic acid.

37. By the end of six months, one patient each had a neurological, haematological or combined relapse. By one year there were a further 3 and 4 haematological and combined relapses respectively, followed by 2 combined relapses in year 2, and 5 haematological relapses in year 3. During the 10 years of the study, there were a total of 7 neurological relapses, 9 combined relapses and 8 haematological relapses. There were no further relapses after year 7. The patients who had been well controlled on liver extract tended to relapse later than those who had been treated with folic acid since diagnosis. Nine patients developed glossitis (which can indicate B_{12} deficiency) before or at the same time as haematological relapse. Patients who developed hematologic relapse alone, were treated with increased doses of folic acid up to 50 or 100 mg/day. One or two haematological

remissions and relapses usually occurred within the 3 months following the increased dose; eventually neurologic relapse occurred in these patients. In all, a total of 21 patients eventually developed neurologic relapse. The authors state in their conclusions that only 3 of 36 patients were maintained in a satisfactory haematological and neurologic condition when maintained on folic acid alone. Even where patients did not relapse, they were stated to have extremely low vitamin B_{12} levels.

38. The authors considered that folic acid did not directly affect B_{12} metabolism since their additional studies did not show B_{12} levels being lowered when both folic acid and B_{12} were given together; however, the data suggested that B_{12} could affect folic acid metabolism.

Best, 1959

39. A 46 year old male was admitted to hospital with paraesthesia in the feet and hands and a tightness and burning sensation in the feet and weakness in the knees and ankles, following a 3 month period of increasing visual fatigues and having become increasingly irritable, depressed and disinterested. He also reported a sore tongue and an inability to feel the ground with his feet. Some years prior to admission, he had undergone extensive intestinal resection (including major portions of the ileum) due to complications arising from Crohn's disease. He was subsequently readmitted with weight loss, anaemia and soft pale stools and steatorrhea which was treated with a low fat high protein diet and 10 mg/day folic acid, following which his red cell count and haemoglobin levels increased. Folic acid was then continued for 26 months until presentation with neurological symptoms as above. Examination indicated that there was a loss of sensation but reflexes were present. Haemoglobin levels were low (just outside normal range) and bone marrow smears showed active megaloblastic erythropoiesis with normal leucopoiesis. Further investigations showed that B_{12} could not be absorbed even in the presence of intrinsic factor. Folic acid treatment was replaced with i.m injections of B_{12} and the patient began to recover, with the neurological manifestation resolving by 3 months. The author concluded that the case illustrated the dangers of folic acid being too readily prescribed to patients with megaloblastic anaemia secondary to lesions of the small intestine.

Crosby, 1960

40. In the first of two cases reported, a multivitamin containing folic acid was prescribed to an individual presenting with mild gastrointestinal complaints and a history of tiredness. The supplement improved his symptoms but a year later he presented with difficulty in walking and occasional urinary incontinence. Physical examination revealed evidence of spinal cord disease; tests for pernicious anaemia indicated that he could not absorb vitamin B_{12} but he was not anaemic and his bone marrow was not megaloblastic. The author concluded that the folic acid improved his anaemia symptoms but permitted the spinal cord disease to progress, had he not been given folic acid, the development of obvious anaemia would have led to earlier diagnosis. No details of the folic acid dosage are given. In a second case, a 62 year old woman was diagnosed with pernicious anaemia, for 11 years she received

injections of liver extract or vitamin B_{12} and both her anaemia and early neurologic symptoms improved. The patient then moved cities and her new physician gave her only occasional vitamin B_{12} injections and none for 2 years. During that time, she took two multivitamins containing a total of 0.35 mg/day folic acid. The patient's ataxia became increasingly severe until she was unable to walk; she was not anaemic and her bone marrow was not megaloblastic and she was unable to absorb B_{12}. As with the previous case, the folic acid allowed improvement of the anaemia but the changes to the spinal cord progressed. The author concluded that folic acid was potentially dangerous and should not be included in multivitamin preparations.

Ellison, 1960

41. In the first of two case reports, a 58 year old woman presented with a number of neurological symptoms, but no anaemia or megaloblastic changes. The patient had taken two vitamin supplements containing a total of 4.4 mg folic acid/day for 2 years. Pernicious anaemia was diagnosed and the patient was treated with liver extract and subsequently vitamin B_{12}. After some months, the neurological symptoms improved, but the paresthesia did not completely resolve. In the second case, a 70 year old woman was admitted to hospital with a number of neurological symptoms including numbness of the fingers, paresthesia of the legs and difficulty in walking. Haemoglobin levels were normal as were red blood cell counts and peripheral blood smears. Some years previously, the woman had been anaemic and treated with B_{12}. For some months prior to admission, the patient had taken a supplement containing 0.33 mg/tablet between once and "at least 3 times" a day – total dose being 0.33 to ≥ 1 mg. It was concluded that diagnosis had been obscured by the consumption of multi-vitamins containing folic acid and recommended that it should not be included in such supplements.

Baldwin and Dalessio, 1961.

42. In the first case of spinal cord degeneration reported by these authors, a 61 year old woman had been given folic acid treatment for recurring anaemia (which had been previously treated with liver extract) before presenting at hospital. She had been given a supplement of 5 mg folic acid plus a 0.2 mg and 1 mg supplement which also contained vitamin B_{12} and other B group vitamins, total dose 6.2 mg folic acid/day. The anaemia had responded to the therapy and the patient took the supplements for 5 years. A year prior to hospital admission, she became aware of tingling and numbness in her lower extremities, which progressed such that she had difficulty in walking. Haemoglobin and red cell count were in the normal range as was a bone marrow aspiration. The patient was then treated with B_{12} and the neurological symptoms resolved. This case was not included by IOM in their review.

43. In a second case, a 73 year old man presented with tingling and loss of sensation in his hands and feet. He was considered to have peripheral neuropathy of "the undetermined type" and given vitamin supplement containing a daily dose of 0.5 mg folic acid which were taken for 16 months. The symptoms initially resolved slightly but then deteriorated. On admission, haemoglobin levels and bone marrow examination were unremarkable but haematocrit and red cell count was low with macrocytosis being observed. Pernicious anaemia was diagnosed and the patient was treated with B_{12}. The authors concluded that the folic acid dose in this case had

not restored the haematological parameters to normal but had prevented profound anaemia occurring, but in both cases the use of folic acid had obscured the diagnosis of pernicious anaemia.

Allen et al., 1990

44. This study reported on a number of patients [?] with cobalamin deficiency who were treated erroneously with folic acid. Of these, 4 were given oral doses of 0.4 or 1 mg/day for folic acid for 3-18 months. Two of the patients had pernicious anaemia, while the others had an ileal resection or jejunal diverticulosis. Three of the patients had positive haematological responses to the treatment of which three deteriorated neurologically, while one of the patients did not respond to folic acid but also deteriorated neurologically. The intention of the study was to assess whether the serum cobalamin assay was adequate to diagnose deficiency since the clinical picture was diverse and whether measurement of metabolites would help to distinguish B_{12} (cobalamin) from folate deficiency.

Data from animal studies

Agamanolis et al., 1976.

45. Severe vitamin B_{12} deficiency was produced in 9 Rhesus monkeys by feeding them a vitamin B_{12} depleted diet for 5 years; 3 control animals were supplemented with vitamin B_{12}. Three of the vitamin deficient monkeys were supplemented with folic acid, initially a 5 mg i.m. dose weekly, followed by 5 mg/day in drinking water 5 days/week. The monkeys were periodically observed and filmed in a “room sized cage”; they also underwent regular neurological and ophthalmological examinations. The examinations included evaluation of muscle strength, bulk and tone, deep tendon reflexes, pupillary and ocular function and response to pinprick. Ophthalmoscopic examination and retinal photography were carried out regularly following the 43rd month of the study when an autopsy of one of the animals revealed pathological changes to the optic nerve. Full autopsies were performed on two monkeys that died at 39 and 53 months (both B_{12} deficient) and five animals who were sacrificed at 43, 44 (folate supplemented only), 47, 48 (B_{12} supplemented control) and 50 months.

46. Spinal cord disease was reported in two B_{12} deficient monkeys at 48 and 52 months and in two B_{12} deficient monkeys who were supplemented with folate at 37 months. Spinal cord disease was absent in four B_{12} deficient monkeys, one of the three monkeys who were supplemented with folate only and in the three B_{12} supplemented controls. Degeneration of the visual pathway was observed in the B_{12} deficient animals at 39*[8], 43*, 45, 45 and 53* months and in the three folate only supplemented animals at 33 months. Pathological examination showed marked loss of myelin and axons in the peripheral visual pathways in a discrete patch occupying 30-50% of the cross-sectional surface corresponding to the papillomacular bundle. Severe trans-synaptic degeneration occurred in the lateral geniculate bodies of the three most severely affected monkeys, characterized by marked loss of neurons and degenerative changes in the remaining ones. Degeneration of the visual pathways

[8] Where marked with an asterisk, the degeneration was first observed at autopsy.

was not seen in the controls. Damage to the white matter of the spinal cord was seen in 4 monkeys, damage to the cranial nerves in five animals and damage to the central white matter in four. No effects were seen in the peripheral nerves. The authors noted that the lesions in the optic nerve and spinal cord appeared 10-11 months earlier in the 3 B_{12} deficient monkeys who had been supplemented with folic acid. They stated that this was consistent with the suggestion that folic acid treatment worsened the symptoms of neurological damage in patients with pernicious anemia (though no specific reference is given). It was noted that all the animals received physiological does of folate in the diet. The neuropathologic changes were consistent with those seen in human SCD. The deficient monkeys showed a progressive decrease in serum and liver B_{12} levels and red blood cell count.

47. With regard to the study in monkeys, it was noted by the SCF amongst others, that the visual lesions observed in the monkeys are only rarely seen in humans.

Van der Westhuyzen et al., 1982

48. Nitrous oxide inactivates B_{12}[9] when administered to fruit bats, resulting in severe neurological impairment leading to ataxia, paralysis and death. This occurs after 6 weeks in B_{12} depleted animals and 10 weeks in B_{12} replete animals. The bats were caught in the wild and maintained in captivity on an all fruit diet, washed or peeled to ensure that no insects or micro-organisms were consumed. Vitamin supplements were also provided. Bats who were not supplemented with B_{12} became deficient after 9-12 months. The bats were exposed to nitrous oxide for 90 minutes per day. Two sets of experiments were conducted; in the first, the bats were already B_{12} deficient and were given approximately 1.54 mg/kg bw folic acid, 1.15 mg/kg bw formyl tetrahydrofolate, 0.77 g/kw bw methionine or were controls. The fruit diet provided 22 µg folate/day. In the 12 control animals, neurological impairment became apparent an average of 5 weeks after N_2O exposure. This was characterized by a reluctance to use the hindlimbs, inability to use a clawhold and inability to fly. The animals became immobile and died within 1-2 days of that occurring. The animals were moribund by 6-8 weeks. In the 5 or 6 animals receiving either folic acid or formyl folate respectively, the onset of neurological impairment was more rapid, being 4.3 and 4.5 weeks respectively. This was not statistically significant (method uncertain). However, the time in which flying was reduced to hopping was also significantly decreased from 6.2 weeks in the controls to 4.8 weeks in the bats given folic acid or formyl folate. In bats supplemented with methionine, there was no indication of neurological impairment and flight was normal.

49. In the second set of experiments, the bats were B_{12} replete before treatment and were controls or treated with methionine or methionine and folic acid for up to 12 weeks. In the 8 controls, neurological impairment was evident after an average of 9.2 weeks, with flight being reduced to hops around 9.6 weeks; half of the animals were moribund by 11 weeks. In the 5 or 6 bats treated with methionine or methionine + folic acid respectively, neurological impairment was not apparent and flight was not affected. None of the N_2O treated bats showed histological changes in the brain or spinal cord. In particular, the neurons appeared healthy and there was no evidence

[9] The nitrous oxide oxidises the active reduced cob(I)alamin to from cob(III)lamin. The cobalamin (B_{12}) requiring enzyme methionine synthetase is also inactivated).

of demyelination. It was suggested that as bats shown signs of early cord damage after prolonged B_{12} deprivation, there had been insufficient time for the exposure to N_2O to be apparent as histological damage.

50. It was concluded that supplementation of the diet with folic acid caused acceleration of the neurological lesions. Administration of formyltetrahydrofolate produced a similar aggravation of the neurological lesions. Supplementation with methionine protected the bats from neurological impairment but failed to prevent death. The methionine protected against the exacerbating effects of folate, preventing the development of neurological changes. This suggested that the lesions arising from B_{12} deficiency may be related to a deficiency in the methyl donor S-adenosylmethionone which follows diminished synthesis. The mechanism for the exacerbation of neurological symptoms by folic acid was considered uncertain but has been suggested to be due to a "folate trap" where folate becomes trapped in an unusable form due to the lack of the B_{12} dependent methionine synthetase activity. However, the authors propose that the accumulation of a methylated folic acid intermediate may be responsible for the neurological damage.

51. The SCF noted there were methodological flaws in the initial fruit bat study, notably that the control animals were not given sham injections and the observations of flight being reduced to hops, was very subjective. The B_{12} deficient bats receiving the oral folic acid reached the same stage of neurological impairment slightly, but not significantly, earlier than the untreated ones.

Van der Westhuyzen and Metz 1983

52. In a follow up experiment to assess S-adenosyl methionine levels, wild caught fruit bats were treated with N_2O for up to 13 weeks as above. Of these, the 6 controls who received no other treatment developed ataxia and paralysis leading to death in an average of 9.8 weeks, those also receiving a folic acid supplement of approximately 1.4 mg/kg bw became ataxic earlier at a mean 8.8 weeks, while the bats receiving a methionine supplement survived for a significantly longer period. Mean brain weights were increased in all the animals receiving the N_2O treatment compared to untreated bats. S-adenosyl methionine levels were also higher in the brains of N_2O treated animals and only slightly lower in the liver, suggesting that depletion of S-adenosyl methionine was not related to the neuropathy.

Additional studies used by EVM

Marshall, 1960,

53. It has not been possible to obtain this paper and no abstract is available. It is unclear if it a study or discussion.

Vilter et al, 1950

54. This study reported on 36 of an original group of 42 patients with pernicious anaemia who had been treated with 30 mg folic acid, three times weekly. Of these patients, 11 were maintained satisfactorily, 4 died from disease unrelated to their anaemia but were maintained satisfactorily up to their deaths, 5 experienced haematological relapse, 7 experienced neurological relapse, 7 showed

manifestations of haematological and neurological relapse concomitantly and 2 developed manifestations of haematological relapse followed shortly by neurological relapse. 14 patients in all experienced haematological relapse an average of 30 months after the treatment was changed from liver extract to folic acid. Patients experiencing relapses were then investigated for their responses to other treatments. For example, 4 patients with haematological relapse but little or no neurological deterioration were given increased doses of folic acid, 50 mg daily. Peripheral blood cell counts rose but the macrocytosis generally persisted. After 2-11 months on the treatment, the values fell again accompanied by the appearance of SCD. Patients were subsequently treated with parenteral liver extract or B_{12}.

Vilter et al, 1960

55. This paper discusses the background to megaloblastic anaemias and the influence of vitamin deficiencies. Data are reported on 18 patients and their vitamin status and in some instances their response to folic acid is noted.

Hansen and Weinfeld, 1962

56. Patients with pernicious or megaloblastic anaemia were given small doses of folic acid or B_{12} to examine the idea that a “mass action” effect resulted in a different response when high doses rather than low doses of folic acid were given. Some of the pernicious anaemia patients were given 0.1-0.4 mg/day i.m. while others received a multi-vitamin preparation with 1-3 mg/day folic acid. Of the patients receiving the injections, 4 had increased numbers of reticulocytes but not to the extent that their haemoglobin and red cell count would have implied. In the patients receiving the multi-vitamin the responses were similar to patients that had received “pharmacological” doses, with an increased in reticulocyte number 15 days after therapy began. It was concluded that the dose affected the response in both types of anaemia and that a small amount of B_{12} was necessary even for folic acid dependent anaemia.

57. Two other 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. These have not been considered here.

Additional studies used by SCF

58. No additional studies were cited by SCF.

Other studies not used by IOM, SCF or EVM

59. Meyer (1946). Eight cases were reported, these had been treated with oral doses of up to 50 mg folic acid. The folic acid failed to prevent the development or progression of neurological symptoms. However, improvements in red cell count and haemoglobin did occur. Liver extract was found to enhance the effect of folic acid.

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