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Scientific Advisory Committee on Nutrition

Folate and Disease Prevention

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Preface

The purpose of this report is to update the report by the Committee on Medical Aspects of Food Policy (COMA) 'Folic acid and the prevention of disease (2000). The COMA report reviewed the available evidence linking folate with human health, particularly the need for adequate intakes of folate at the time of conception to reduce the risk of a pregnancy affected by a neural tube defect (NTD). The report concluded that fortification of flour with folic acid would reduce the number of NTD-affected pregnancies. COMA did not find sufficient evidence of other health benefits of folate, e.g. reducing the incidence of cardiovascular disease, to justify fortification for that purpose. After considering this advice and listening to the views of stakeholders, the Food Standards Agency Board recommended to Health Ministers in 2002, that mandatory fortification should not be introduced at that time. This was due to concerns about the impact on older adults with vitamin B12 deficiency who might have diagnosis and treatment delayed, and also because of concerns about limiting consumer choice.

A significant quantity of evidence has emerged on the health effects of folate since COMA reported in 2000. The evidence can broadly be divided into two types: scientific research studies on the potential benefits of folic acid; and impact assessments on the effect of mandatory fortification polices. The availability of this data led to the decision by the Scientific Advisory Committee on Nutrition (SACN) in 2003, to assess the evidence that had arisen since the COMA report. This decision coincided with a request from the Minister for Health in 2004, to consider the wider implications of folic acid fortification.

SACN's draft report on folate and disease prevention was made available for comment in November 2005 and I would like to thank all those who responded. All the comments from stakeholders were carefully considered by the Committee before finalisation of the report. SACN's risk assessment is now available for consideration by the Food Standards Agency in providing advice to the Health Ministers regarding the implications of fortifying flour with folic acid.

In order to draw its conclusions and make recommendations, SACN had to consider a large body of evidence of variable type and clarity. Clear, strong

evidence from randomised controlled trials (RCTs), has shown large benefits of folic acid supplementation in reducing the risk of pregnancies affected by NTDs. This evidence is supported by the results of mandatory fortification polices in the USA, Canada and Chile where substantial reductions in the incidence of NTD-affected pregnancies have been observed.

Against this strong evidence of known benefit, SACN had to weigh the evidence of potential harm of folic acid fortification, particularly in relation to the possibility of delaying diagnosis of vitamin B12 deficiency and increasing the progression of pre-existing polyps to bowel cancer. Data to support these associations are much less clear than those on folate and NTDs. For example, the evidence relating to the masking of B12 deficiency by folic acid is limited to often poorly described case reports, while the evidence on cancer risk indicates a potential dual effect of the nutrient, depending on age, possibly genotype, and intake of other nutrients.

SACN also assessed current folate intakes of the UK population using the National Diet and Nutrition Survey data set. This has shown wide variation in folate intakes. Some people, particularly women of reproductive age and the elderly, have low intakes while others have intakes close to or exceeding the upper level of folic acid intake considered to be safe (1mg/day for adults). These high intakes are associated with consumption of foods, such as fat spreads and breakfast cereals, which are fortified with folic acid on a voluntary basis and/or folic acid containing supplements. The Committee also explored the potential impact of fortifying flour with folic acid at different levels on population intakes of folate and on reducing NTD risk.

Following a detailed assessment of the evidence, SACN is recommending mandatory fortification of flour with folic acid as the most effective way to increase folate intakes of women most at risk of NTD-affected pregnancies, provided voluntary fortification is controlled and advice is given about supplement use. Such a policy would also be of net benefit to the UK population as it would lead to a redistribution of folic acid intakes, improving the folate intake of low consumers while reducing the intakes of high consumers.

Because of the uncertainties relating to potential harmful effects of folic acid, SACN agreed that the recommendation for mandatory fortification should include the proviso that population average folic acid intakes, and numbers of

people consuming more than the upper levels considered to be safe, should not increase substantially if mandatory fortification is introduced.

SACN is also recommending that women should continue to follow current advice to take folic acid supplements prior to and 12 weeks after conception to minimise the risk of NTDs. It is clear however that this advice alone is unlikely to be successful at reducing NTD rates in the UK. In this country about half of all pregnancies are unplanned, so by the time the pregnancy is recognised the developmental window for the neural-tube, when folate requirements are high, has passed. No other countries that have implemented policies which rely only on advice to take folic acid supplements have been effective at reducing NTD rates.

SACN also considered other beneficial effects that have been proposed for folic acid, such as reduced risk of cardiovascular disease. The Committee concluded that there is currently insufficient evidence to support mandatory fortification of flour with folic acid on these grounds.

Since the report was finalised, two major manufacturers of fat spreads have announced a significant reduction in the level of folic acid fortification. Although it has not been possible to publish an assessment of how this will affect high consumers of folic acid, there are indications that these actions will reduce the number of people exceeding intakes considered to be safe. However, this reduction is unlikely to affect folate levels of women most at risk of NTD-affected pregnancies.

I should like to thank members of SACN, especially Professor Sheila Bingham and the other members of the SACN Folate Subgroup. I would also like to thank Dr Chris Bates (MRC Human Nutrition Research, Cambridge), Dr Christine Pfeiffer (Centre for Disease Control and Prevention [CDC], Atlanta, USA) and Professor Elaine Gunter (Advisor to NHANES Laboratory, CDC) for their very helpful comments regarding measurements of folate status.

Professor Alan Jackson
Chair of the Scientific Advisory Committee on Nutrition
December 2006

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1 Summary

Background

1. In its report, *Folic Acid and the Prevention of Disease* (Department of Health, 2000), the Committee on Medical Aspects of Food and Nutrition Policy (COMA) concluded that universal fortification of flour with folic acid (240µg/100g) would significantly reduce the number of conceptions and births affected by neural tube defects (NTDs). In July 2002, after consultation, the Food Standards Agency advised the Health Ministers not to introduce mandatory fortification at that time, due to outstanding concerns about vitamin B12 deficiency. In 2004, the Scientific Advisory Committee on Nutrition (SACN) was asked by Health Ministers to consider the wider impact of folic acid fortification, particularly in relation to the elderly.

Methodology

2. The SACN *Framework for the Evaluation of Evidence* (2002) was used as the basis to identify and assess evidence published since the COMA report.

3. The main issues considered were: UK dietary intakes of folate and other B vitamins; trends in rates of NTD-affected pregnancies in the UK and in countries with fortification policies; possible effects of fortification on people aged 65 years and over with vitamin B12 deficiency; the relationship between folate and cardiovascular disease, cancer, cognitive function, depression, and bone health. The report also explored the potential impact of mandatory fortification of flour with folic acid on: reducing NTD risk; total folate intakes of the population; the intakes of people aged 65 years and over with low vitamin B12 status.

Dietary intakes and status of folate, vitamins B2, B6, and B12 in the UK

4. Data from the National Diet and Nutrition Survey (NDNS) series was used to assess the intakes and status of the UK population.

5. Although average daily folate intakes were above the recommended nutrient intake (RNI) in all age groups, there was some evidence of marginal folate status in young women and people aged 65 years and over. Values for folate

status and normal ranges are, however, dependent on assay method which makes it difficult to assess adequacy and deficiency determined by one assay method against reference ranges determined by another method.

6. Lower intakes of vitamin B2 and marginal vitamin B2 status were widespread in all age groups, particularly in women, girls, and boys. Most age and sex groups had adequate vitamin B6 intakes but a relatively high proportion of females aged 15-24 years, and over 65 years, had intakes below the lower reference nutrient intake (LRNI). Marginal vitamin B6 status was found in 10% of people aged 4-64 years with indications of higher prevalence in adults aged 65 years and older. Although there was no evidence of inadequate vitamin B12 intakes, serum concentrations indicated poor vitamin B12 status in older adults.

Folate

7. The term *folate(s)* describes a family of B-group vitamins and includes naturally occurring folates found in foods and *folic acid*, a synthetic form used in supplements and food fortification.

Recommended upper intake level for folic acid in the USA, Europe, and the UK

8. In the USA and Europe, the tolerable upper intake level (UL) of 1mg/day for adults has been set for folic acid. ULs set for children were extrapolated from the UL for adults on the basis of body weight. In the UK, a guidance level (GL) of 1mg/day of folic acid has been set for adults. As GLs were not set for children, ULs were used for the modelling exercise on the potential impact of folic acid fortification of flour in the UK (see paragraphs 28-32).

Folate and NTD

9. Although supplementation with folic acid is advised prior to conception until the 12th week of pregnancy approximately half of all pregnancies are unplanned, which limits the value of recommendations. European Union countries with policies recommending women to consume folic acid supplements to reduce NTDs have observed no effect on reducing the number of NTDs.

10. Available data on the number of NTD-affected pregnancies are insecure due to under-reporting. Taking account of under-reporting in England and Wales,

but not in Scotland and Northern Ireland, there were approximately 700-900 NTD-affected pregnancies in the UK in 2003.

Folic acid fortification strategies and the incidence of NTD in other countries

11. Countries that have introduced mandatory fortification (USA, Canada, Chile) have reported significant reductions in NTD-affected pregnancies of 27% to over 50%. As a result of the practice of *overage*, the impact of fortification on folate status has been greater than predicted.

Possible adverse effects of mandatory fortification of flour with folic acid

Risks to older people with vitamin B12 deficiency

12. There are concerns that mandatory fortification of flour with folic acid might have adverse effects on neurological function in people aged 65 years and over with vitamin B12 deficiency. Clinical signs of vitamin B12 deficiency are anaemia and/or neurological impairment. Treatment with folic acid can alleviate or *mask* the anaemia and therefore delay the diagnosis of vitamin B12 deficiency, which can lead to the irreversible and serious condition of subacute combined degeneration of the spinal cord.

13. The assessment of vitamin B12 deficiency is complicated by the limitations of current diagnostic techniques. Low serum concentrations of vitamin B12 are not always predictive of a clinical response to vitamin B12 therapy. The prevalence of low vitamin B12 status in the UK has been estimated to be 5% in people aged 65-74 years and 10% in people aged 75 years and over.

14. Evidence suggests that *masking* of vitamin B12 deficiency is not associated with doses of folic acid up to 1mg/day. There are no reports from countries that have introduced mandatory fortification indicating deleterious effects on older people with low vitamin B12 status.

Epilepsy

15. It has been suggested that folic acid modifies the pharmacokinetics of phenytoin, an anti-epileptic drug, and may lower serum phenytoin concentrations leading to poorer seizure control. Evidence from Canada has shown that mandatory fortification, estimated to provide an average of

200µg/day of folic acid, did not lower serum phenytoin concentrations in epileptic patients.

Multiple births

16. There is no substantive evidence to suggest that folic acid fortification is associated with multiple births resulting from natural conception. However, high intakes of folic acid may increase the likelihood of twin births in women undergoing multiple embryo transplant fertility treatment.

Embryo selection

17. It has been proposed that the use of folic acid in pregnancy could increase the survival of embryos with genotypes that are associated with deleterious effects. There is no substantive evidence from countries where supplementation is advised or mandatory fortification has been introduced to support this.

Anti-folate chemotherapy

18. There are concerns that folic acid may reduce the efficacy of anti-folate drugs such as methotrexate (MTX), which is widely used in chemotherapy regimens and for treatment of autoimmune diseases. There are insufficient human data on the effect of folic acid on antifolate medication or the doses at which folic acid might affect their action to conclude that mandatory folic acid fortification would modify their efficacy.

Unmetabolised folic acid in the systemic circulation

19. The appearance of unmetabolised folic acid in the systemic circulation has raised concerns regarding the long-term effects of high intakes of folic acid. Overall, there are insufficient data in humans to assess the long-term effects of exposure to unmetabolised folic acid in the systemic circulation.

Folate, B vitamins, and chronic disease

Folate and cardiovascular disease

20. Observational studies have suggested a protective effect of increasing folate intake, but not circulating folate concentrations, on CVD risk. No randomised controlled trials have demonstrated a beneficial or harmful effect of folic

acid supplements on CVD risk. One RCT found an increased CVD risk with supplementation of folic acid in combination with vitamins B12 and B6.

Folate and cancer

21. Some animal studies suggest that folic acid may have dual modulatory effects on cancer: inhibiting tumour development in normal tissues but promoting the progression of established neoplasms. The doses of folic acid used in these studies were considerably higher than the amounts that would be consumed by humans as a result of fortification.

22. Although evidence from prospective studies in humans suggests a trend towards a protective effect of folate intake on colon cancer risk, some studies did not adjust for all confounding factors. No RCTs designed to investigate the relationship between folic acid and cancer incidence have yet reported.

23. Time trends for colorectal cancer (CRC) incidence in the USA and Canada show that mandatory fortification of foods with folic acid occurred at around the same time as non-significant increases in CRC incidence. If this was caused by folic acid fortification, the effect of folic acid on cancer progression would have to have been immediate, which may not be plausible. The increase in rates occurred at different times for men and women and in different age groups. The timing of changes in average blood folate concentrations of the USA population was also not clearly consistent with changes in CRC incidence.

24. The evidence for an association between folic acid and increased or reduced cancer risk in humans is equivocal.

Folate and cognitive function

25. No RCTs have shown an effect of folic acid or vitamin B12 on cognitive function in older people, which might be due to insufficient sample size and duration. Overall, the evidence for either beneficial or deleterious effects of folic acid or vitamin B12 therapy on cognitive function in older people is presently inconclusive.

Folate and depression

26. There is insufficient evidence from prospective studies and RCTs to suggest an association between folate and depression.

Folate and bone health

27. There is insufficient evidence to suggest beneficial effects of folic acid on bone health and no evidence to suggest any deleterious effects.

The potential impact of mandatory fortification of flour with folic acid

28. The potential effect of fortifying flour with different doses of folic acid (100-450µg/100g) on the total folate intake (including current levels in fortified foods, supplement use, processing losses, overage) of different population age groups was investigated by modelling intake data from the NDNS series. The purpose of the modelling exercise was to explore the effect of mandatory folic acid fortification of flour on: risk of NTD-affected pregnancies; the number of people with folic acid intakes above the UL/day; and the number of people aged 65 years and over, with low vitamin B12 status, exceeding folic acid intakes of 1mg/day.

29. AS GLs were not set for children in the UK, ULs were used for the purpose of the modelling exercise (see paragraph 8).

30. Results from the modelling exercise showed that at current levels of folic acid intake (including intake from voluntarily fortified foods and supplements), mandatory fortification of flour with folic acid would progressively: reduce NTD risk; increase the proportion of people in the population with folic acid intakes above the UL/day and the number of people aged 65 years and over with low vitamin B12 status consuming more than 1mg/day of folic acid.

31. If no foods were voluntarily fortified, the option that would provide the optimum balance between benefits and possible risks is mandatory fortification at a level of 300µg of folic acid per 100g flour (excluding wholemeal flour). At this level it is estimated that compared to current levels:

- 77-162 NTD-affected pregnancies/year could be prevented (11-18% risk reduction);
- the proportion of the population with intakes below the RNI would be reduced from 23% to 5%;
- the number of people with folic acid intakes above the UL/day would be reduced by 12,000;
- there would be no change in the number of adults aged 65 years and over, with low vitamin B12 status, exceeding intakes of 1mg/day.

32. Without intakes of folic acid from voluntary sources, mandatory fortification of flour with folic acid would confer a more even distribution of folic acid intakes across the population compared to current voluntary fortification and supplement use. This means that women at greatest risk of NTD-affected pregnancies, i.e. those with the lowest folate intakes, would be reached through mandatory fortification.

Conclusions

33. The current recommendation is that women planning a pregnancy should supplement their diet with 400µg/day of folic acid (5mg/day for women with a previous pregnancy affected by NTD) prior to conception until the 12th week of pregnancy. About half of all pregnancies are unplanned which limits the value of recommendations for preconceptional supplementation. Policies in the EU, recommending folic acid supplementation to reduce NTD-affected pregnancies, have been ineffective.

34. Introduction of mandatory fortification of flour with folic acid at current levels of folic acid intake (including intake from voluntary fortification and supplements) would reduce the risk of NTD-affected pregnancies in the UK. However, it would also increase the proportion of people in the population at risk of exceeding folic acid intakes above the UL/day and the number of people aged 65 years and over with low vitamin B12 status at risk of consuming more than 1mg/day of folic acid.

35. There are approximately 127,000 people in the UK who are currently consuming intakes of folic acid above the UL/day from voluntarily fortified

foods and supplements. As there are presently no controls on the levels of folic acid which can be added to foods, the number of people with intakes above the UL/day could be higher if levels in these foods are increased in the future.

36. Without the contribution of folic acid from voluntarily fortified foods, mandatory fortification would reduce risks of intakes exceeding the UL/day for folic acid relative to the current practice of voluntary fortification. This is because voluntary fortification of foods with folic acid and inappropriate supplement use are harder to quantify and control and, unlike flour, their consumption is very variable.

37. Replacement of voluntary folic acid fortification of certain foods with mandatory fortification of flour would result in a redistribution of folic acid intakes within the population and would be the most effective way to reach those sections of the population with the lowest folate intakes, i.e., younger women from the most socioeconomically deprived areas.

38. Without the contribution of folic acid intakes from voluntarily fortified foods, the optimal level for mandatory fortification of flour with folic acid would be 300µg/100g flour. This level would be effective in reducing NTD risk without increasing the number of people with intakes of folic acid above the UL/day or the number of adults aged 65 years and over, with low vitamin B12 status and folic acid intakes above 1mg/day. Exempting wholemeal flour from fortification would have little effect on NTD risk but would further reduce numbers with intakes of folic acid above the UL/day.

39. There is currently insufficient evidence from RCTs examining chronic disease risk (cardiovascular disease, certain cancers, bone disease and age-related cognitive decline) to either support or advise against mandatory fortification of flour with folic acid on these grounds.

40. There is currently insufficient evidence for an adequate risk assessment of folic acid and cancer risk or the intake levels which might be associated with risk. A substantial increase in current average population intakes of folic acid and the numbers consuming more than the GL/UL per day for folic acid should therefore be avoided.

Recommendations

41. As previously recommended by COMA (DH, 2000), all women who could become pregnant should take 400µg/day folic acid as a medicinal or food supplement prior to conception and until the twelfth week of pregnancy. Women with a history of a previous NTD-affected pregnancy are advised to take 5mg/day of folic acid prior to conception and until the twelfth week of pregnancy. This recommendation is applicable even if mandatory fortification of flour with folic acid is introduced.

42. Individual long-term intakes of folic acid from fortified foods and supplements above the GL/UL per day for folic acid should be avoided. The risk currently posed by voluntary fortification of food with folic acid and supplement use in contributing to intakes above the GL/UL per day for folic acid needs to be addressed.

43. Mandatory fortification of flour with folic acid would improve the folate status of women most at risk of NTD-affected pregnancies. However, if mandatory fortification is combined with the current practice of voluntary fortification of foods with folic acid and inappropriate supplement use, the numbers of people consuming levels of folic acid above the GL/UL per day would be substantially increased.

44. Mandatory fortification should only be introduced in the UK if it is accompanied by:

- Action to reduce folic acid intakes from voluntarily fortified foods to ensure that the numbers of people with intakes above the GL/UL per day do not exceed current levels and there is no substantial increase in mean intakes or in the folate status of the UK population;

- Measures for careful monitoring of emerging evidence on the effects of long-term exposure to intakes of folic acid above the GL/UL per day and postulated adverse effects, including neurological damage, CVD, and cancer.

45. The establishment of a new baseline for folic acid intakes and blood folate concentrations will be required prior to fortification to ensure that mandatory fortification does not lead to substantial increases in folic acid

intake or folate status and so that trends can be monitored in future surveillance programmes. The adoption of a common standard analytical method to measure folate status at baseline and all future surveillance studies will also be required as well as the establishment of suitable reference ranges to predict folate adequacy and deficiency.

46. If mandatory fortification is introduced:

- Careful consideration should be given to the issue of overage;
- The evidence on benefits and postulated adverse effects should be reviewed after a period of five years.

47. Clear guidance is needed on the use of folic acid containing supplements by the general population.

48. More reliable diagnostic indices to identify vitamin B12 deficiency should be developed. The development of a clinical strategy to manage issues related to vitamin B12 is necessary irrespective of a decision on future mandatory fortification of flour with folic acid.

49. The prevalence of poor vitamin B2 status in the UK needs to be addressed.

2 Introduction

50. In 2000, the Committee on Medical Aspects of Food and Nutrition Policy (COMA) published its report on *Folic Acid and the Prevention of Disease* (Department of Health [DH], 2000). In September 2003, the Scientific Advisory Committee on Nutrition (SACN) requested an update on the evidence that had arisen since the COMA report was published. This was followed, in 2004, by a request from Health Ministers to consider the wider impact of folic acid fortification, particularly in relation to the elderly.

Terms of Reference

51. The SACN Folate Subgroup was established in February 2004 with the following terms of reference:

- Consider the evidence since the COMA report, *Folic Acid and the Prevention of Disease* (DH, 2000);
- Advise on any gaps in the evidence base, with particular reference to the issue of folic acid masking the diagnosis of vitamin B12 deficiency;
- Consider when and how to review the previous COMA risk assessment.

52. After initial consideration of evidence published since the COMA report, it was agreed there were sufficient data to warrant a full risk assessment.

Background

53. In its report, *Folic Acid and the Prevention of Disease* (DH, 2000), COMA concluded that universal fortification of flour with folic acid would significantly reduce the number of conceptions and births affected by neural tube defects (NTDs). Following a formal public consultation in 2000, a stakeholder meeting on folic acid fortification was convened by the Food Standards Agency (FSA) and the Health Departments in March 2002.

54. Fortification of flour with folic acid was considered by the Agency's Board in May 2002 (FSA, 2002a). In July 2002, the Agency advised the Health Ministers responsible for making a decision on folic acid fortification not to introduce

mandatory fortification at that time, due to outstanding concerns about vitamin B12 deficiency (see section 6) (FSA, 2002b).

55. The Health Ministers responded in June 2004 and agreed with the Agency's advice. The letter stated:

- The wider impact of folic acid fortification, in particular the benefits and risks to older adults, should be assessed.
- The matter will be reassessed following SACN's consideration and as the evidence becomes available from overseas.
- At the same time, options will be considered to increase the usage of preconceptual supplements, and increase dietary intakes of folate, for example, through the Healthy Start and 5-A-DAY Programmes and to address the concerns relating to prevalence and identification of vitamin B12 deficiency.

56. Both Healthy Start and 5-A-DAY are ongoing and management of vitamin B12 deficiency is under discussion.

57. The COMA report, *Folic Acid and the Prevention of Disease* (DH, 2000), concluded that fortifying flour with 240µg folic acid/100g would have a significant effect in preventing NTD-affected pregnancies without resulting in unacceptably high intakes in any population group. This was based on the assumption that the flour content of bread was over 70%. After publication of the COMA report, it was realised that bread actually comprised about 60% flour and that to achieve the increase in folate intake estimated by COMA, would require fortification of flour with 280µg folic acid/100g (FSA, 2002a).

58. In addition to advising on folic acid fortification of flour, COMA concluded that all women who could become pregnant should continue to be advised to take 400µg folic acid per day as a medicinal or food supplement prior to conception until the twelfth week of pregnancy (DH, 2000).

Methodology

59. The SACN *Framework for the Evaluation of Evidence* (SACN 2002) was used as the basis to identify and assess evidence published since the COMA report (DH, 2000). The evidence base for this report is mainly restricted to prospective cohort studies and randomised controlled trials (RCTs) in humans although some evidence from cell studies and animal data was considered.

60. The report considers: UK dietary intakes of folate and other B vitamins involved in folate metabolism; trends in the rates of NTD-affected pregnancies in the UK and in countries where fortification with folic acid has been introduced; the possible effects of fortification on people aged 65 years and over with vitamin B12 deficiency; the relationship between folate and cardiovascular disease, cancer, cognitive function, bone health; and other proposed health benefits and risks to the UK population of fortifying flour with folic acid.

61. The potential impact of mandatory fortification of flour, at different levels of folic acid, on the total folate intakes of the population was also explored. This information was used to estimate the reduction in NTD risk associated with different levels of fortification. Additionally, the effects of different levels of fortification on the intakes of people aged 65 years and over with low vitamin B12 status were considered.

62. The relationship between folate intake and neural tube defects was not considered as the evidence had previously been reviewed by COMA (DH, 2000).

63. The procedures for finalisation of the report can be found in Annex 1.

3 Folate

64. The term *folate(s)* describes a family of B-group vitamins comprising an aromatic pteridine ring linked to p-aminobenzoic acid and one or more glutamate residues. There are large numbers of naturally occurring folates but methyl- and formyl- tetrahydropteroylpolyglutamates are the main forms found in foods.

65. *Folic acid* (pteroylmonoglutamic acid) is the synthetic form commonly used in supplements and food fortification. Folic acid is more stable in foods than natural folates and is better absorbed.

66. The bioavailability of natural folates found in food is approximately 50% lower than that of folic acid (Gregory, 1997). Consumption of natural food folate has been found to result in a significantly smaller increase in red blood cell folate concentration compared to folic acid supplements or folic acid in fortified cereals (Cuskelly *et al*, 1996).

67. The following terms are used in this report:

- *Folate(s)* refers to all compounds comprising an aromatic pteridine ring linked to *p*-aminobenzoic acid and one or more glutamate residues, and includes natural folates and folic acid;
- *natural folate(s)* refers only to naturally occurring folate(s) found in foods;
- *folic acid* refers only to the synthetic form used in supplements and food fortification.

Function

68. Folate is required for cell division and cell maintenance. It acts as a co-enzyme in the transfer and processing of one-carbon units and also plays an important role in nucleotide synthesis, methylation, and gene expression; for example: in the synthesis of thymidine, which is essential for the *de novo* construction or repair of DNA, the re-methylation of plasma homocysteine

to methionine, and the 'site-specific' methylation of the cytosine base in DNA, which regulates gene expression.

69. The complex metabolic pathways governing the functions of folate are controlled by a number of enzymes such as methylene tetrahydrofolate reductase (MTHFR), methionine synthase (MTR), methionine synthase reductase (MTRR), and thymidylate synthate (TS). Inherited variants of the genes controlling these enzymes affect their activity and have been associated with folate status, inherited susceptibility to NTDs, as well as other conditions such as cardiovascular disease (see paragraphs 272-274) and cancer (see paragraphs 326–329).

Metabolism of folate and folic acid

70. Folates are broken down in the gut by folate conjugase in the mucosal brush borders from folate polyglutamates to the monoglutamate form. Upon absorption, the monoglutamates are converted in the mucosal cells to 5-methyl tetrahydrofolic acid (5-MTHF), which is usually the only form found in plasma. 5-MTHF is taken up by cells but cannot be retained intracellularly unless it is metabolised to tetrahydrofolate (THF). THF can be conjugated into polyglutamate forms, which prevents it leaving the cell.

71. Folic acid is not conjugated and is therefore more bioavailable. Before it can become active, folic acid has to be reduced to THF and methylated in the gut mucosa. The reduction is catalysed by dihydrofolate reductase (DHFR); THF is then converted to 5-MTHF, the form found in the circulation. The capacity for this conversion by gut mucosa is limited and single doses of oral folic acid, in excess of about 260µg, can lead to the appearance of unmetabolised folic acid in the systemic circulation (Kelly *et al*, 1997). The long term biological effects of exposure to unmetabolised folic acid in humans are unknown. The presence of unmetabolised folic acid in the systemic circulation is considered further in section 6, paragraphs 257 to 263.

Dietary sources of folate and folic acid

72. Folates are found in a wide variety of foods. Rich sources are liver, yeast extract, and green leafy vegetables such as spinach, kale, and Brussels sprouts. Lower amounts are found in other vegetables and fruit, including broccoli, spring greens, cabbage, parsnips, and oranges (DH, 2000).

73. The main sources of folic acid are supplements and fortified foods. In the UK, fortification of food products with folic acid is on a voluntary basis and unregulated. Foods which are voluntarily fortified with folic acid include a number of breakfast cereals (FSA, 2002c) and some brands of reduced/low-fat spreads (Annex 2). These foods and supplements make up a large proportion of the total folate intake of some individuals (see Annex 2).

74. The NDNS of adults aged 19-64 years (Henderson *et al*, 2003) shows that breakfast cereals provided 15% of total folate intake for men and women; supplements containing folic acid contributed 4% to the total folate intake of men and 14% to the total folate intake of women.

75 All folic acid fortified spreads are reduced or low-fat spreads. Information regarding the contribution of folic acid fortified reduced/low-fat spreads to folic acid intakes is not available from the NDNS of adults (Henderson *et al*, 2003) as the fortification of reduced/low-fat spreads was only introduced in 2002 (Unilever; personal communication, 2005), after the completion of field work for the NDNS. Data from the Expenditure and Food Survey show that reduced/low-fat spreads accounted for about 60% of yellow fat consumption in 2003-2004 (DEFRA[1] , 2005). Spreads fortified with folic acid presently occupy approximately 20% of the market for yellow fat spreads with one brand (currently fortified with 1000µg/100g) occupying 18.5% of the market share (Annex 2, Appendix 2). A few other yellow fat spreads are fortified at the same or lower levels.

Other B vitamins involved in folate metabolism

76. Several other B vitamins are involved in folate metabolism, including vitamin B2 (riboflavin), vitamin B6 and vitamin B12.

77. Vitamin B2 functions prosthetically in the forms flavin mononucleotide and flavin adenine dinucleotide in flavoprotein enzymes involved in oxidation-reduction reactions, e.g. the conversion of 5, 10-methylenetetrahydrofolate to 5-methyltetrahydrofolate by methylenetetrahydrofolate reductase.

78. Vitamin B6 functions as a co-enzyme in its pyridoxal phosphate form in amino acid metabolism, e.g. the conversion of homocysteine to cystathionine by cystathionine b-synthase.

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79. Vitamin B12 acts as a co-enzyme in the methylcobalamin and 5'-deoxyadenosylcobalamin forms in the recycling of folate co-enzymes, e.g. the re-methylation of plasma homocysteine to methionine by methionine synthase, and the degradation of valine through methylmalonyl CoA. Vitamin B12 is also required for nerve myelination.

Current recommendations on folate intake

80. In 1991, revised dietary reference values (DRVs) for food energy and nutrients were agreed by COMA (DH, 1991). The reference nutrient intake (RNI) is the amount of a nutrient that is considered sufficient to meet the requirements of 97.5% of the population. The lower reference nutrient intake (LRNI) is the amount of a nutrient that is considered sufficient to meet the requirements of 2.5% of the population.

81. For adults, the RNI for folate is 200µg/day and the LRNI is 100µg/day. For infants and children, the RNIs are: 0-12 months, 50µg/day; 1-3 years, 70µg/day; 4-6 years, 100µg/day; 7-10 years, 150µg/day; the RNI for children 11 years and above is the same as that for adults. The LRNIs for infants and children are: 0-12 months, 30µg/day; 1-3 years, 35µg/day; 4-6 years, 50µg/day; 7-10 years, 75µg/day; the LRNI for children 11 years and above is the same as that for adults.

82. Details of how the DRVs for folate were derived and a comparison with the USA values are given in Annex 3. The DRVs for riboflavin, vitamin B6, and vitamin B12 are also provided in Annex 3.

83. Although most pregnancies in women consuming folate at the RNI will not be affected by NTDs, evidence from clinical trials has shown that folate intakes above the RNI during early pregnancy reduce the risk of NTD-affected pregnancies. All women who could become pregnant are therefore advised to take 400µg/day folic acid as a medicinal or food supplement prior to conception and until the twelfth week of pregnancy (DH, 1992).

84. To prevent recurrence of NTD in the offspring of men or women with spina bifida themselves or women with a previous pregnancy affected by NTD, folic acid supplements of 5mg/day are recommended[2] (DH, 1992).

2 Diabetes UK recommend that all women with diabetes planning a pregnancy should also take a 5mg dose of folic acid daily.

Recommended upper intake levels for folic acid in the USA, Europe, and the UK

85. No adverse effects have been reported with consumption of high amounts of natural folates found in food (Butterworth and Tamura, 1989). Concerns regarding the safety of high intakes are restricted to folic acid.

USA

86. In 1998, the Food & Nutrition Board (FNB) of the Institute of Medicine (IOM) in the USA reviewed the potential hazards of high intakes of folic acid (FNB, 1998) on: neurological impairment in individuals with vitamin B12 deficiency, general toxicity, reproduction and development, carcinogenicity, hypersensitivity, and intestinal zinc absorption. The most serious potential adverse effect of excessive intakes of folic acid was considered to be progression of neurological symptoms in individuals with vitamin B12 deficiency and this was used as the critical endpoint to set a Tolerable Upper Intake Level[3] (UL).

87. Case-reports of patients with vitamin B12 deficiency who showed development or progression of neurological complications following oral administration of folic acid were used to evaluate a dose-response relationship. A *lowest observed adverse effect level* (LOAEL) of 5mg was set, based on evidence that at doses of 5mg/day of folic acid there were over 100 reported cases of neurological progression; at doses below this amount, only 8 documented cases were found. An uncertainty factor (UF) of 5 was applied to the LOAEL because of the severity of the neurological complications observed and because a LOAEL rather than a *no observed adverse effect level* (NOAEL) was used to derive the UL. The LOAEL of 5mg/day was divided by the UF of 5 to obtain the UL of 1mg/day for adults. As no data were available on adverse effects for other life stage groups, ULs for children were extrapolated from the UL for adults on the basis of relative body weight (see Table 22, Annex 4).

Europe

88. In 2000, the European Scientific Committee on Food (SCF) set a UL for folic acid of 1mg/day for adults (SCF, 2000). The effects of high intakes of folic acid were considered in relation to the following safety issues: modification of

3 The Tolerable Upper Intake Level represents the highest level of daily nutrient that is likely to pose no risk of adverse health effects for almost all individuals in the general population.

vitamin B12 deficiency (masking of haematological symptoms/exacerbation of neurological symptoms), epileptogenic and neurotoxic effects, efficacy of folate antagonists used in chemotherapy, zinc absorption/status, carcinogenicity, and hypersensitivity.

89. The UL of 1mg/day for adults was based on the risk of progression of neurological symptoms in vitamin B12 deficient patients as the most serious adverse effect. It was concluded that in nearly all studies showing neurological relapse, dose levels of folic acid were above 5mg/day and data on the effect of dose levels of 1-5mg/day were limited to a few cases. The SCF set a LOAEL of 5mg/day and concluded that doses up to 1mg/day were unlikely to cause masking of the haematological signs seen with pernicious anaemia. ULs for children were set on the basis of body weight (see Table 23, Annex 4).

UK

90. In 2003, the Expert Group on Vitamins and Minerals (EVM) reported on safe levels of intake for vitamins and minerals in food supplements and fortified foods in the UK (EVM, 2003). Safe Upper Levels[4] (SULs) were set when supported by adequate data. A Guidance Level (GL), which represents an approximate indication of intakes that would not be expected to cause adverse effects, was set when there were insufficient data to determine a SUL. The EVM did not set a SUL for folic acid intake as the available evidence on adverse effects of folic acid were not considered to be sufficiently robust. A GL of 1mg/day was set for folic acid based on concerns that intakes above this level may mask signs of vitamin B12 deficiency. GLs were not set for children as there were no data reporting adverse effects in children.

4 The Safe Upper Level represents intakes that can be consumed over a lifetime without significant risk to health.

4 Dietary intakes and status of folate, vitamin B2, vitamin B6 and vitamin B12 in the UK

91. Data on dietary intakes and status were obtained from the British National Diet and Nutrition Surveys (NDNS) of adults aged 19-64 years (Henderson *et al*, 2003; Ruston *et al*, 2004), people aged 65 years and older (Finch *et al*, 1998), young people aged 4 to 18 years (Gregory *et al*, 2000), and children aged 1 ½ to 4 ½ years (Gregory *et al*, 1995).

92. In the NDNS series, dietary intake was assessed by weighed records of all foods consumed over: 7 consecutive days for adults aged 19-64 years and young people aged 4-18 years; 4 consecutive days for adults aged 65 years and over and children aged 1 ½ to 4 ½ years.

93. Data from these surveys were compared against the DRVs for the different age groups of the population.

Folate intake and status

Measurement of folate status

94. Red cell folate concentration is considered to be a better measure of long term folate status than serum folate because it reflects body stores at the time of red cell synthesis; serum folate concentrations reflect recent dietary intake. Analytical methods used to assess folate status are usually based on microbiological or radioimmunoassay; other methods include chemiluminescence, magnetic separation, ion capture and tandem mass spectrometry.

95. For all age groups in the NDNS series (from 4 years and above), red cell folate concentration below 230nmol/L (101µg/L) was considered severely deficient and concentrations between 230-345nmol/L (101-152µg/L) were considered to be indicative of marginal status (Sauberlich, 1974). The normal range for serum folate concentration in adults was considered to be between 7-46nmol/L (3-20µg/L) (Dacie & Lewis, 1995). These cut-offs and ranges were

originally derived from studies using a microbiological assay. A normal range of values has not been defined for children under 4 years.

96. In the NDNS series, different analytical methods were used to assess folate status in the different surveys: for adults aged 19-64 years and young people aged 4-18 years, the Abbot IMx fluorescence polarization method was used; for people aged 65 years and older, a microbiological assay was used to measure serum folate concentrations and a radioassay (Biorad Quantaphase II) was used for red cell folate concentrations; for children aged 1 ½ - 4 ½ years, a radioassay (Biorad Quantaphase I) was used to measure blood folate concentrations.

97. There is substantial analytical variation between the different assay methods. Several studies have reported different results between microbiological and radioassays (McGown *et al*, 1978; Shane *et al*, 1980; Brown *et al*, 1990). Interlaboratory comparisons of serum and red cell folate concentrations using 4 different assay methodologies showed considerable variation between the different methods (overall coefficient of variation 18-41%) and between different laboratories using a similar assay kit/protocol (van den Berg *et al*, 1994). Gunter *et al* (1996) also showed large intra- and intermethod variations among 20 participating laboratories using 7 different types of assay analysing a common series of serum and whole blood pools. The greatest variations were found at low and high concentrations of folate.

98. The agreement between the different assays is also dependent on the composition of the blood sample used for folate measurement and the genotype of the individual. It has been demonstrated that the radioassay under-recovers 5-MTHF but correctly recovers folic acid (Blackmore *et al*, 2005); differential recovery was also observed for other reduced folate forms.

99. As folate status measurements are dependent on the assay method used and because different methods have been used for different population samples, it is not possible to make direct comparisons of folate status or population mean levels between different age groups in the UK or with population samples from other other countries, for example, in the USA.

Adults aged 19-64 years

100. The mean daily intakes of folate were above the RNI in both men (359µg/day) and women (292µg/day). Less than 0.5% of men and 2% of women had intakes below the LRNI.

101. Average serum folate concentrations were 20.8nmol/L (9.2µg/L) for men and 22.1nmol/L (9.8µg/L) for women; average red blood cell folate concentrations were 694nmol (306µg/L) for men and 685nmol/L (302µg/L) for women.

102. Although the average folate intakes were greater than the RNI, 5% of all men and women, 8% of women aged 19-24 years, 4% of women aged 25-34 years, and 5% of women aged 35-49 years had red blood cell folate concentrations less than 350nmol/L (154µg/L) indicating an increased risk of marginal status.

103. A woman's risk of having a child with an NTD has been reported to be associated with red cell folate concentrations during early pregnancy (15 weeks gestation), in a continuous dose-response relationship (Daly *et al*, 1995). Concentrations below 340nmol/L (150µg/L) were associated with a relatively high risk and concentrations above 906nmol/L (400µg/L) were associated with a relatively low risk. In the NDNS, the average red blood cell folate concentrations of women aged 19-24 years, 25-34 years and 35-49 years were 575nmol/L (254µg/L), 630nmol/L (278µg/L) and 691nmol/L (305µg/L) respectively. The incidence of NTD-affected pregnancies in England and Wales is higher in younger women, which corresponds to the lower folate status in this group (Botting, 2001).

104. Comparison of NDNS levels with those reported by Daly *et al* (1995) might suggest that women of childbearing age in the UK are at intermediate risk of NTD-affected pregnancies. However, the microbiological assay was used by Daly *et al* (1995) and the Abbot IMx fluorescence polarization method was used in the NDNS. In addition, red cell folate concentrations in the Daly *et al* (1995) study were measured in pregnancy at 15 weeks gestation, when they are lower because of haemodilution and may therefore underestimate the risk of NTD affected pregnancies. The magnitude of the association between red cell folate and NTD risk may also be confounded by the link between red cell folate and general nutritional status (Kirke *et al*, 1992). For these reasons, it is not possible to make direct comparisons of red cell folate concentrations

of non-pregnant women in the NDNS with those of pregnant women reported by Daly *et al* (1995).

105. The estimated numbers of NTD-affected pregnancies ascertained in the UK are shown in Tables 24-27 (Annex 5).

Adults aged 65 years and over

106. In free-living people aged 65 years and over, mean daily intakes of folate were 279µg/day for men and 220µg/day for women. One percent of men and 5% of women had intakes below the LRNI (100µg/day). In institutionalised people mean daily intakes of folate were 235µg/day for men and 200µg/day women; 4% of men and 5% of women had intakes below the LRNI.

107. Average serum folate concentrations for free-living people were 15.3nmol/L (6.8µg/L) for men and 16.5nmol/L (7.3µg/L) for women; for institutionalised people, average serum folate concentrations were 9.7nmol/L (4.3µg/L) for men and 12.5nmol/L (5.5µg/L) for women. Average red blood cell folate concentrations for free-living people were 495nmol (219µg/L) for men and 501nmol/L (221µg/L) for women; in institutions, red blood cell folate concentrations were 449nmol/L (198µg/L) for men and 542nmol/L (239µg/L) for women.

108. Among free-living older people, 21% had red blood cell folate concentrations indicative of marginal status, and 8% had red blood cell folate concentrations indicating folate-deficiency. In the institutionalised people, 19% had red blood cell folate concentrations indicative of marginal status, and 16% had a red blood cell folate concentration indicating folate-deficiency. However, these values need to be interpreted with caution because of assay differences (see paragraphs 95-99).

Young people aged 4-18 years

109. For young people aged 4-18 years, mean daily intakes of folate (242µg boys; 197µg for girls) were above the RNI. Less than 1% of boys and 3-4% of girls over 11 years of age had intakes below the LRNI; at ages 4-11 years, only girls between 7-10 years had intakes below the LRNI. In the age range 4-11 years, 2% of girls aged 7-10 years had intakes below the LRNI.

110. Average serum folate concentrations were 21.7nmol/L (9.6µg/L) for boys and 20.6nmol/L (9.1µg/L) for girls; average red blood cell folate concentrations were 626nmol (276µg/L) for boys and 573nmol/L (253µg/L) for girls.

111. Seven percent of boys and 9% of girls had red blood cell folate concentrations indicative of marginal status. No more than 1% of any age/sex group had red blood cell folate concentrations indicating folate deficiency.

Children aged 1 ½ - 4 ½ years

112. For children aged 1 ½ -4 ½ years, the mean daily intakes of folate (132µg) were above the RNI in all age and sex groups. One percent of boys and girls under 4 years of age had intakes below the LRNI.

113. Average plasma folate concentrations were 20.9nmol/L (9.2µg/L) for boys and 21.3nmol/L (9.4µg/L) for girls; average red blood cell folate concentrations were 934nmol (412µg/L) for boys and 894nmol/L (395µg/L) for girls.

114. Two percent of children had red blood cell folate concentrations of less than 400nmol/L (177µg/L).

Summary

115. The average daily folate intakes were above the RNI in all age groups. Evidence of marginal folate status in young women and people aged 65 years and over has been reported but there is considerable variability in the analytical methods used to assess blood folate concentrations. Values for folate status and normal ranges are dependent on the assay method used, which makes it difficult to make direct comparisons between different studies and surveys. In the NDNS series, different methods were used to assess folate status for the various age groups. Results determined by different methods may not be comparable and should be interpreted with caution.

Vitamin B2 (riboflavin) intake and status

116. The *in vitro* activation coefficient of erythrocyte glutathione reductase (EGRAC) is a measure of red blood cell glutathione reductase saturation with its vitamin B2 derived cofactor, flavin adenine dinucleotide. An activation

coefficient between 1-1.3 is generally regarded as normal and an activation coefficient greater than 1.3 is indicative of a marginal status.

117. An activation coefficient of >1.2 was originally proposed to define marginal vitamin B2 status (Glatzle *et al*, 1970). Subsequent revisions to the methodology (Thurnham *et al*, 1972; Thurnham & Rathakette, 1982) may have resulted in a systematic increase in activation coefficients and the adoption of an activation coefficient of >1.3 to define marginal vitamin B2 status. Further increases in activation coefficients may have also occurred as a consequence of methodological changes to the EGRAC assay.

Adults aged 19-64 years

118. Mean daily vitamin B2 intakes (men 2.3mg; women 2.0mg) were above the RNI (men, 1.3mg/day; women, 1.1mg/day) in all age and sex groups. Daily vitamin B2 intakes below the LRNI (0.8mg/day) were found in 7% and 13% of men and women aged 19-24 years respectively, and in 10% of women aged 25-34 years. In older age groups, 2% of men and 5% of women aged 35-64 years had daily vitamin B2 intakes below the LRNI.

119. Based on the EGRAC, two-thirds of adults had marginal vitamin B2 status. The proportion of men and women with marginal status decreased with age: from 82% in men aged 19-24 years to 54% in those aged 50-64 years; and from 77% in women aged 19-24 years to 50% in those aged 50-64 years.

Adults aged 65 years and over

120. Mean daily intakes of vitamin B2 were above the RNI in free-living (1.78mg) and institutionalised (1.69mg) people. In free living people daily intakes were below the LRNI (0.8mg/day) in 5% of men and 9% of women. In women over 85 years of age, 15% had intakes below the LRNI. In institutionalised people daily intakes below the LRNI were found in 3% of men and women. In women over 85 years of age 6% had intakes below the LRNI. Based on the EGRAC, 41% of free-living adults had marginal status for vitamin B2 and in institutionalised people, 41% of men and 32% of women had marginal vitamin B2 status.

Young people aged 4-18 years

121. Mean daily intakes of vitamin B2 (boys 1.7mg; girls 1.4mg) were above the RNI (0.8-1.3mg/day) in all sex/age groups. Intakes were below the LRNI (0.4-0.8mg/day) in 6% of boys and over 20% of girls in the 11-18 year age group. Marginal status for vitamin B2, based on EGRAC, was found in 75% of boys and 87% of girls.

Children aged 1 ½ -4 ½ years

122. Mean daily intakes of vitamin B2 (1.2mg) were above the RNI (0.6-0.8mg/day) in all sex and age groups. Less than 1% of children had intakes below the LRNI (0.3-0.4mg/day) for vitamin B2. Marginal status for vitamin B2, based on EGRAC, was found in 14% of children aged 1 ½ -2 ½ years, 29% of those aged 2 ½ -3 ½ years and 38% of those aged 3 ½ -4 ½ years.

Summary

123. Evidence from NDNS data indicates that lower intakes of vitamin B2 and marginal vitamin B2 status are widespread in all age groups, particularly in women, girls, and boys. A relatively high proportion of females aged 11-34 years and adults over 65 years had daily vitamin B2 intakes below the LRNI. A relatively high proportion of males aged 11-24 years also had daily vitamin B2 intakes below the LRNI.

124. There was a strong inverse correlation between riboflavin intakes and EGRAC values in the NDNS. Both dietary riboflavin intakes and EGRAC values highlight the high proportion of young people with marginal riboflavin status.

125. There has been some uncertainty about the most appropriate level to set the activation coefficient in defining marginal status. The activation coefficient used to define marginal vitamin B2 status needs to be re-evaluated.

Vitamin B6 intake and status

126. The erythrocyte aspartate aminotransferase activation coefficient (EAATAC) is a measure of red blood cell aspartate aminotransferase saturation with its vitamin B6-derived cofactor, pyridoxal phosphate. In the NDNS, EAATAC values above 2.00 were considered to be indicative of marginal status in adults.

Adults aged 19-64 years

127. Mean daily vitamin B6 intakes (men 3.3mg; women 2.9mg) were above the RNI (men, 1.4mg/day; women, 1.2mg/day) in all age and sex groups. Intakes below the LRNI (men, 1.0mg/day; women, 0.8mg/day) were found in 1% of men and 2% of women; 5% of women aged 19-24 years had an average intake below the LNRI. Ten per cent of men and 11% of women had marginal status for vitamin B6 based on the EAATAC.

Adults aged 65 years and over

128. Mean daily intakes of vitamin B6 were above the RNI in free-living (2.1mg) and institutionalised (1.7mg) older people. For free-living people, daily intakes below the LRNI were found in 1% of men and 6% of women aged over 85 years. For institutionalised people, daily intakes below the LRNI were found in 1% of men and less than 0.5% of women aged over 85 years. The EAATAC was not measured. However, two other biochemical markers of vitamin B6 status, pyridoxal phosphate and pyridoxic acid, were measured in plasma samples from this survey (Bates *et al* 1999). The results suggest a high prevalence of poor vitamin B6 status, with 47% of free-living people and 75% of those living in institutions having a pyridoxal phosphate concentration below a range considered normal from other studies.

Young people aged 4-18 years

129. Mean daily intakes of vitamin B6 (boys 2.2mg; girls 1.8mg) were above the RNI (0.9-1.5mg) in all sex and age groups. Intakes were below the LRNI (0.6-1.1mg) for vitamin B6 in 1% of boys aged 11-14 years, 1% of girls aged 7-14 years and 5% of girls aged 15-18 years. Marginal vitamin B6 status, indicated by raised EAATAC, was found in 10% of boys and girls.

Children aged 1 ½ -4 ½ years

130. Mean daily intakes of vitamin B6 (1.2mg) were above the RNI (0.7-0.9mg/day) in all sex and age groups. Intakes were below the LRNI (0.5-0.7mg/day) for vitamin B6 in 1% of children aged under 4 years and 5% in those aged over 4 years. The EAATAC was not measured.

Summary

131. Most age and sex groups had adequate vitamin B6 intakes, but a relatively high proportion of females aged 15-24 years, and over 65 years, had intakes below the LRNI. Evidence of poor vitamin B6 status was found in 10% of people aged 4-64 years. A higher prevalence of poor vitamin B6 status, of at least 47%, was indicated for adults aged 65 years and over.

Vitamin B12 (cobalamin) intake and status

132. In the NDNS series, poor vitamin B12 status was defined as a serum vitamin B12 concentration of <118pmol/L.

Adults aged 19-64 years

133. Mean daily vitamin B12 intakes (men 6.8µg; women 5.1µg) were above the RNI (1.5µg) in all age and sex groups. Intakes below the LRNI (1.0µg/day) were found in less than 0.5% of men and 1% of women. Mean serum vitamin B12 concentrations were 298pmol/L and 288pmol/L in men and women respectively. About 4 % of women, and 2% of men aged 35-49 years and 3% of men aged 50-64 years, had serum vitamin B12 concentrations below 118pmol/L.

Adults aged 65 years and over

134. Mean daily intakes of vitamin B12 were above the RNI in free-living (5.2µg) and institutionalised (4.7µg) older people. In the free-living people less than 0.5% of men and 1% of women had a daily intake below the LRNI. None of the institutionalised older people had a daily intake below the LRNI.

135. Mean serum vitamin B12 concentrations were 237pmol/L and 239pmol/L in the free-living and institutionalised groups respectively. Six percent of the free-living group and 9% of the institutionalised group had serum vitamin B12 concentrations below 118pmol/L. A subsequent analysis of the same samples (Bates *et al*, 2003) which measured plasma concentrations of methylmalonic acid (MMA), another marker of vitamin B12 status, suggested that 24% of the free-living and 46% of the institutionalised particpants were at risk of low vitamin B12 status (see paragraphs 204 and 206).

Young people aged 4-18 years

136. Mean daily intakes of vitamin B12 (boys 4.4µg; girls 3.4µg) were above the RNI (0.8-1.5µg) in all sex and age groups. Intakes were below the LRNI (0.5-1.0µg) for vitamin B12 in 1% of girls aged 7-14 years and 2% of girls aged 15-18 years. Mean serum vitamin B12 concentrations were 403pmol/L in boys and 395pmol/L in girls. One percent of boys aged 15-18 years and 8% of girls aged 15-18 years had serum vitamin B12 concentrations below 118pmol/L.

Children aged 1 ½ -4 ½ years

137. Mean daily intakes of vitamin B12 (2.8µg) were above the RNI (0.7-0.8µg) in all sex and age groups. There were no intakes below the LRNI (0.3-0.5µg). Mean serum vitamin B12 concentrations were 632pmol/L in boys and 639pmol/L in girls. Two percent of children had serum vitamin B12 concentrations below 250pmol/L.

Summary

138. There was no evidence of inadequate vitamin B12 intakes. However, serum concentrations of this vitamin indicated poor vitamin B12 status in older adults, which may reflect an increased prevalence of malabsorption of vitamin B12 in this age group (see section 6).

5 Folate and neural tube defects

139. NTDs include anencephaly, spina bifida, and encephalocele. The development and closure of the neural tube, which forms into the brain and spinal cord, is normally completed within 28 days after conception. NTDs are thought to be caused by failure of the neural tube to close.

140. There is conclusive evidence from RCTs that folic acid supplementation can prevent NTDs (MRC Vitamin Study Research Group, 1991; Czeizel & Dudas, 1992). Folate status is associated with risk of NTDs in an inverse dose-response relationship (Daly *et al*, 1995). The available evidence for the relationship between folate status and NTDs is reviewed in the COMA report, *Folic acid and the prevention of disease* (DH, 2000).

Genetic aspects of NTD risk

141. NTD risk has also been associated with variants in a number of the genes coding for enzymes involved in folate metabolism.

142. A relatively common autosomal recessive gene variant (677 C→T) affects the enzyme methylene tetrahydrofolate reductase (MTHFR) resulting in reduced activity. Homozygosity (TT) for this mutation is associated with increased risk of NTDs (Ou *et al*, 1996; Botto *et al*, 1999) and an approximate doubling of the risk for spina bifida (Botto & Yang, 2000). There is considerable ethnic and geographical variation in the frequency of the C677T variant. The highest frequency (20% or more) is found in US Hispanics, Italians, Columbians and American Indians in Brazil and lowest (only a few percent) in black Africans and African-Americans (Botto & Yang, 2000). In Caucasian populations in Europe, Australia, and North America, the frequency of the homozygous TT genotype, is 8-20%, (Sharp & Little, 2004). In the UK, the prevalence of homozygosity for the MTHFR TT variant has been reported as 10% for whites, 1% in people of African origin, and 2% in South Asians (Cappuccio *et al*, 2002).

143. Variants in methylenetetrahydrofolate dehydrogenase 1 (MTHFD1) G1958A and human folate carrier (RFC-1) A80G have also been reported to contribute to NTD susceptibility, for example, in the Italian population (De Marco *et al*,

2001; De Marco *et al*, 2003; De Marco *et al*, 2006) and in an Irish population (Brody *et al*, 2002). In addition, the risk of NTD was found to be elevated in infants carrying mutant alleles for both methionine synthase reductase (MTRR) and methionine synthase (MTR) (Zhu *et al*, 2003). It has also been proposed that interactions between genes involved in folate metabolism may be important in determining genetic susceptibility to NTD and three genotype combinations (MTRR/GCPII; MTHFR 677/CbetaS; MTHFR 677/MTRR) have been reported to increase NTD risk (Relton *et al*, 2004).

Other factors associated with NTD risk

144. Women with a history of a previous pregnancy affected by an NTD have a higher risk of another NTD-affected pregnancy. Women with diabetes are at increased risk of NTD-affected pregnancies (Becerra *et al*, 1990) and increased maternal weight has also been associated with greater risk of NTD-affected pregnancies (see paragraphs 182-183).

145. It is estimated that approximately 30% of NTD-affected pregnancies may not be responsive to changes in folic acid intake (MRC Vitamin Study Research Group, 1991).

The use of folic acid supplements in pregnancy in the UK

146. The critical period for folic acid supplementation is before closure of the neural tube, i.e. in the first 28 days following conception.

147. To prevent first occurrence of NTD, the Department of Health recommends that all women who could become pregnant should take 400µg folic acid per day as a medicinal or food supplement prior to conception until the twelfth week of pregnancy (DH, 1992). To prevent recurrence of NTD in the offspring of women or men with spina bifida themselves, or with a history of a previous child with an NTD, a dose of 5mg/day is advised. Diabetes UK also recommends that all women with diabetes should take 5mg/day before conception and during the first 12 weeks of pregnancy.

148. The Health Survey of England 2002 (Blake *et al*, 2003) provides the most up-to-date information on the use of folic acid supplements prior to and during pregnancy. Information on folic acid supplement intake prior to pregnancy

was collected from mothers who had planned their pregnancy and comprised two-thirds of the interviewed sample.

149. Of those mothers who reported planning their pregnancy, over half (55%) reported taking supplements or modifying their diet to increase folate intake before they became pregnant. Seventy-nine percent of mothers reported increasing their folate intake during pregnancy. The proportion of mothers taking action to address folate intake increased with age from 32% (16-24 years) to 60% (35 years and over). Only 43% of mothers in the most socio-economically deprived areas were likely to increase their folate intake compared to 70% of mothers from the least socio-economically deprived areas.

150. The Infant Feeding Survey 2000 (Hamlyn *et al*, 2002), which covers the UK, is based on retrospective postpartum interviews and has reported similar findings to the Health Survey. Seventy-three per cent of mothers questioned (compared to 50% in 1995) indicated that they had taken folic acid supplements and 31% (compared to 26% in 1995) had modified their diets to increase their folate intake in early pregnancy. Information was not available, however, on when action to increase folate intakes had been taken (pre/post-conception). The Infant Feeding Survey also indicates that the uptake of folic acid supplements did not differ between countries, although mothers in Scotland were more likely to modify their diets to increase folate intakes (34% compared to 31% in England & Wales and 28% in Northern Ireland).

151. The COMA review of the Welfare Food Scheme in the UK (DH, 2002), which provides free vitamin supplements to beneficiary population groups, considered whether free provision of periconceptional folic acid supplements might improve uptake in pregnant women from these groups. It was noted that the uptake of other supplements by groups eligible under the scheme was poor. COMA recommended that the composition of free vitamin supplements (vitamins A, C & D) available to pregnant women through the Scheme should be reviewed, that a supplement providing folic acid, vitamins D and C and omitting vitamin A would be preferable to the current preparation, and steps should be taken to improve the uptake of vitamin supplements.

152. The efficacy of European Union (EU) policies recommending women to consume folic acid supplements to reduce NTD-affected pregnancies has been questioned (Botto *et al*, 2005; Busby *et al*, 2005). Botto *et al* (2005) examined the rates of NTD-affected pregnancies between 1988 and 1998 in 11 EU countries and observed that they were unaffected by recommendations for women to consume folic acid supplements. In England and Wales, a decline in NTD-affected pregnancies was observed between 1988 and 1998 but the recommendation in 1992, for women to consume folic acid supplements, had no detectable impact on the rate of NTD-affected pregnancies. Analysis of NDNS data (Henderson *et al*, 2003) indicates that uptake of supplements is greater in people with higher intakes of micronutrients from foods. It is possible that folic acid supplements are taken during pregnancy by women with the best folate status who are also at lowest risk of an NTD-affected pregnancy.

NTD-affected pregnancies in the UK

153. Reported numbers of NTD-affected pregnancies in England and Wales (1992-2004), Wales (1998-2004), Scotland (1992-2003) and the reported number of NTD-affected births in Northern Ireland (1992-2003) are provided in Tables 24-27, Annex 5.

Ascertainment in England and Wales

154. In England and Wales, data for NTD-affected live and stillbirths are collated through the National Congenital Anomaly System (NCAS) which is managed by the Office for National Statistics (ONS). NCAS is a composite of data from two different reporting systems, use of which varies within regions. Notification to NCAS is voluntary and most information is supplied by local community trusts or health authorities; since 1998, however, local congenital anomaly registers have started to provide data to NCAS. Local registers ascertain cases from multiple sources resulting in a notable increase in the notification rates for areas that are covered by them. The two main problems with data reported to NCAS are under-notification and diagnostic misclassification (Botting, 2001). Completeness of reporting varies according to the type of condition being notified; for example, cases of anencephaly and spina bifida, which are readily visible conditions, tend to be more completely reported compared to some less visible conditions. The ONS

(2004) have estimated that approximately two-thirds of anencephaly and spina bifida cases were notified through NCAS.

155. Levels of reporting also vary according to region. Boyd *et al* (2005) compared NCAS data with four local congenital anomaly registers, covering about 109,000 births annually, and found that NCAS registered 68% of NTD-affected births. From 1991-1999, the four local registers recorded 1041 cases of NTD-affected pregnancies (including therapeutic terminations), which included 176 NTD-affected births compared to 119 NTD-affected births recorded by NCAS. In areas where notification has been assigned to the local register, e.g. in Wales, substantial improvements in ascertainment have been achieved since 1997 (Botting, 2000).

156. One limitation of NCAS is that it does not include therapeutic termination data. Termination of pregnancy for fetal handicap was not legally possible when NCAS started (1964) so terminations were not included. This could be one reason why notifications of congenital anomalies fell in the 1980s and 1990s. Termination data are collected by the Department of Health but these data have not been validated for their completeness or accuracy of diagnosis (Boyd *et al*, 2005). Since the Abortion Act of 1967, the ONS, and its predecessors, have included both NCAS data and therapeutic termination data reported from the Department of Health in summary tabulations of congenital anomalies. In England and Wales in 2003, there were 418 notified births, stillbirths and therapeutic terminations due to NTD anomalies; this comprised 121 NTD-affected births and 297 therapeutic terminations (see Table 24, Annex 5).

157. Another issue is the under-reporting of NTD terminations. Murphy *et al* (1996) compared the national NTD-affected pregnancy rates (birth and termination rates combined), based on data from the Office of Population Censuses and Surveys (OPCS) with those in Oxfordshire and Berkshire, where rates were fully reported, between 1974 and 1994. They estimated that, between 1974-1990, under-reporting of NTD-affected pregnancy rates (birth and termination rates combined) to OPCS was 34%.

158. Morris & Wald (1999), making a number of assumptions, including a constant rate of NTD-affected pregnancies between 1976-1980, estimated the level of under-reporting of NTD-affected pregnancy terminations to be 56%.

159. Data from the Congenital Anomaly Register and Information Service for Wales (CARIS) are only available from 1998 (see Table 25, Annex 5). Data reported to CARIS is much more complete as multiple sources are used to collect information on NTD-affected pregnancies. Comparison of the separate data for NTD reported terminations in Wales shows that termination rates are much higher than suggested by the combined NCAS data for England and Wales (Table 24, Annex 5).

160. Assuming under-reporting of 34% for NTD affected pregnancies (births and terminations combined) (Murphy *et al*, 1996) it can be estimated that there were 633 NTD-affected pregnancies in England and Wales in 2003.

161. Assuming under-reporting of NTD-affected births (32%) (Boyd *et al*, 2005) and NTD-reported terminations (56%) (Morris & Wald, 1999) it can be estimated that there were 853 NTD-affected pregnancies (178 NTD-affected births and 675 NTD-affected terminations) in England and Wales in 2003.

162. Taking account of both the above estimates for under-reporting, there were approximately 630-850 NTD-affected pregnancies in England and Wales in 2003. Approximately 70% of these were managed by terminations.

Ascertainment in Scotland

163. In Scotland the Information and Statistics Division of the National Health Service Scotland collates data on NTD-affected pregnancies from a number of independently recorded patient databases. Only singleton births are currently included and multiple births are excluded. The Scottish Birth Record, which will include data on multiple births, is in the process of being introduced.

164. The rate of NTD-affected pregnancies is higher in Scotland compared to England and Wales.

165. In Scotland there were 49 NTD-affected pregnancies in 2003 (see Table 26, Annex 5). Approximately 50% of these pregnancies were managed by terminations. The completeness of ascertainment of NTD-affected pregnancies in Scotland has not been reported.

Ascertainment in Northern Ireland

166. Data for Northern Ireland are collected by 4 separate regional health boards. Data are only available for NTD-affected births as terminations are not legalised. The rate of NTD-affected births is much higher in Northern Ireland compared to England and Wales, and Scotland. It is not clear how far this is a result of genetic factors or because abortions are not permitted in Northern Ireland.

167. In Northern Ireland, there were 11 NTD-affected births in 2003 (see Table 27, Annex 5). The extent of under-reporting in Northern Ireland is unknown.

Summary

168. The available data on the number of NTD-affected pregnancies (births and terminations) are insecure. This is mainly because there are major difficulties in obtaining accurate ascertainment due to under-reporting of births and terminations affected by NTDs.

169. The available data on NTD-affected pregnancies represent baseline estimates. The actual numbers of NTD-affected pregnancies are likely to be higher than these estimates because of under-reporting.

170. Using different methods to adjust for under-reporting, it can be estimated that there were 630-850 NTD-affected pregnancies in England and Wales in 2003. There were 49 NTD-affected pregnancies in Scotland and 11 NTD-affected births in Northern Ireland in 2003. The extent of under-reporting in Scotland and Northern Ireland is unknown.

171. In the UK, there were approximately 700-900 NTD-affected pregnancies in 2003. This figure takes account of potential under-reporting in England and Wales but does not take account of under-reporting in Scotland and Northern Ireland.

172. In 2003, approximately 70% of the NTD-affected pregnancies in England and Wales and 50% of the NTD-affected pregnancies in Scotland were managed by terminations.

Trends in NTD-affected pregnancies

173. The ONS has reported trends in NTD-affected births in England and Wales from 1975-1999 (Botting, 2001). There has been a constant decline in NTD-affected births since the 1970s until the 1990s from 26.9 per 10,000 live and stillbirths in 1975-79, 5.1 per 10,000 live and stillbirths in 1985-89, to 1.5 per 10,000 live and stillbirths in 1995-1999. Regional congenital anomaly registers detected no decline in NTD-affected pregnancies during the 1990s (Abramsky *et al*, 1999).

174. From 1975-1999, NTD rates for women under 20 years have been consistently higher than for older age groups (Botting, 2001). By 1995-99 NTD rates were lowest for women 40 years and over which is probably due to improved prenatal diagnosis in this age group.

175. NTDs account for about 70% of central nervous system (CNS) anomalies. Improved prenatal screening has resulted in a higher proportion of NTD-affected pregnancies being identified and terminated. Separate data for NTD-reported terminations were not routinely available until 1995 (see Table 24, Annex 5). In England and Wales, the proportion of pregnancies terminated for all notified CNS anomalies increased from 1.5% in the 1970s to 40% in the mid-1980s and over 60% from 1991 onwards (Botting, 2001). In 1997, CNS anomaly-affected live births were one tenth of those in the mid-1960s; this was due to both an increase in terminations of CNS anomaly-affected pregnancies and also a decrease in the prevalence of CNS anomaly-affected pregnancies. Based on their estimates of under-reporting of NTD-affected pregnancies, Morris & Wald (1999) estimated that 40% of the decline in NTD-affected births between 1970 and 1997 was due to therapeutic terminations and 56% was due to a decline in incidence.

176. Statistics reporting trends in NTD-affected pregnancies in Scotland (see Table 26, Annex 5) show that rates were constant during the 1990s although there may have been a downward trend from the late 1990s onwards in the numbers of NTD-affected births. Prior to the 1990s, registers in Scotland reported a constant decline in NTD-affected pregnancies since the 1960s, which is similar to that observed in England and Wales (Murphy *et al*, 2000).

177. There was no observed trend in NTD-affected births during the 1990s in Northern Ireland (Table 27, Annex 5).

Relation between trends in NTD-affected pregnancies and folate intake

178. Increased folate intake has been suggested as one possible explanation for the fall in NTD-affected pregnancies in England and Wales between the 1980s and 1990s (Murphy *et al*, 2000). Estimates of average daily folate intakes from the National Food Survey (based on household food purchase patterns for England, Scotland, Wales and Northern Ireland) show that between 1980 and 1990, average intakes increased by about 30-40µg/day in the UK, from approximately 200µg/day to 240µg/day. The increase in average folate intakes in the UK during the mid-1980s coincided with the increased fortification of breakfast cereals during the period 1985-1991. There are no estimates of average daily folate intakes prior to 1980.

179. The NDNS of adults shows an increase in average folate intakes of women, from 219µg/day in 1986/7 (Gregory *et al*, 1990) to 292µg/day in 2000/1 (Henderson *et al*, 2003).

180. The decline in rates of NTD-affected pregnancies in England and Wales predates government advice about the benefits of periconceptional supplementation with folic acid to prevent NTDs (DH, 1992). Despite surveys showing an increased awareness of the benefit of periconceptional supplementation with folic acid among women of childbearing age (particularly among older women) during the 1990s, there has been no corresponding decline in the incidence of NTD-affected pregnancies (Botting, 2001). This may be because folic acid supplements were not consumed during the periconceptional period which is necessary if they are to be efficacious. Supplement consumption may also be greater for women with higher intakes of micronutrients from foods (Henderson *et al*, 2003) who are also at lowest risk of an NTD-affected pregnancy.

181. It is estimated that half of all pregnancies in the UK are unplanned (DH, 2000), which limits the value of recommendations for pre-conceptional supplementation.

Maternal weight and NTD-affected pregnancies

182. Case-control studies have suggested that pre-pregnancy Body Mass Index (BMI) greater than 30 is associated with an increased risk of NTD-affected pregnancies (Werler *et al*, 1996; Watkins *et al*, 1996; Shaw *et al*, 1996). In

Canada, a retrospective population-based study of 420,362 women who underwent antenatal screening between 1994 and 2000 detected 292 open NTDs (Ray *et al*, 2005a). Maternal weight was positively associated with risk of NTDs. When comparing the highest with the lowest quartile of maternal weight, the adjusted odds ratio for NTD-affected pregnancies was 2.6 (95% CI 1.8-4.0). Women of increased weight remained at significantly higher risk of NTD-affected pregnancies than those in lower weight categories, despite the introduction of mandatory fortification of staple foods with folic acid.

183. Lower serum folate concentration has been suggested as a possible reason for the link between higher maternal BMI and increased risk of NTD-affected pregnancies. In cross-sectional National Health and Nutritional Examination Surveys (NHANES) in the USA, after controlling for intake of folate in food and nutritional supplements, an increase in BMI in women of childbearing age was associated with a lower concentration of serum folate (Mojtabai, 2004).

Folic acid fortification strategies and the incidence of NTD in other countries

184. Details of some countries that have introduced folic acid fortification strategies are outlined in Table 28, Annex 6. In countries where mandatory fortification with folic acid has been introduced (Canada, Chile and the USA) evidence is available of the incidence of NTD-affected pregnancies pre- and post-fortification. The changes in rates of NTD-affected pregnancies following mandatory fortification in these countries are shown in Table 1. Although this information is useful to illustrate the corresponding position in each country, comparison between countries is complicated because some countries include termination data and report on all NTD-affected pregnancies and others report only the number of NTD-affected births. Within each country, there is also some degree of limitation in the ascertainment of NTD data and the completeness of the data can be affected by the method of collation.

185. In the USA and Canada mandatory fortification was introduced in 1998. In the USA, Honein *et al* (2001) estimated that the prevalence of NTD-affected births decreased by 19% following fortification. The Centers for Disease Control and Prevention (CDC) (2004) compared the numbers of NTD-affected pregnancies (live births, still births, fetal deaths and elective

terminations) in a 24-month pre-fortification period (1995-1996) with those in a 24-month post-fortification period (1999-2000). The estimated rate of spina bifida and anencephaly affected pregnancies was 10.6 per 10,000 live births pre-fortification compared with 7.6 per 10,000 live births post-fortification indicating an approximate 27% decline in NTD-affected pregnancies following mandatory fortification.

186. Mills and Signore (2004) noted that the incomplete ascertainment of prenatally diagnosed NTD cases in the USA had probably led to under-reporting of the number of NTD cases prevented by mandatory fortification. The ascertainment of prenatally diagnosed NTD cases in Canada is more complete and Mills and Signore (2004) suggest that the actual reduction in NTD-affected pregnancies in the USA is likely to be similar to those observed in Canada, i.e. approximately 50%.

187. More complete figures are available from four provinces in Canada. Table 1 shows that in Quebec there was a 32% reduction in the incidence of NTD-affected pregnancies (live and still births, elective terminations) post-fortification (de Wals *et al*, 2003) and a 51% decline in Ontario (Ray *et al*, 2002). In Nova Scotia, there was a decrease of 54% (Persad *et al*, 2002) and in Newfoundland a 78% decrease after the implementation of folic acid fortification (Liu *et al*, 2004). Prior to mandatory fortification there was no significant change in the average rates in Newfoundland between 1991-93 and 1994-97.

188. In Chile, mandatory fortification of flour with folic acid was introduced in 2000. Average spina bifida rates fell from 9.32 per 10,000 births during 1990-2000 to 4.77 per 10,000 births during 2001-2002 and average anencephaly rates fell from 8.19 per 10,000 births during 1990-2000 to 3.18 per 10,000 births during 2001-2002; induced pregnancy terminations, which are illegal in Chile, were not reported. The rates of spina bifida and anencephaly-affected births in Chile thus declined by approximately 51% and 42% respectively, after the implementation of folic acid fortification (Lopez-Camelo *et al*, 2005). Prior to fortification there was no decrease in the mean annual rates between 1982-1989 and 1990-2000.

Table 1. Comparison of NTD rates in countries pre- and post-fortification

Country	NTD-affected pregnancy rate (per 10,000 live births)*	Reduction in NTD risk post fortification (%)**
USA (Centers for Disease Control and Prevention, 2004)		
Pre-fortification	10.6	
Post-fortification	7.6	27
CANADA		
Ontario (Ray *et al*, 2002)		
Pre-fortification	11.3	
Post-fortification	5.8	51
Nova Scotia (Persad *et al*, 2002)		
Pre-fortification	25.8	
Post-fortification	11.7	54
Quebec (de Wals *et al*, 2003)		
Pre-fortification	18.9	
Post-fortification	12.8	32
Newfoundland (Liu *et al*, 2004)		
Pre-fortification	43.6	
Post-fortification	9.6	78
CHILE (Lopez-Camelo *et al*, 2005)		
Pre-fortification (live and still births only)	17.5	
Post-fortification (live and still births only)	8.0	47

*Includes live/still births, prenatally diagnosed cases and elective terminations unless otherwise stated.
**The reduction in NTD risk may not correspond to the left hand column as the percentage is sometimes an average of separate % risk reductions for spina bifida and anencephaly cases.

Overage of folic acid in fortified foods

189. Measurements of the total folate content in folic acid enriched cereal-grain products in the USA following fortification have shown that a considerable number of these products contain total folate levels that are higher than the amount required by regulations (Rader *et al*, 2000). It has been estimated that, as a consequence of fortification, typical folic acid intakes in the USA have increased by 215-240µg/day which is more than twice the originally intended increment of approximately 100µg/day (Choumenkovitch *et al*, 2002; Quinlivan & Gregory, 2003).

190. A comparison of food intake data from two National Health and Nutrition Surveys (NHANES) carried out 1988-1994 (NHANES III) and in 1999-2000 estimated that mean dietary intakes of total folate increased by 76µg/day (from 275µg/day to 351µg/day) after mandatory fortification (Dietrich *et al*, 2005). However, the changes in blood folate levels of the study population were substantially higher: mean serum folate concentrations increased more than twofold following the introduction of mandatory fortification from 11.4nmol/L (5µg/L) in 1988-1994 to 26.9nmol/L (11.9µg/L).

191. Overage is the practice employed by manufacturers of adding nutrients above the recommended level to allow for losses during manufacture and storage. It is estimated that overage for folic acid supplements could be 30% more than the amount expressed on the label (EVM, 2003). The experience in the USA where, post-fortification, typical folic acid intakes appear to have increased by more than twice that originally intended, is that overage is likely to have been a major contributor to the increased intake, possibly together with an increase in the availability of foods voluntarily fortified with folic acid. This illustrates that overage is an important consideration in determining the level of folic acid used for fortification of flour.

192. In the COMA report on folic acid (DH, 2000), the effects of folic acid fortification of flour on the dietary intakes of different population groups were estimated by modelling dietary intake data from the NDNS. The models were based on the levels of folic acid as consumed and may not have fully accounted for overage. The COMA report also noted that consideration should be given to controlling levels of folic acid fortification of breakfast cereals. The issue of overage, folic acid content of vitamin supplements and the control of fortification of other foods would also need to be taken into account in considering the level of fortification.

Summary

193. Countries that have introduced mandatory fortification with folic acid have consistently reported significant reductions in NTD-affected pregnancies (27% to over 50%).

194. As a result of overage, the impact of fortification on folate status has been greater than predicted. The issue of overage, folic acid content of vitamin supplements and control of voluntary fortification of other foods are important considerations regarding the level of mandatory fortification.

6 Possible adverse effects of mandatory fortification of flour with folic acid

195. The clear benefit of folic acid supplementation on reducing the risk of NTDs has been established (DH, 2000). Postulated adverse effects of increased folic acid intake include: masking symptoms of vitamin B12 deficiency in older people; modifying the effects of drugs used to treat epilepsy which could lead to an increase in seizures, increased prevalence of multiple births, increasing the survival of embryos with genotypes associated with deleterious effects, and modifying the effects of anti-folate drugs. There are also concerns regarding the presence of unmetabolised folic acid in the systemic circulation. Evidence relating to postulated adverse effects is considered below.

196. Additional concerns include possible effects of high folic acid intakes on increasing the rate of cognitive decline and increasing cancer risk. These concerns are considered in section 7.

Risks to older people

197. The metabolism of folate is inter-related with the metabolism of vitamin B12. Clinical signs associated with vitamin B12 deficiency are anaemia, identical to that of folate deficiency, and neurological impairment. Treatment with folic acid can alleviate the anaemia (the 'masking' of vitamin B12 deficiency) and therefore delay the diagnosis of vitamin B12 deficiency, which can lead to irreversible neurological damage. The possibility of increased intakes of folic acid delaying the diagnosis of vitamin B12 deficiency in older people was the main risk previously considered by COMA (DH, 2000).

Vitamin B12 deficiency

198. Vitamin B12 deficiency is rarely caused by inadequate dietary intakes, although dietary deficiency is more common in vegetarians (Herrmann *et al*, 2003), particularly vegans (Lloyd-Wright *et al*, 2003), because vitamin B12 is only found in foods of animal origin. A more important cause of vitamin B12 deficiency is malabsorption of food-bound vitamin B12 or pernicious anaemia.

199. Food-bound vitamin B12 malabsorption is associated with gastric atrophy and is characterised by the inability to release vitamin B12 from food or from intestinal transport proteins, particularly in the presence of hypochlorhydria. Pernicious anaemia is an autoimmune disease characterised by the destruction of the gastric mucosa and subsequent intrinsic factor (required for vitamin B12 absorption) deficiency (Carmel, 1997).

200. Clinical signs of vitamin B12 deficiency are macrocytic anaemia and/or neuropathy (Lindenbaum *et al*, 1988) which may be reversible. In some cases, deficiency leads to the irreversible and serious condition of subacute combined degeneration of the spinal cord.

201. Historically, macrocytic anaemia was the basis for diagnosis of vitamin B12 deficiency because recognition of anaemia preceded the finding of vitamin B12 deficiency (Carmel, 2000). Neurological symptoms were also thought to be later manifestations of vitamin B12 deficiency, occurring after development of anaemia (Woltmann, 1919; Victor & Lear, 1956). Macrocytic anaemia is the most common presentation of vitamin B12 deficiency, although haematological and neurological manifestations are often dissociated (Savage & Lindenbaum, 1995): haematological symptoms do not always occur and neurological symptoms are sometimes the only clinical expression of vitamin B12 deficiency. Neurological symptoms in the absence of anaemia have been reported to occur in 20-30% of cases (Lindenbaum *et al*, 1988).

Biochemical markers of vitamin B12 deficiency

202. The assessment of vitamin B12 deficiency is complicated by the limitations of current diagnostic techniques. There is also considerable inter-laboratory and inter-assay variation in the different methods used to determine serum/plasma concentrations of vitamin B12 and/or its metabolites.

203. Measurements of serum vitamin B12 concentrations have poor specificity and sensitivity in diagnosing deficiency, as low serum concentrations do not always indicate vitamin B12 deficiency and patients with vitamin B12 deficiency do not always have low serum concentrations (Lindenbaum *et al*, 1990; Joosten *et al*, 1993).

204. Vitamin B12 is required as a cofactor in the enzymatic conversion of methylmalonyl-CoA to succinyl-CoA. In the absence of vitamin B12,

methylmalonyl-CoA accumulates and is hydrolysed to methylmalonic acid (MMA). Vitamin B12 is also required for remethylation of plasma homocysteine (tHcy) to methionine by methionine synthase. Serum or plasma concentrations of MMA and tHcy are therefore increased in vitamin B12 deficiency and are considered more sensitive diagnostic indicators than plasma or serum vitamin B12 since they are almost always elevated in persons with biologically significant vitamin B12 deficiency (Lindenbaum *et al*, 1990). Measurement of plasma tHcy concentration has poor specificity as elevated tHcy concentration may result from a number of causes including folate or vitamin B6 deficiency (Joosten *et al*, 1993) as well as renal impairment (Hultberg *et al*, 1993). Raised MMA concentration is a more specific marker for vitamin B12 deficiency although concentrations are also raised with renal impairment (Hvas *et al*, 2000). Elevated concentrations of plasma or serum creatinine and/or urea, which are indicative of renal impairment, need to be taken into account in the interpretation of raised concentrations of MMA and tHcy. Combined measures are also used but have been found not to reliably predict the later development of clinical disease (see paragraphs 214-217).

205. Vitamin B12 circulating in blood is bound to two transport proteins, haptocorrin and transcobalamin. Vitamin B12 attached to transcobalamin (holotrancobalamin) represents the functionally active fraction available to cells and it has been suggested that a decrease in plasma holotranscobalamin concentration is the earliest indication of vitamin B12 deficiency (Herzlich & Herbert, 1988). Plasma holotranscobalamin concentration has been shown to be a sensitive diagnostic indicator of vitamin B12 deficiency (Hvas and Nexo, 2005) however concentrations are raised in renal disease (Herrmann *et al*, 2003) and other influences on holotranscobalamin concentrations have not been systematically investigated (Miller *et al*, 2006).

Prevalence of low vitamin B12 status

206. In the COMA report (DH, 2000), poor vitamin B12 status was defined as serum vitamin B12 concentration ‹118pmol/L or mean corpuscular volume (MCV) ›101fl. Application of these criteria to the NDNS of adults aged over 65 years (Finch *et al*, 1998) suggests that 5-10% (based on serum vitamin B12 concentration ‹118pmol/L) or 2-4% (based on MCV ›101fl) have poor vitamin B12 status. A subsequent analysis of the NDNS data (Bates *et al*, 2003) with low status defined as serum vitamin B12 concentrations ‹150pmol/L or

plasma MMA concentrations >0.5µmol/L suggested that 20% and 24% respectively of adults over 65 years had low status.

207. In an analysis of the Oxford Healthy Ageing Project (OHAP) (n=1562, age 65 years or over) participants with serum vitamin B12 concentration <150pmol/L or serum B12 concentration 150-200pmol/L together with a plasma MMA concentration >0.35µmol/L (80th percentile in subset with normal renal function) were classified as being at high risk of vitamin B12 deficiency. Based on these criteria 10% of people aged 65-74 years and 20% of people over 75 years were at high risk of vitamin B12 deficiency (Clarke *et al*, 2003).

208. A subsequent analysis combined data (n=3511, age 65 years or over) from the OHAP, NDNS of people aged 65 years and over, and the Medical Research Council nutrition study. After adjustment for creatinine concentration, the prevalence of low vitamin B12 status (whether defined as serum vitamin B12 concentrations <150pmol/L or serum vitamin B12 concentrations <200pmol/L and tHcy concentrations >20µmol/L) increased with age from 5% among people aged 65-74 years to 10% or greater among people aged 75 years or more (Clarke *et al*, 2004).

Response to vitamin B12 therapy

209. The most definitive indication of vitamin B12 deficiency would be improvement of haematological and/or neurological symptoms in response to vitamin B12 therapy.

210. In a study in the USA (Solomon, 2005) the records of ambulatory patients (n=95; age not reported) with haematological and/or neurological findings consistent with vitamin B12 deficiency, and who had received at least 3 months of vitamin B12 therapy, were evaluated for haematological and/or neurological response to treatment. Serum vitamin B12 concentrations were defined as low (<150pmol/L), intermediate (150-220pmol/L) or normal (>220pmol/L); serum MMA concentrations were defined as normal (<0.25µmol), moderately increased (0.25-0.38µmol), elevated (>0.38µmol); and plasma tHcy concentrations were defined as normal (<12µmol), moderately increased (12-13.6µmol), and elevated (>13.6µmol).

211. After 3 months of vitamin B12 therapy, 37 patients (39%) had haematological (n=8) or neurological (n=30) responses (responders), 25 (26%) patients showed

no improvement (non-responders), and 33 patients (35%) were considered possible responders because of less marked improvements. Vitamin B12 concentrations were above 150pmol/L in 84% of the responders and above 220pmol/L in 54% of responders. MMA concentrations were normal (<0.25µmol) in 23% of responders and were elevated (>13.6µmol) in only 37%. Concentrations of tHcy were normal (<12µmol) in 50% of the responders. If vitamin B12 therapy had been limited to patients with both vitamin B12 concentrations below 220pmol and MMA concentrations above 0.25µmol, 63% of responders would not have received any treatment. In the group of non-responders, 20% had vitamin B12 concentrations below 150µmol, 60% had MMA concentrations above 13.6µmol and 43% had tHcy concentrations greater than 13.6µmol. Results from this study suggest that low vitamin B12 and elevated metabolite concentrations are not always predictive of a subsequent clinical response to vitamin B12 therapy. This study did not report whether renal impairment had been considered as a cause of elevated metabolite concentrations.

212. Hvas *et al* (2001) assessed the effect of vitamin B12 treatment in reducing elevated concentrations of plasma MMA and tHcy in an RCT. Participants (n=140; median age 75 years) with mild to moderately elevated plasma MMA (0.40-2.00µmol) were treated weekly with intramuscular injections of either 1mg of cyanocobalamin or placebo for 4 weeks. After 3 months, significant decreases in MMA and tHcy concentrations were observed in the treatment group compared to the placebo group. Although concentrations of MMA decreased to below 0.29µmol in 69% of the treatment group they remained above this level in 31% of participants (which could not be explained by renal impairment).

213. In a study of 80 patients (median age 66 years) diagnosed with food-bound malabsorption of vitamin B12 with clinical manifestations of neurological impairment (Andrès *et al*, 2003), 48 patients were treated with intramuscular vitamin B12 and 22 with oral vitamin B12 (500-1000µg/d). Although correction of serum vitamin B12 and tHcy concentrations occurred in all patients within 2-3 months, symptom recovery was only observed in 43% of patients.

Clinical relevance of low vitamin B12 status

214. The relationship between low vitamin B12 status and clinically manifest B12 deficiency is uncertain, i.e. whether low vitamin B12 status will progress to clinical disease (e.g. symptomatic anaemia or neuropathy), fluctuate, or resolve.

215. Hin *et al* (2006) examined the association between peripheral neuropathy and low vitamin B12 concentrations (<133pmol/L) and related markers in a population-based study of older people (n=1000; ≥75years). Low serum vitamin B12 concentrations were identified in 13% of the participants. No association was found between concentrations of vitamin B12, MMA, tHcy or HoloTC and symptoms of neuropathy. A significantly increased risk of missing ankle tendon jerks (a more sensitive marker of peripheral neuropathy) was found in individuals in the top 2 quartiles of MMA concentration (mean 0.32-0.68µmol) compared to the bottom quartile (mean 0.18µmol) and in individuals with tHcy concentration in the top quartile (mean 22.34µmol/L) compared to the bottom quartile (mean 9.92µmol/L). No adjustment was made for elevated concentrations of MMA and tHcy due to renal impairment as a previous analysis of the data had indicated that this was not an important factor in this population (R Clarke; personal communication, 2006). Individuals with serum vitamin B12 concentration below 133pmol/L (n=100) were treated for 3 months with hydroxycobalamin injections (1mg/month). Although treatment was associated with substantial reductions in concentrations of MMA and tHcy in 99% of the participants, there were no significant effects on any clinical manifestations.

216. Hvas *et al* (2001) followed up individuals (n=432; median age 72 years) with an elevated concentration of plasma MMA (>0.28µmol), without evidence of renal impairment, for 1-4 years. Over time, a high variation was found in MMA concentrations (coefficient of variation, 34%), which increased (more than 20%) in 16% of participants but decreased (more than 20%) in 45% of participants. No association was found between a high MMA concentration and clinical manifestations related to vitamin B12 deficiency.

217. Solomon (2005) observed large intra-individual variations in pre-therapy concentrations of vitamin B12, MMA and tHcy of ambulatory patients (n=304; age not reported) evaluated for vitamin B12 deficiency, which varied by 23%, 23%, and 17% respectively over 2-6 weeks.

Oral supplementation with vitamin B12 to correct deficiency

218. The active absorption of protein-bound vitamin B12 is impaired in individuals with vitamin B12 deficiency although approximately 1% of orally administered crystalline cynocobalamin is absorbed by passive diffusion (Baik & Russell, 1999). Consequently, vitamin B12 deficiency is usually treated with intramuscular injections of hydroxycobalamin or cynocobalamin (1mg/month). Oral supplements of vitamin B12 (1-2mg/day) have been shown to be as effective as intramuscular injections in correcting biochemical markers of vitamin B12 deficiency (Hathcock & Troendle, 1991).

219. In the Netherlands, Eussen *et al* (2005) conducted a dose-finding trial in older people with low vitamin B12 status (serum vitamin B12 concentrations 100-300pmol/L; plasma MMA concentrations ≥ 0.26μmol/L and serum creatinine ≤120μmol/L) to determine the lowest oral dose of vitamin B12 associated with maximum reductions in serum MMA concentrations. Participants (n=120, age ≥ 70 years) received vitamin B12 in daily oral doses of 2.5, 100, 250, 500, or 1000μg, for 16 weeks which resulted in reductions to below 0.26μmol/L in 21%, 38%, 52%, 62%, and 76% of the participants respectively. Oral doses which were associated with 80-90% of the maximum reduction in plasma MMA concentration (33%) varied between 647-1032μg/day, which is about 400 times the RNI for vitamin B12 (1.5μg/day). This study did not attempt to distinguish the extent to which differences in individual responses were due to active rather than passive absorption of vitamin B12.

Prevalence of clinically manifest vitamin B12 deficiency

220. The prevalence of clinically manifest vitamin B12 deficiency due to pernicious anaemia or due to food-bound malabsorption is uncertain. Pernicious anaemia has traditionally been regarded as the most common cause of vitamin B12 deficiency (Carmel, 1997). Lindenbaum *et al* (1988) found that pernicious anaemia was the underlying cause in 68-83% of patients (n=40) with neurological symptoms due to vitamin B12 deficiency. Carmel (1997) reported that 8 out of 9 studies found food-bound malabsorption of vitamin B12 was the cause of vitamin B12 deficiency in more than 30% of patients. In a study of 172 patients in France (› 65 years) with a confirmed vitamin B12 deficiency, 57% were diagnosed with malabsorption of food-bound vitamin B12 and 33% were diagnosed with pernicious anaemia (Andrès *et al*, 2005). In over 200 elderly patients (age not reported) in France, vitamin B12 deficiency

was attributed to malabsorption of food-bound vitamin B12 in 60-70% of cases and pernicious anaemia in 15-20% of cases. Other causes included dietary deficiency in less than 5%; malabsorption in less than 5% and hereditary vitamin B12 metabolism diseases in less than 1% (Andrès *et al.*, 2004). In a UK based population study of older people (n=1000; ≥ 75years), only 3 out of the 125 (2.4%) people identified with low vitamin B12 status (<133pmol/L) had pernicious anaemia (Hin *et al*, 2006).

221. Tables 2-5 show the number of patients seen in hospitals in the UK (2000-2002/3), by country, and give an indication of clinical disease associated with vitamin B12 deficiency. The numbers do not include patients seen in primary care settings. The number of cases reported might be an under-representation of the actual frequency of vitamin B12 associated neurological impairment since some cases may remain unrecognised, especially when clinical symptoms and signs are less well defined. The subacute combined degeneration of the spinal cord category only includes cases associated with biochemical evidence of vitamin B12 deficiency; the peripheral neuropathy category includes all causes of peripheral neuropathy, not just those associated with vitamin B12. Diabetes mellitus is the most common cause of neuropathy and the second most common is alcohol abuse (Williams, 2003).

Table 2. Count of finished admission episodes for selected diagnoses in NHS hospitals in England

	Finished Admission Episodes		
Diagnosis	2000/01	2001/02	2002/03
Subacute combined degeneration of spinal cord	18	18	25
Pernicious anaemia	543	581	623
Peripheral neuropathy	9,244	9,739	11,545

Table 3. Count of finished admission episodes for selected diagnoses in NHS hospitals in Wales

	Finished Admission Episodes		
Diagnosis	2000	2001	2002
Subacute combined degeneration of spinal cord	2	1	1
Pernicious anaemia	31	55	35
Peripheral neuropathy	352	323	354

Table 4. Count of deaths and discharges (equivalent to finished admission episodes) for selected diagnoses in NHS hospitals in Northern Ireland

	Deaths and Discharges		
Diagnosis	2000	2001	2002
Subacute combined degeneration of spinal cord	0	0	0
Pernicious anaemia	46	29	49
Peripheral neuropathy	182	195	273

Table 5. Count of continuous inpatient stay (equivalent to finished admission episodes) for selected diagnoses in NHS hospitals in Scotland

	Continuous Inpatient Stay		
Diagnosis	2000	2001	2002
Subacute combined degeneration of spinal cord	3	1	2
Pernicious anaemia	173	123	148
Peripheral neuropathy	958	1080	1133

Source: Hospital Inpatients System. A finished admission episode is the first period of in-patient care under one consultant within one healthcare provider. Please note that admissions do not represent the number of in-patients, as a person may have more than one admission within the year.

218. The General Practice Research Database (GPRD) contains longitudinal medical records from approximately 420 primary care practices throughout the UK (representing 4% of the total number in the UK) and is geographically representative. Tables 6 to 9 show the number of patients in the GPRD, in 2005, with at least: one record of vitamin B12 deficiency or serum vitamin B12<150pmol/L; one prescription for vitamin B12 supplements; one record of pernicious anaemia caused by vitamin B12 deficiency; and one record of neuropathy caused by vitamin B12 deficiency (UK Medicines and Healthcare Products Regulatory Agency; Personal Communication, 2006). Information regarding vitamin B12 was extracted from 369 primary care practices, which represents 3.5% of the total in the UK.

Table 6: Number of patients (aged 65 years and over) in the GPRD (2005) with a record of being vitamin B12 deficient or with vitamin B12 levels < 150pmol/L

Country	Number of patients	As percentage of total number of patients aged 65 years and over registered in the GPRD for 2005*
England	2731	0.76
Wales	185	0.83
Scotland	157	0.97
Northern Ireland	78	0.61
Total	**3151**	**0.76**

Table 7: Number of patients (aged 65 years and over) in the GPRD (2005) with a record of at least one prescription for vitamin B12

Country	Number of patients	As percentage of total number of patients aged 65 years and over registered in the GPRD for 2005*
England	1671	0.46
Wales	86	0.38
Scotland	180	1.11
Wales	33	0.26
Total	**1970**	**0.48**

Table 8: Number of patients (aged 65 years and over) in the GPRD (2005) with at least one record of pernicious anaemia and at least one prescription for vitamin B12

Country	Number of patients	As percentage of total number of patients aged 65 years and over registered in the GPRD for 2005*
England	406	0.11
Wales	23	0.10
Scotland	44	0.27
Northern Ireland	15	0.12
Total	**488**	**0.12**

Table 9: Number of patients (aged 65 years and over) in the GPRD (2005) with at least one record for peripheral neuropathy and at least one prescription for vitamin B12

Country	Number of patients	As percentage of total number of patients aged 65 years and over registered in the GPRD for 2005*
England	34	0.009
Wales	2	0.009
Scotland	3	0.19
Northern Ireland	3	0.023
Total	**42**	**0.010**

*Total number of patients aged 65 years and over registered in the GPRD (369 practices) for 2005: England, 361 377; Wales, 22 367; Scotland, 16 196; Northern Ireland, 12 814; UK, 412 754.

The impact of folic acid on individuals with undiagnosed vitamin B12 deficiency

223. Folic acid supplementation can improve anaemia among persons with vitamin B12 deficiency, but does not prevent damage to the nerves, spinal cord, or brain, and it has been suggested that the neurological manifestations may even be aggravated (Reynolds, 2002). Treatment of undiagnosed vitamin B12 deficient individuals with folic acid may, therefore, delay diagnosis of the deficiency (the 'masking' of vitamin B12 deficiency) and result in the insidious progression of neurological symptoms leading to irreversible neurological damage.

224. Evidence suggests that folic acid intakes up to 1mg/day are not associated with delayed diagnosis of vitamin B12 deficiency in older people. The UL (i.e. the highest level likely to pose no risk of adverse health effects for almost all individuals in the population) set for folic acid intake in the USA (FNB, 1998) and in Europe (SCF, 2000) of 1mg/day, is based on the progression of neurological symptoms in individuals with undiagnosed vitamin B12 deficiency (see paragraphs 86 to 89). In the UK, a GL (which represents an approximate indication of levels that would not be expected to cause adverse effects) of 1mg/day was set for folic acid intake (EVM, 2003) based on concerns regarding the masking of vitamin B12 deficiency (see paragraph 90).

Epidemiological evidence of clinically manifest vitamin B12 deficiency pre- and post-fortification

225. The incidence of clinically manifest vitamin B12 deficiency before and after fortification does not appear to have been systematically considered in countries that have introduced mandatory fortification. A number of studies have assessed biochemical markers of status in some population groups. In the USA, Mills *et al* (2003) compared the prevalence of megaloblastic anaemia in 1573 patients (53-75 years) at the Veterans Medical Center in Washington (between 1992 and 2000), with low serum vitamin B12 concentrations (<258pmol/L), before and after fortification (implemented 1996-1998). There were no significant differences in the proportions without anaemia during the pre-fortification period (39.2%), the period when fortification was being implemented (45.5%), and the period following mandatory fortification (37.6%). These findings did not change when the analysis was limited to patients aged over 60 years or when low serum vitamin B12 concentration was defined as <150pmol/L. About 55-60% of patients (70-80% of those aged over 74 years) had low serum vitamin B12 concentrations with anaemia.

226. Another post-fortification study in the USA (Johnson *et al*, 2003) observed a prevalence for vitamin B12 deficiency (serum vitamin B12 <258pmol/L and MMA >0.27µmol/L) of 23% in an elderly group at risk of nutritional problems (mean age 76 years; n=103); these subjects were three times more likely to have impaired cognition than those who were not deficient. The study reported only one non-anaemic vitamin B12 deficient subject with elevated MCV; however, it was also noted that coexisting iron deficiency was quite common in the vitamin B12 deficient subjects, several of whom exhibited microcytic anaemia or low-normal MCV. Vitamin B12 deficiency was associated with poor cognition, anaemia and hyperhomocysteinemia. The authors noted that people aged 65 years and over are prone to many chronic illnesses and anaemia associated with chronic disease is likely to be due to multiple nutritional deficiencies and multifactorial in origin.

227. Data from hospital discharge surveys in the USA suggest that the number of cases of pernicious anaemia or subacute combined degeneration of the spinal cord have not increased post-fortification (see paragraphs 457-458).

228. A study of women in Canada, aged 65 years and older (n=4572), reported an increase of 64% in mean serum folate concentrations with a concomitant decrease in folate deficiency (serum folate < 6.0nmol/L) from 6.3% to 0.88% after fortification (Ray *et al*, 2003). Average serum vitamin B12 concentrations increased from 280 to 300pmol/L, and the prevalence of low vitamin B12 concentrations (serum vitamin B12<150pmol/L) in combination with supraphysiological concentrations of serum folate (>45nmol/L) increased significantly from 0.09% before fortification to 0.61% following fortification.

229. Six months after folic acid fortification of flour in Chile, Hirsch *et al* (2002a) reported an increase in mean serum folate concentrations of low-income people over 70 years (n=149), from 16.2 to 32.7nmol/L. Twenty eight per cent had low vitamin B12 status when defined as serum B12 concentration <165pmol/L; and 31% had vitamin B12 deficiency when defined as serum tHcy >14µmol/L. The fortification of flour with folic acid increased their dietary intake of folic acid by about 400µg/day. A moderate reduction in tHcy concentrations was observed (12.95 to 11.43µmol/L), but there was no change in serum vitamin B12 concentrations.

230. It has been proposed that a controlled field trial is carried out to determine the likelihood of side effects, of which delaying the diagnosis of neurological symptoms associated with vitamin B12 deficiency is of most concern (Wharton and Booth, 2001). A trial with folic acid intakes at the levels recommended (less than 1mg/day) would not be adequately powered to give clear evidence unless the study population was very large. A trial with intakes of folic acid above 1mg/day across the entire study population might expose susceptible individuals to an unacceptable risk and could be considered unethical.

Summary

231. Clinical manifestations of vitamin B12 deficiency are macrocytic anaemia and/or neuropathy. Although anaemia is the most common presentation, haematological symptoms do not always occur and neurological symptoms are sometimes the only clinical expression of vitamin B12 deficiency. Neurological symptoms have been reported to occur in the absence of anaemia in 20-30% of cases.

232. There are a number of uncertainties in the biochemical measures used to diagnose vitamin B12 deficiency. Low serum concentrations of vitamin B12 or

elevated concentrations of the metabolites, MMA and tHcy, are not always predictive of a clinical response to vitamin B12 therapy. Intra-individual variation in concentrations of vitamin B12, MMA, and tHcy can also affect the detection of vitamin B12 deficiency. Furthermore, there is no consensus regarding cut-off points for serum or plasma concentrations of vitamin B12, MMA, or tHcy to define deficiency.

233. Estimates of the prevalence of low vitamin B12 status are uncertain and have ranged from 5% to over 20% depending on the biochemical marker and criteria used. A combined analysis of 3 studies (which measured serum concentrations of vitamin B12 and tHcy) has estimated the prevalence of low vitamin B12 status to be 5% in people aged 65-74 years and 10% in people aged 75 years and over (Clarke *et al*, 2004).

234. The relationship between low vitamin B12 status and clinically manifest vitamin B12 deficiency has not been clarified. It is uncertain whether low vitamin B12 status will always progress to clinical disease. The presence of low vitamin B12 concentrations and elevated MMA and tHcy concentrations is not always predictive of a clinical response to vitamin B12 therapy.

235. It has been hypothesised that mandatory fortification of flour with folic acid might have adverse effects on neurological function in people aged 65 years and over with low vitamin B12 status. Evidence suggests that adverse effects are not associated with doses of folic acid up to 1mg/day. (See section 8 for estimations of the number of people aged 65 years and over, in the UK, who might be exposed to levels of folic acid above 1mg/day at different levels of fortification.)

236. There are no reports from countries that have introduced mandatory fortification indicating deleterious effects on older people with low vitamin B12 status.

237. It has been estimated that a dietary intake 400 times greater than the RNI for vitamin B12 may be needed to correct vitamin B12 deficiency caused by food-bound malabsorption of vitamin B12. The fortification of flour with vitamin B12 to improve status in people aged 65 years and over may not be a feasible option as the effect of population exposure to such high doses is unknown.

Other possible adverse effects of folic acid

Epilepsy

238. Phenytoin, an anti-epileptic drug, decreases serum folate concentrations in half of epileptic patients, thus increasing the risk of folate depletion (Berg *et al*, 1992). Supplementation with folic acid prevents deficiency but also modifies phenytoin pharmacokinetics and may lower serum phenytoin concentrations. At pharmacological doses, folic acid supplementation may lead to poorer seizure control (Reynolds, 2002).

239. In a population-based study of 7103 epileptic patients in Canada (Ray *et al*, 2005b) no significant change in serum phenytoin concentrations was observed after mandatory fortification, which was estimated to provide an average of 200µg/day of folic acid. This suggests that the dose of folic acid provided by mandatory fortification is unlikely to have an adverse impact on anticonvulsant drug metabolism. This study does not, however, provide any information on the impact of higher doses of folic acid.

Multiple births

240. Multiple pregnancies result in more complications and poorer outcomes compared to singleton pregnancies (Kinzler *et al*, 2000). Several studies have reported an association between consumption of multivitamins (including folic acid) or folic acid alone by women during pregnancy and an increase of almost 40% in multiple births (Czeizel *et al*, 1994; Werler *et al*, 1997; Ericson *et al*, 2001).

241. A number of large studies have investigated whether folic acid fortification or supplementation is associated with an increased prevalence of multiple births. In a population-based cohort study in China, of women who either did (n=127,018) or did not (n=114,997) receive folic acid supplements (400µg/day) during pregnancy, no association was observed between folic acid consumption and multiple births (Li *et al*, 2003). Two studies in the USA investigated rates of twinning pre- and post-fortification of foods with folic acid (fortification was introduced 1 January 1996 and became mandatory by 31 December 1998). Shaw *et al* (2003) examined the prevalence of twinning among more than 2.5 million births between 1990-1999 and found no change in twinning prevalence associated with folic acid fortification. Waller *et al* (2003) examined over 1 million births between 1 January 1996 and 31 December

1998. Although an increase in twinning frequency was found, this was consistent with the increase of 1-4% per year observed prior to fortification. However, as both these studies only include births up to 1999 there are not enough data after mandatory fortification to assess the long-term association with increased twinning.

242. Signore *et al* (2005) investigated whether twinning rates had increased in the USA since folic acid fortification was permitted in 1996 by examining birth and fetal death records from 1990-2000. To exclude the influence of fertility treatments (see paragraph 243), the analysis was restricted to nulliparous women aged 16-19 years. A total of 25,065 twin and 3,362,245 singleton pregnancies were included in the analysis. Twin gestation rates were found to be stable from 1990-1996 (7.2 per 1000) then steadily increased (averaging 2.4% per year) until 2000 (8.2 per 1000). The authors concluded that although twin gestation rates had increased following fortification the effect was less than predicted from intervention studies and the increase was difficult to separate from the long-term trend to increased twinning.

243. *In vitro* fertilisation (IVF) has been shown to be a strong confounder in studies examining the relationship between folic acid fortification/supplementation and increased prevalence of multiple births. IVF is associated with both use of folic acid and twinning (Berry *et al*, 2005). In a Swedish study (Ericson *et al*, 2001), almost 2% of the women had used *in vitro* fertilisation and it has been shown, on the basis of modelling, that failure to account for this could explain all of the apparent association between folic acid consumption and twinning (Berry *et al*, 2005).

244. The effect of folate status (plasma and red cell folate concentration) on fertility treatment outcome was assessed in a cohort of women (n=602; mean age 34 years) in the UK undergoing IVF treatment (Haggarty *et al*, 2006). Eighty-nine percent of the women had two embryos transferred. The likelihood of twin births following double embryo transfer IVF treatment was significantly associated with increased plasma/red cell folate concentrations and low age. The authors suggest that the high incidence of twin births associated with IVF treatment could be reduced by identifying women at increased risk of multiple embryo transfer based on plasma folate concentrations and age.

245. Haggerty *et al* (2006) also reviewed data from the USA on multiple birth rates arising from IVF treatment following mandatory fortification and observed that IVF treatment was associated with an 11-13% increase in the incidence of multiple births.

Embryo selection

246. Mutations such as MTHFR 677 TT are associated with CVD and stroke (see section 7). It has been proposed that the use of folic acid in pregnancy could interfere with the process of natural selection by increasing the survival of embryos with genotypes which have relatively common mutations in the MTHFR gene (C677T and A1298C) (Munoz-Moran *et al*, 1998; Reyes-Engel *et al*, 2002). This study reported an increase in individuals with both mutations who were born in Spain between 1977 and 1982 and noted that folic acid supplementation in pregnancy was recommended in Spain in 1982. However, the authors noted that it was difficult to accept the size of the apparent effect they observed. To date, there are no other reports of this finding in Spain or in countries where supplementation is advised or mandatory fortification has been introduced.

Anti-folate chemotherapy

247. Concerns have been raised that the efficacy of anti-folate drugs could be reduced by mandatory fortification of flour with folic acid. The basis of anti-folate chemotherapy is to limit folate availability to tumour cells by competing for intracellular folate enzymes. Inhibition of these enzymes affects the pool of folate derivatives available for single carbon unit transfer for nucleotide synthesis; rapidly dividing tumour cells are therefore unable to replicate DNA and undergo apoptosis (Robien, 2005).

248. Methotrexate (MTX) is an anti-folate drug widely used in chemotherapy regimens and also for treatment of autoimmune diseases such as rheumatoid arthritis. MTX reduces the activity of dihydrofolate reductase (DHFR) which is required for the reduction of folic acid to THF before it can be converted to 5-MTHF and acquire activity.

249. Anti-folate drugs also affect other rapidly replicating normal cells, e.g., haematopoietic and intestinal mucosal cells, which can lead to neutropenia and diarrhoea. Protocols for some anti-folate drugs recommend folic acid

supplementation to optimise the patient's folate status before and between treatment to minimise treatment-related toxicity in normal tissues (Robien, 2005).

250. Studies which have examined the interactions between folates and anti-folate drugs have generally used mouse and cell culture models. A review of studies which evaluated interactions between folates and anti-folates used for the treatment of cancer reported that most studies had focused on the use of folates as a rescue agent against toxic effects of anti-folate drugs and that no human studies had evaluated dietary folate intake as a potential effect modifier (Robien, 2005). One cross-sectional study (Schroder *et al*, 1986) which evaluated the effect of folic acid supplementation (75-200µg/d) before (preceding 3 months) and during MTX treatment in children (n=53) with acute lymphocytic leukemia found no difference in erythrocyte MTX concentrations between children who had or had not received folic acid supplements.

251. Studies on the effect of folic acid supplementation during MTX treatment for rheumatoid arthritis have generally reported an improvement in drug tolerance and minimal interference with drug effectiveness (Robien, 2005). A post-hoc analysis of 2 RCTs comparing MTX with leflunomide (another drug used to treat rheumatoid arthritis) reported that folic acid (1-2mg/day) significantly reduced the response to treatment. Neither of these RCTs, however, was designed to test the effect of folic acid on MTX efficacy and there were a number of differences between the studies, including disease duration and use of non-steroidal anti-inflammatory drugs. One of the studies also did not have a placebo group.

252. The interaction of folic acid with anti-folate drugs is a theoretical concern. There are insufficient human data regarding the effect of folic acid on anti-folate medication and the doses at which folic acid might affect their action to conclude that mandatory folic acid fortification would modify their efficacy.

Summary

253. There is no evidence to suggest that mandatory fortification of flour at levels which would increase folic acid intakes by 200µg/day would adversely modify the pharmacokinetic effects of phenytoin, a drug used for anticonvulsant treatment of epilepsy. The impact of higher levels of folic acid fortification on anticonvulsant effects of phenytoin is unknown.

254. There is no substantive evidence to suggest that folic acid fortification is associated with multiple births resulting from natural conception; however, high intakes of folic acid may increase the likelihood of twin births in women undergoing multiple embryo transplant fertility treatment.

255. There is no substantive evidence to suggest that folic acid fortification is associated with increased survival of embryos with genotypes associated with deleterious effects.

256. There is no evidence to suggest that folic acid fortification would reduce the efficacy of anti-folate chemotherapy.

Unmetabolised folic acid in the systemic circulation

257. The appearance of unmetabolised folic acid in the systemic circulation has raised concerns regarding the long-term effects of high intakes of folic acid.

258. A study that examined the acute appearance of unmetabolised folic acid in the systemic circulation in response to the consumption of folic acid fortified foodstuffs by young (age 18-42 years; n=14) and old subjects (62-83 years; n=30), reported a threshold intake of 266µg folic acid/test meal (Kelly *et al*, 1997). At doses lower than the threshold only 5-MTHF, the form normally present in plasma, was found.

259. In a small pilot study (n=4), Sweeney *et al* (2006) compared the acute effects of consuming 1mg of folic acid as a single dose and as two doses of 500µg, three doses of 333µg, five doses of 200µg, and ten doses of 100µg over 8-hour periods. All studies were carried out 2 weeks apart. Unmetabolised folic acid was not detected in the serum of any participants pre-prandially but was detected post-prandially in the serum of all subjects at all test doses.

260. A study in the USA (Troen *et al*, 2006), where mandatory fortification has been introduced, found unmetabolised folic acid in fasting plasma samples of 78% of participants (n=105; age 50-75 years) (see paragraph 263). In most cases the amount of unmetabolised folic acid in the plasma represented only a small fraction (5%) of total folate compared to the concentration of 5-MTHF (95%). The authors noted that consumption of supplements in the study population was higher than for the average US population.

261. Another study in the USA (Pfeiffer *et al*, 2004) analysed the distribution of folate forms in non-fasting serum samples (n=42). In 32 samples with folate concentration below 50nmol/L (22µg/L), unmetabolised folic acid comprised 2.3% of the total folate concentration. In 10 samples with total folate concentration above 50nmol/L (22µg/L), the proportion of unmetabolised folic acid was 16%. Non-fasting serum samples were used in this analysis and no information was available on dietary intakes of the subjects who had provided the samples.

262. In Ireland, unmetabolised folic acid was found in the cord blood of newborn infants (n=11; 8 full-term, 3 premature) at birth (Sweeney *et al*, 2005). The mean concentration of unmetabolised folic acid was 0.185µg/L, however no information was provided on how much this represented as a proportion of total folate concentration in the cord blood. None of the mothers were consuming folic acid supplements; further details regarding their diet were not provided.

263. A cross-sectional study of postmenopausal overweight/obese women in the USA (n=105; 50-75 years) investigated the relationship between unmetabolised folic acid in the blood and natural killer (NK) cell cytotoxicity (Troen *et al*, 2006). NK cells are part of the immune response and it has been suggested that low NK cytotoxicity may increase cancer risk (Imai *et al*, 2000). Unmetabolised folic acid was found in fasting plasma samples of 78% of the women. NK cytotoxicity was significantly lower in women with unmetabolised folic acid found in the plasma compared to those without detectable unmetabolised folic acid. After stratification by age, a significant linear decrease in NK cytotoxicity was found with higher concentrations of unmetabolised folic acid in women aged 60-75years (25% lower NK cytotoxicity with plasma concentrations > 3.1nmol/L).

Summary

264. Unmetabolised folic acid has been detected in the systemic circulation following oral doses of folic acid above 260µg.

265. Evidence from one cross-sectional study (Troen *et al*, 2006) suggests an association between the presence of unmetabolised folic acid in the blood and altered immune function.

266. Overall, there are insufficient data in humans to assess the long-term effects of exposure to unmetabolised folic acid in the systemic circulation.

7 Folate, B vitamins, and chronic disease

Folate and cardiovascular disease

267. The relationship between folate intake and cardiovascular disease (CVD) risk was previously considered by COMA (DH, 2000). COMA concluded that in the absence of evidence supporting a causal association between improved folate status and reduction in the incidence of CVD, there was no basis for recommending fortification with folic acid in relation to decreasing CVD risk.

268. Studies considered in relation to CVD risk are detailed in Tables 29-31, Annex 7.

Plasma homocysteine

269. Elevated plasma tHcy concentration is a risk factor for CVD (Homocysteine Studies Collaboration, 2002). Plasma tHcy concentrations are inversely associated with measures of folate status (plasma and red blood cell folate concentrations). The remethylation of homocysteine to methionine, by methylene tetrahydrofolate reductase (MTHFR), is dependent on an adequate supply of folate; low folate status, therefore, results in elevated plasma tHcy concentrations. A supplement of folic acid in the range 200 to 400µg/day has been reported to give a near-maximal lowering of plasma tHcy concentration (Ward *et al*, 1997, 2002; van Oort *et al*, 2003).

270. Vitamins B12, B6 and B2 are also required for the remethylation of homocysteine. The dietary supply of vitamins B6 (McKinley *et al*, 2001) and B12 (Quinlivan *et al*, 2002) has also been shown to affect plasma tHcy concentrations. Population subgroups such as vegetarians and, particularly vegans, who have a limited dietary supply of vitamin B12, tend to have raised tHcy concentrations despite an adequate supply of dietary folate (Herrmann *et al*, 2003; Lloyd-Wright *et al*, 2003).

271. Other factors may also affect plasma tHcy concentrations. Lifestyle factors, such as alcohol consumption, physical inactivity and cigarette smoking (de Bree *et al*, 2001; Mennen *et al*, 2002; Ganji & Kafai, 2003; Husemoen *et al*, 2004; Nurk *et al*, 2004) have been associated with elevated plasma tHcy concentrations. Intervention studies have suggested that coffee

consumption can raise plasma tHcy concentrations (Verhoef *et al*, 2002; Strandhagen *et al*, 2003) and betaine (trimethylglycine, which can be metabolised endogenously from choline) consumption can lower plasma tHcy concentrations (Olthof *et al*, 2003; Steenge *et al*, 2003). Modification of dietary patterns, for example, a diet rich in fruits, vegetables, low-fat dairy products and with reduced saturated and total fat, may be more effective in lowering plasma tHcy concentrations than increasing consumption of folate rich foods alone (e.g. diet rich in fruit and vegetables) (Appel *et al*, 2000).

272. A number of gene variants affect folate blood concentrations and plasma tHcy concentration (see paragraph 69). The most commonly studied genetic variant is the MTHFR 677 *C→T* polymorphism, which is associated with elevated plasma tHcy concentrations (Sharp & Little, 2004). The homozygous form (*TT*) is associated with plasma tHcy concentrations typically 25% higher (Engbersen *et al*, 1995).

273. An RCT of vitamin B2 supplementation found no effect on plasma tHcy concentrations in older subjects (McKinley *et al*, 2002). Some evidence from observational studies, however, suggests that vitamin B2 status may affect plasma tHcy concentrations in persons with the *TT* genotype, but the reported associations are not consistent (Hustad *et al*, 2000; Jacques *et al*, 2002; McNulty *et al* 2002). Hustad *et al* (2000) observed an inverse association between vitamin B2 status and plasma tHcy concentration in persons with the *CT* or *TT* genotype regardless of their folate status. Jacques *et al* (2002) observed an inverse association between vitamin B2 status and plasma tHcy concentration in subjects with the *TT* genotype only if their plasma folate concentrations were low, while McNulty *et al* (2002) found an inverse association between vitamin B2 status and tHcy concentration only in persons with the *TT* genotype, regardless of their folate status. Moat *et al* (2003) observed an association between vitamin B2 status and lowering of plasma tHcy concentration that was not restricted to the *TT* genotype and not related to folate status.

274. A meta-analysis of observational studies concluded that elevated concentrations of plasma tHcy were a modest independent risk factor for cardiovascular disease in healthy populations (Homocysteine Studies Collaboration, 2002). Another meta-analysis of observational studies concluded that individuals with the C677T MTHFR genotype had a

significantly higher risk of coronary heart disease, particularly in the presence of low folate status (Klerk *et al*, 2002). A meta-analysis, which combined both the prospective studies of plasma tHcy concentration and disease risk and C677T MTHFR genotype case-control studies, concluded there was strong evidence for a causal relationship between elevated concentrations of plasma tHcy and cardiovascular disease (Wald *et al*, 2002). However, these results were disputed by a later meta-analysis which combined prospective and case-control studies of the association between C677T MTHFR genotype and coronary heart disease and found no strong evidence of an association in Europe, Australia and North America (Lewis *et al*, 2005). A meta-analysis of cross-sectional, case-control and cohort studies investigating the association between plasma tHcy concentrations and the MTHFR polymorphism (Casas *et al*, 2005), concluded that the observed increase in risk of stroke among individuals homozygous for the MTHFR *TT* variant was close to that predicted from the differences in plasma tHcy concentration (Homocysteine Studies Collaboration, 2002).

275. It has been suggested that elevated plasma tHcy concentration may induce endothelial dysfunction (Chambers *et al*, 2000), which is a risk factor for CVD (Widlansky *et al*, 2003). High doses of folic acid (5-10mg/day) have also been shown to improve flow-mediated dilatation in coronary artery disease patients (Doshi *et al*, 2001; Title *et al*, 2000) and smokers (O'Grady *et al*, 2002), forearm blood flow, but not arterial elasticity, in smokers (Mangoni *et al*, 2002), and volumetric coronary blood flow in hyperhomocysteinemic patients with coronary artery disease (Willems *et al*, 2002). In one study, the effect of folic acid (5mg/day) on flow-mediated dilatation was shown to be independent of a plasma tHcy concentration lowering effect (Doshi *et al*, 2002). Lower doses of folic acid (e.g. 400µg/day), which result in maximal, or near maximal, plasma tHcy concentration reductions have not been shown to affect flow-mediated dilatation (Pullin *et al*, 2001; Hirsch *et al*, 2002b).

Epidemiological studies of folate and cardiovascular disease

Cross-sectional studies

276. Yang *et al* (2006) compared age-adjusted stroke mortality data (for people aged ≥40 years) in the USA and Canada between 1990-2002, with similar data from England and Wales. A decline in stroke mortality rates during this

period was observed in all countries. In the USA, a significant acceleration in the overall rate of decline was observed after 1998 (2.9% per year) compared to 1990-1997 (0.3% per year) which could not be explained by changes in other risk factors for stroke (cigarette smoking, hypertension, diabetes, total serum cholesterol). A significant accelerated decline in stroke mortality was also found in Canada after 1998 (5.4% per year) compared to 1990-1997 (1% per year). An improvement in the decline of stroke mortality was not observed in England and Wales after 1998. The authors noted that although the timing of the accelerated decline in stroke mortality was consistent with the timing of fortification in the USA and Canada, it was not possible to establish how much of the decline was due to reduced incidence and how much was due to reduced case-fatality rate. Improved health care may also have contributed to the decline in stroke mortality rates.

Prospective studies

277. Prospective epidemiological evidence for the association of folate status and dietary intakes of folate with CVD risk in the general population is shown in Tables 29 and 30 (Annex 7).

278. In two studies, higher circulating folate concentrations were associated with reduced CHD risk (Morrison *et al*, 1996; Voutilainen *et al*, 2004). Folsom *et al* (1998) found increasing plasma folate concentrations were associated with reduced CHD in women but not men.

279. In three studies, no association was found between the concentration of circulating folate and the risk of CHD (Chasan-Taber *et al*, 1996; Ford *et al*, 1998; Hung *et al*, 2003). Ford *et al* (1998) suggested that their study lacked adequate power to examine the association between circulating folate concentrations and disease-specific endpoints.

280. The results from studies investigating an association between dietary folate intake and risk of CVD are more consistent. In two studies, higher dietary intakes of folate were associated with reduced risk of CHD (Rimm *et al*, 1998; Voutilainen *et al*, 2001). He *et al* (2004) found increased dietary intake of folate was associated with a lower risk of ischemic stroke.

Randomised controlled trials

281. A number of RCTs have investigated the effect of treatment designed to lower plasma tHcy concentration on the risk of CVD.

282. In a trial of patients with atherosclerotic vascular disease (n=101) supplementation with 2.5mg folic acid, 250µg B12, and 25mg B6, for an average of over 2.5 years (range 0.9–6.0 years) reduced atherosclerotic plaque progression, assessed by two-dimensional B-mode ultrasound measurement of carotid plaques (Hackam *et al*, 2000).

283. Vermeulen *et al* (2000) measured the development or progression of subclinical atherosclerosis, estimated from exercise electrocardiography, among 158 healthy siblings of patients with premature atherothrombotic disease. Subjects received supplements of either 5mg folic acid and 250mg vitamin B6 or placebo for two years. A significant decrease in both plasma tHcy concentrations and abnormal exercise electrocardiography was found in those receiving folic acid/vitamin B6. The choice of primary outcome measure used in this study has been criticised because of its very low positive-predictive value when used in symptom-free populations (Bostom & Garber, 2000). Supplementation with folic acid/vitamin B6 was also associated with a reduction in blood pressure but there was no effect on measures of vascular function (van Dijk *et al*, 2001).

284. Schnyder *et al* (2001) examined the effect of B vitamin supplementation in 205 patients, following coronary angioplasty. Subjects received supplements of either 1mg folic acid, 400µg vitamin B12, and 10mg vitamin B6 or placebo for 6 months and the effect on restenosis and major adverse events was determined. After six months of vitamin treatment there was a significant decrease in plasma tHcy concentration and a significant decrease in the frequency of restenosis (19.6% vs. 37.6%).

285. A follow-up study (Schnyder *et al*, 2002) reported the outcome six months after the supplementation period had been completed and included data for a larger group of 553 patients. The need for repeated revascularization was lower in the group which had received supplements of folic acid/vitamin B6 (10.8% vs. 22.3%) and there was a non-significant trend toward fewer deaths and non-fatal myocardial infarctions in this group.

286. In another trial (Lange *et al*, 2004), 636 patients who had undergone successful coronary stenting were randomly assigned to receive 1mg of folic acid, 1mg of vitamin B12, and 5mg of vitamin B6 intravenously, followed by daily oral supplementation with either 1.2mg folic acid, 60µg vitamin B12 and 48mg vitamin B6, or placebo, for 6 months; in-stent restenosis was assessed using coronary angiography. B vitamin supplementation had an adverse effect on the risk of restenosis (34.5% vs. 26.5%) and a higher percentage of patients in the B vitamin group required repeat target-vessel revascularization (15.8% vs. 10.6%). B vitamin supplementation significantly reduced plasma concentrations of tHcy.

287. Till *et al* (2005) conducted a trial in 50 patients with atherosclerotic disease, who were supplemented with either 2.5mg folic acid, 500µg vitamin B12 and 25mg vitamin B6, or placebo, for 12 months. Atherosclerosis was assessed using ultrasound examination of carotid intima-media thickness (IMT). B vitamin supplementation significantly reduced plasma concentrations of tHcy and significantly decreased carotid IMT.

288. A number of secondary prevention trials have investigated the effect of supplemental folic acid in people with a previous vascular event. Some of these trials are ongoing (see Table 31, Annex 7).

289. In the first large intervention trial to report, the second Cambridge Heart Antioxidant Study (CHAOS-2) (Baker *et al*, 2002), 1882 ischemic heart disease patients received either 5mg/day folic acid or placebo. Although plasma tHcy concentrations were reduced by 13%, folic acid supplementation had no effect on the composite end-point of non-fatal myocardial infarction, cardiovascular death, or unplanned revascularization (risk ratio 0.97; 95% CI, 0.72-1.29). The trial was terminated prematurely, after a median treatment period of 1.7 years, because of a perceived lack of power to address the hypothesis.

290. The second large trial to report, the Vitamin Intervention for Stroke Prevention (VISP), examined the effect of B vitamin supplementation and the subsequent lowering of plasma tHcy concentration on recurrent stroke, myocardial infarction and death in patients (n=3680) with ischemic stroke (Toole *et al*, 2004). The control group received 200µg vitamin B6, 6µg vitamin B12 and 20µg folic acid each day; the intervention group received 25mg B6, 400µg vitamin B12 and 2.5mg folic acid each day. A 15% reduction in plasma

tHcy concentration was observed in the intervention group, but there was no effect on vascular outcomes during the two years of follow-up (risk ratio 1.0; 95% CI, 0.8-1.3). An association of baseline plasma tHcy concentration with vascular risk was found. The trial was terminated prematurely, after two years, when the interim analyses showed little possibility of demonstrating any effect of treatment on vascular events.

291. A subsequent subgroup analysis (n=2155) of the VISP data (Spence *et al*, 2005) excluded patients with very low (<250pmol/L) and very high (>637pmol/L) plasma vitamin B12 concentrations to eliminate those with possible vitamin B12 malabsorption and those likely to be taking vitamin B12 supplements. Patients with significant renal impairment were also excluded. For the combined outcome of stroke, coronary disease and death there was a 21% reduction in the risk of events for the intervention group compared to the control group (p=0.056 after adjustment for age, sex, blood pressure, smoking, and vitamin B12 concentration). Patients with median (322pmol/L) or higher baseline vitamin B12 concentrations randomised to the high-dose of vitamins had the best overall outcome and those with vitamin B12 concentration below the median and assigned to the low-dose vitamins had the worst (p=0.02 for combined outcome). As this study was a post hoc subgroup analysis the results should be interpreted with caution.

292. A sub-study of the Vitamins to Prevent Stroke (VITATOPS) trial (Dusitanond *et al*, 2005), examined the effect of B vitamin supplementation on circulating markers of inflammation (high-sensitivity C-reactive protein, soluble CD40L, IL-6) endothelial dysfunction (ICAM-1, VCAM-1, von Willebrand factor) and hypercoagulability (P-selectin, prothrombin fragment-1 and –2, D-dimer). Patients (n=285) with recent stroke or transient ischemic attack were randomised to receive either placebo or B vitamin supplementation (25mg vitamin B6, 500µg vitamin B12 and 2mg folic acid per day) for six months. A 30% reduction in plasma tHcy concentrations was observed, but there was no effect on any of the circulating markers of inflammation.

293. In the Norwegian Vitamin (NORVIT) trial (Bonaa *et al*, 2006), 3749 myocardial infarction patients (age 30-85 years) were randomised to receive one of the four following treatments: 800µg/day of folic acid, 400µg/day of vitamin B12 and 40mg/day vitamin B6; 800µg/day of folic acid and 400µg/day of vitamin B12; 40mg/day of vitamin B6; or placebo, for an average of three years. In the two

groups that received folic acid and vitamin B12, mean plasma tHcy concentration was reduced by 27% but there was no significant effect on CVD risk compared to the placebo group. Treatment with vitamin B6 also had no effect on CVD risk. An increased CVD risk was reported in the group who received folic acid, vitamin B12, and vitamin B6 (RR, 1.22; 95% CI, 1.00-1.5; p=0.05).

294. In the Heart Outcomes Prevention Evaluation (HOPE) 2 study, patients with a history of vascular disease or diabetes (n=5522; age ≥55 years) were randomised to receive either placebo or 2.5mg folic acid, 1mg vitamin B12, 50mg vitamin B6, for an average of 5 years (HOPE 2 investigators, 2006). Patients were recruited from countries with mandatory fortification (USA, Canada) and without mandatory fortification (Brazil, western Europe, Slovakia). The primary outcome measure was death from cardiovascular causes, myocardial infarction, and stroke. Although there was a significant reduction in plasma tHcy concentration in the intervention group, no significant differences were found between the groups in rates of death from cardiovascular causes or myocardial infarction.

295. The earlier secondary prevention trials were initiated in the late 1990s and were designed to detect 30% reductions in the risk of CHD or stroke, based on results of epidemiological studies (Boushey *et al*, 1995; Danesh & Lewington, 1998). Since then, a meta-analysis of observational studies suggests that a 25% lowering of plasma tHcy is associated with a 10% lower risk of CHD and a 20% lower risk of ischemic stroke (Homocysteine Studies Collaboration, 2002). With improvements in CVD mortality rates, the cardiac event rates in ongoing trials have been lower than predicted. Several ongoing trials have, therefore, extended the duration of the follow-up period in order to accumulate sufficient number of cases (R Clarke; personal communication, 2005).

296. The B-Vitamin Treatment Trialists' Collaboration (2006) reviewed the design and statistical power of 12 randomised trials assessing the effects of lowering plasma tHcy with B vitamin supplements on the risk of CVD (see Table 31, Annex 7) and concluded that these trials may not involve enough vascular events or be of sufficient duration to detect reliable plausible effects of plasma tHcy lowering on CVD risk. The authors suggest that a meta-analysis of all the trials, involving 52,000 patients, should have adequate power to determine whether reducing tHcy concentration by 20-25% reduces the risk of CVD by at least 10%, within a few years.

Summary

297. Observational studies investigating the relationship between dietary folate intake and CVD risk have observed a protective association. Studies investigating the relationship between circulating folate concentrations and CVD risk have generally not shown a significant association.

298. One cross-sectional study (Yang *et al*, 2006) which examined trends in stroke mortality rates before and after mandatory folic acid fortification in the USA and Canada observed a significant decrease in stroke mortality following fortification in both countries. It is unclear how much of the decline is due to reduced incidence and how much to reduced case-fatality rate.

299. A number of RCTs have investigated whether folic acid supplementation reduces CVD risk. To date, no RCTs have demonstrated a beneficial or harmful effect of folic acid supplements on CVD risk. One RCT found an increased CVD risk with supplementation of folic acid in combination with vitamins B12 and B6.

Folate and cancer

DNA methylation

300. DNA methylation is important for epigenetic control of gene expression and maintenance of genomic stability (Jones & Baylin, 2002). DNA methylation occurs at cytosine bases within the cytosine-guanine (CpG) dinucleotide. CpG sites are unevenly distributed and are deficient in extensive sections of the human genome. In contrast, CpG islands are short regions of the genome which are rich in CpG nucleotides and are often found in the promoter regions of genes. Methylation of these CpG islands is thought to be important for gene expression.

301. Global DNA hypomethylation, together with hypermethylation of promoter regions, is a common observation in tumours (Jones & Baylin, 2002). The precise way in which methylation occurs is highly complex and poorly understood, with methylation changes in one gene resulting in hypo- and hypermethylation in other genes and gene specific hypermethylation occurring within the context of global hypomethylation.

302. Folate plays a central role in DNA methylation since it is critical for the remethylation of homocysteine to methionine which is the precursor of S-adenosylmethionine, the primary methyl group donor for DNA methylation (Selhub & Miller, 1992). On the basis of animal studies, concerns have been raised that folate status may alter DNA methylation, the pattern of gene expression, and ultimately disease risk. Genetic and nutritional factors which influence folate status have been correlated with human global DNA methylation (Stern *et al*, 2000; Friso *et al*, 2002). The extent to which folate influences DNA methylation is not clear and requires further investigation.

303. Animal studies suggest that altering the maternal diet in pregnancy can influence the methylation status of the offspring (Cooney *et al*, 2002; Wolff *et al*, 1998). Dietary supplementation of mice with folic acid, vitamin B12, choline, and betaine, was found to alter the phenotype of their offspring (Waterland & Jirtle, 2003) leading the authors to suggest that higher intakes of folic acid could influence epigenetic gene-regulatory mechanisms during human embryonic development.

304. It has also been suggested that folic acid supplementation significantly increases methylation within CpG islands raising concerns that fortification of flour with folic acid may methylate normally unmethylated promoter regions of tumour suppressor genes leading to their inactivation and promoting the development of cancer (Kim, 2004).

305. Whilst it is possible that early exposure of the human egg and conceptus to altered methylating environments may influence the risk of long-term methylation patterns, there are no reports of increased cancer incidence in the offspring specifically linked to the introduction of advice on folic acid use in pregnancy or mandatory fortification.

Folic acid and animal models of cancer

306. Studies using young animal models of colorectal cancer suggest that folic acid suppresses the development of tumours (Kim, 2003). In older animal models of colorectal and breast cancer, however, high levels of folic acid may promote the development of tumours (Kim, 2003; Kim 2004; Ulrich & Potter, 2006).

307. Based on the evidence from animal models it has been suggested that folic acid may have dual modulatory effects on cancer development depending

on the time and dose of the intervention (Ulrich & Potter, 2006): inhibiting tumour development in normal tissues but promoting the progression of established neoplasms.

308. Findings from animal studies have a number of limitations and cannot always be extrapolated to humans. This is because animal models given large doses of carcinogens or genetically modified to induce tumour development differ in a number of histologic, clinical and genetic aspects from cancer development in humans (Rogers & Nauss, 1985; Banerjee & Quirke, 1998).

Human studies of folate and cancer

309. The relationship between folate intake and cancer risk was previously considered by COMA (DH, 1998; DH, 2000). COMA found there was insufficient evidence at that time to draw conclusions on any specific links between folate intake and the development of cancer.

Prospective epidemiological studies

310. Human prospective studies considered in relation to cancer risk are detailed in Tables 32-35, Annex 7.

311. Most studies have investigated the relationship between folate intake and risk of colorectal (CRC) and breast cancer.

Colorectal cancer

312. A meta-analysis of 7 prospective cohort and 9 case-control studies (Sanjoaquin *et al*, 2005) examined the association between folate intake and CRC risk. In the cohort studies, a significantly lower risk of CRC (RR=0.75; CI, 0.64-0.89) was observed for those in the highest category of folate intake from foods compared to those in the lowest category. Total folate intake (from foods and folic acid supplements) was associated with a non-significant 5% lower risk of CRC (RR=0.95; CI, 0.81-1.11). The pooled estimates from the case-control studies showed a significantly lower CRC risk associated with high folate intakes from foods compared to low intakes (RR=0.76; CI, 0.60-0.96) but not for total folate from food and supplements. However, as there was considerable heterogeneity between the case-control studies, the authors suggested that the pooled estimates should be interpreted with caution. The meta-analysis concluded that higher folate intake may be

associated with lower CRC risk but confounding by other dietary factors could not be ruled out. The protective association observed was stronger for intakes of folate from foods alone than total folate from foods and supplements.

313. In an analysis of data from the Nurses' Health Study and the Health Professionals Follow-Up Study, total folate intake was not associated with colon cancer risk in men but higher intakes were associated with a lower risk in women (Wei *et al*, 2004). Larsson *et al* (2005) also observed a reduced risk of colon cancer in women with higher total folate intake. Other studies have observed no association between total folate intake and colorectal cancer risk in women (Flood *et al*, 2002; Harnack *et al*, 2002; Terry *et al*, 2002). Su and Arab (2001) and Konings *et al* (2002) observed higher total dietary folate intakes were associated with reduced colon cancer risk in men, but not women. Glynn *et al* (1996) observed no significant association between folate intake and risk of colon cancer in men (odds ratio 0.51; 95% CI, 0.20-1.31).

314. Details of prospective cohort studies examining the association between folate intake and CRC risk can be found in Table 32, Annex 7.

315. Serum folate concentrations were not associated with colon or rectal cancer risk in one study (Glynn *et al*, 1996), but higher concentrations were associated with reduced risk of CRC in another (Kato *et al*, 1999). Van Guelpen *et al* (2006) observed no significant association overall between plasma folate concentration and CRC although they noted that when plasma folate concentrations were divided into fifths, the risk was significantly higher in the third and fourth fifth, but not in the highest, compared to the lowest fifth. This study did not adjust for a number of other factors that might affect CRC risk such as intake of other dietary variables or use of aspirin or non-steroidal anti-inflammatory drugs.

316. Details of prospective studies investigating the association of serum folate with CRC risk can be found in Table 33, Annex 7.

317. Some studies in the USA have suggested an association between multivitamin use, prior to the introduction of fortification, and a reduction in risk of CRC. In the Nurses' Health Study cohort, a reduced risk was observed in colon cancer incidence after 15 or more years since first use of multivitamins

(RR=0.25, 95% CI 0.13-0.51) (Giovannucci *et al*, 1998). In the Cancer Prevention Study II cohort an association was observed with past (10 or more years previously), but not recent, multivitamin use and reduced risk of CRC (RR=0.71, 95% CI 0.57-0.89) (Jacobs *et al*, 2003). In the Health Professionals' Follow-up Study, multivitamin use of 10 or more years was not associated with a significant reduction in colon cancer incidence (Giovannucci *et al*, 1995).

318. Associations between multivitamin use and disease outcomes cannot be attributed to a single nutrient, and confounding by health conscious behaviours among multivitamin consumers (e.g. exercise and dietary factors) may also complicate interpretation.

Breast cancer

319. No studies have reported a significant association between folate intake and breast cancer risk (Zhang *et al*, 1999; Sellers *et al*, 2001, 2004; Feigelson *et al*, 2003). One study (Sellers *et al*, 2004), however, found that lower dietary folate intakes were associated with increased breast cancer risk in women with a family history of breast cancer.

320. Low folate intake combined with alcohol consumption (and in some studies, low methionine intake) has been associated with a higher risk of colon and breast cancers in some epidemiological studies (Giovannucci *et al*, 1995; Glynn *et al*, 1996; Zhang *et al*, 1999, 2003; Su & Arab, 2001; Sellers *et al*, 2001). Alcohol has been shown to act as a folate (or methyl group) antagonist (Halstead, 1995). Other studies have, however, found no association between cancer risk and a combined low folate and high alcohol intake (Flood *et al*, 2002; Harnack *et al*, 2002; Feigelson *et al*, 2003).

321. No studies have reported a significant association between plasma folate concentrations and breast cancer risk (Wu *et al*, 1999; Zhang *et al*, 2003).

Other cancers

322. No relationship has been found between dietary folate intake and squamous or basal cell carcinoma of the skin (Fung *et al*, 2002, 2003; van Dam *et al*, 2000), bladder cancer (Michaud *et al*, 2002), cervical cancer (Alberg *et al*., 2000), non-Hodgkin's lymphoma (Zhang *et al*, 2000), and pancreatic cancer (Skinner *et al*, 2004). Stolzenberg-Solomon *et al* (2001) found an inverse

relationship between folate intake and pancreatic cancer in smokers. Two prospective nested case-control studies that investigated the relationship between serum folate concentrations and prostate cancer risk reported no association (Weinstein *et al*, 2003; Hultdin *et al*, 2005). Hultdin *et al* (2005) found an increased risk for prostate cancer in those with higher serum vitamin B12 concentrations.

323. While no relationship between dietary folate intake and lung cancer was observed in one study (Yuan *et al*, 2003), a protective effect was reported in a nested case-control study (Voorrips *et al*, 2000).

324. Two prospective studies examining the relationship between dietary folate intake and the incidence of ovarian cancer (Larsson *et al*, 2004; Kelemen *et al*, 2004) reported no significant association. Both studies, however, observed that higher dietary folate intakes were associated with reduced risk of ovarian cancer among women consuming alcohol.

325. A study in Canada (French *et al*, 2003) reported that folic acid fortification (which became mandatory in 1998) was associated with a significant decline in the incidence of neuroblastoma among children under 17 years of age. The overall incidence of neuroblastoma declined from 1.57 cases per 10,000 before 1998 to 0.62 cases per 10,000 after 1998 (RR, 0.38; 95% CI: 0.23-0.62) after adjustment for mean age and median disease stage at diagnosis. No significant change was found in the rate of infant acute lymphoblastic leukaemia or hepatoblastoma.

MTHFR gene variants and cancer risk

326. A review of case-control studies investigating the relationship between polymorphic genes involved in folate metabolism and colorectal cancer risk (Little *et al*, 2003; Sharp & Little, 2004) concluded that, in most studies, the gene variants MTHFR 677 TT and 1298 CC, associated with low folate blood concentrations, were also associated with a moderate reduction in risk for colorectal cancer. Relative risks ranged from 0.45-0.9, but most did not reach statistical significance.

327. A prospective study (Van Guelpen *et al*, 2006) found a reduced CRC risk associated with the MTHFR 677 TT genotype which was independent of plasma folate concentrations.

328. Some studies have found an increased colorectal cancer risk associated with the MTHFR 677 TT genotype combined with low folate intake or high alcohol consumption (Kim, 2003).

329. It has also been suggested that the MTHFR C677T polymorphism modifies the relationship between dietary folate intake and breast cancer. A case-control study which examined the effect of MTHFR 677 TT and 1298 CC polymorphisms on the association between folate intake and breast cancer risk observed that lower intakes of folate were associated with increased breast cancer risk among all genotypes but particularly among subjects with the 677 TT genotype (Shrubsole *et al*, 2004)

Randomised controlled trials

330. No RCTs designed to investigate the relationship between folate and cancer risk have yet reported. A number of small RCTs have examined the effect of folic acid supplementation on surrogate endpoint biomarkers of CRC but it is difficult to draw any definitive conclusions from these studies (Kim, 2003).

331. A follow-up study of nearly 3000 women who participated in a folic acid supplementation trial during pregnancy in the 1960s assessed the relationship between folic acid supplementation and death (Charles *et al*, 2004). Women in the RCT received placebo, 200µg/day, or 500µg/day folic acid during pregnancy. In women randomised to the high dose of folic acid, an increased risk of all cancer deaths was observed (RR 1.70; 95% CI, 1.06-2.72) and a trend for an increased risk of breast cancer and all cause mortality. The authors acknowledged that this could be a chance finding as the number of deaths in the cohort was small (n=24 from a total of 2928 randomised) and the confidence intervals wide. The study design and statistical analysis of this trial has been criticised as being inappropriate (Bland, 2005). This study was also not designed to test the hypothesis that folic supplementation could influence cancer risk.

332. Preliminary results (abstract) from an RCT suggest a role of folic acid in the progression of premalignant lesions (Cole *et al*, 2005). Subjects with a recent history of colorectal adenomas were randomly assigned to receive placebo (n=505) or 1mg/day of folic acid (n=516) with or without aspirin, for 3 years. At the end of the intervention period, the incidence of colorectal adenomas was not significantly different between the two groups. In subjects who

continued supplementation (n=488) with placebo or folic acid, a significant increase in adenoma multiplicity was reported among subjects consuming folic acid (RR 1.44; 95% CI, 1.03-2.02). Details are not available of the duration of the second interval of folic acid supplementation or other factors considered in the analysis. As this study has not been published in a peer-reviewed journal, caution should be exercised in its interpretation.

333. Hubner *et al* (2006) investigated effects of variants in folate metabolism genes on colorectal cancer in 546 patients participating in an RCT investigating supplementation with folic acid and aspirin for the prevention of colorectal adenoma recurrence. Participants with a recent history of colorectal adenomas were randomised into 4 groups: folic acid alone (500µg/day); aspirin alone (300µg/day); folic acid and aspirin; and placebo. Colorectal adenoma recurrence was assessed after a period of 3 years. No association was found between folic acid supplementation or aspirin treatment and risk of colorectal adenoma or colorectal carcinoma recurrence. A significantly reduced risk of recurrence was reported for patients heterogygous for the MTRR A66G polymorphism, or heterozygous for the MTHFR A1298C polymorphism.

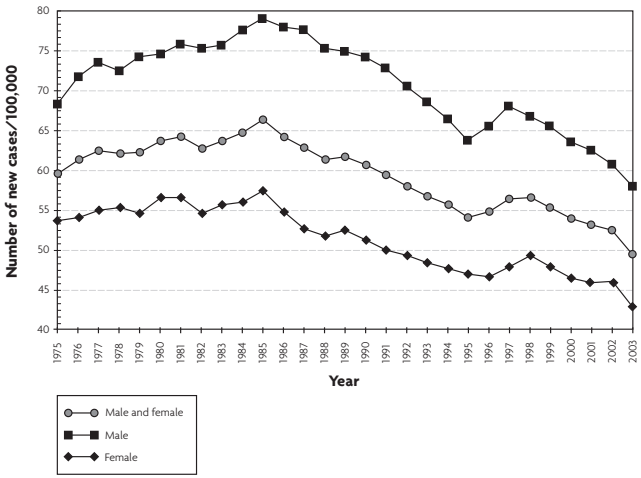
334. Results from the NORVIT (Bonaa *et al*., 2006) (see paragraph 293) and HOPE-2 (HOPE 2 Investigators, 2006) (see paragraph 294) trials, which examined the effect of folic acid supplementation on cardiovascular outcomes, also reported effects on cancer risk. In the NORVIT trial, cancer risk was not increased in patients supplemented with 800µg of folic acid for 3 years; the HOPE-2 trial found no significant differences in incident cancers and deaths from cancer in patients assigned to 2.5mg of folic acid for 5 years or placebo. However, both the NORVIT and HOPE-2 trials were not designed to investigate the effect of folic acid on cancer risk and may not have been adequately powered to detect an association. Additionally, any adverse effects of folic acid on cancer risk may be specific to a certain stage of cancer, for example, individuals with premalignant lesions may be at greater risk and subjects for these trials were not selected on this basis.

Trends in cancer risk in the USA and Canada following mandatory fortification

USA

335. In the USA, voluntary fortification of food products with folic acid was first authorised in March 1996 and mandatory fortification became effective from January 1998. For all populations combined, age-adjusted overall trends in colorectal cancer incidence in the USA[5] between 1975-2003 (National Cancer Institute, 2005) (see Figure 1) show there was a significant increase in colorectal cancer incidence of 0.8% per year from 1975 until 1985, a significant decline of 1.8% per year between 1985-1995, a non-significant increase of 1.2% per year between 1995-1998, followed by a significant decline of 2.1% per year between 1998-2003. The incidence rate after 1998 continued to decline at approximately the same rate as before 1996.

Figure 1: Colorectal cancer incidence rates, USA 1975-2003 (per 100,000/year, age-adjusted to the 2000 USA population)



336. The temporary increase in rates of CRC incidence after several years of decline occurred around the same time as fortification of food products with folic acid, raising the possibility that fortification might be implicated in the increase in CRC incidence between about 1996-1998. Food manufacturers

5 Information on cancer incidence was based on data collected by cancer registries participating in the National Cancer Institute's Surveillance, Epidemiology, and End Results (SEER) Program and represents 10-14% of the population (Edwards et al, 2005).

began fortifying foods in 1996 (when voluntary fortification was permitted) and CRC incidence began to increase in the same year. Colorectal tumours usually develop from benign polyps or adenomas over a period of 10-15 years (Tomeo *et al*, 1999). If the increase was caused by fortification then the effect of folic acid on tumour progression would have to have been instantaneous. Longer periods of exposure are generally expected before an increase in cancer or precancerous lesions can be detected, for example, up to 3 years in polyp recurrence intervention trials (Martinez *et al*, 2006).

337. If the trends are considered by sex, it can be seen from Figure 1, that the increase in CRC incidence occurred in 1996 for men and in 1997 for women. A more detailed consideration of the trends shows that they also varied by age (see Figures 5-8, Annex 8): for ages 20-54 years, CRC incidence increased in 1995 for men and in 1997 for women; for ages 55-64 years, CRC incidence increased in 1996 for men and in 1997 for women; for ages 65-74 years, CRC incidence increased in 1996 for men and in 1997 for women; for ages 75 years and over, CRC incidence increased in 1997 for men and in 1996 for women. The greatest increases occurred in those aged 75 years and over, suggesting that the overall trend (Figure 1) is largely driven by the pattern for 75 years and older age-groups. Precursor high risk polyps are more common in this age group than in younger age groups (Williams *et al*, 1982; Clark *et al*, 1985).

338. Average plasma and red cell folate concentrations in the USA increased following folic acid fortification (Pfeiffer *et al*, 2005) peaking in 1999-2000 before starting to decrease in 2001-2002 (Ganji and Kafai, 2006). However, it can be seen from Figure 1, that CRC incidence rates (for men and women combined) started to decline after 1998, i.e. before the decrease in population blood folate concentrations.

339. If the increase in CRC incidence was caused by folic acid fortification it is not clear why the increase should occur at different times in the various age groups and at different times for men and women or why the change in rates was not clearly consistent with changes in folate status.

340. The increased incidence might be explained by improved screening for colorectal cancer: in 1995, the US Preventive Services Task Force (USPSTF)[6] reversed earlier position statements and endorsed screening with fecal occult blood testing (FOBT) and sigmoidoscopy for people at average risk for

6 The USPSTF is an independent expert advisory panel convened by the US Public Health Service, Department of Health and Human Services, that systematically reviews the evidence of effectiveness and develops recommendations for clinical preventive services.

colorectal cancer (Frame *et al*, 1997; Levin & Bond, 1996). Examination of trends in cancer screening practices (Breen *et al*, 2001) based on data from the National Health Interview Survey (NHIS)[7] , shows that the proportion of the population aged 50 years or older who reported recent use of screening endoscopy[8] increased from 12% in 1992 to 19% in 1997 for men and from 7% to 10% in women; use of FOBT increased from 24% in 1992 to 29% in 1998 for men and from 25% to 26% for women. Use of colorectal cancer screening using either FOBT or endoscopy increased from 29% in 1992 to 37% in 1997 for men; and from 28% to 30% in women.

341. Screening for CRC may lead to increased incidence since cancers are detected earlier than they would be otherwise. It may also lead to decreased incidence since premalignant lesions can be identified and removed before they develop into cancer. This makes it difficult to make inferences about the effect of screening on incidence rates, particularly as different tests are recommended which may have differing effects on incidence, and trends in their use appear to vary for men and women and over time. The overall marked decline in CRC incidence rates in the 1990s has been attributed to increased screening (Cress *et al*, 2006).

342. There are no clear temporal changes in the incidence of other cancers such as breast, lung, and prostate following mandatory folic acid fortification.

Canada

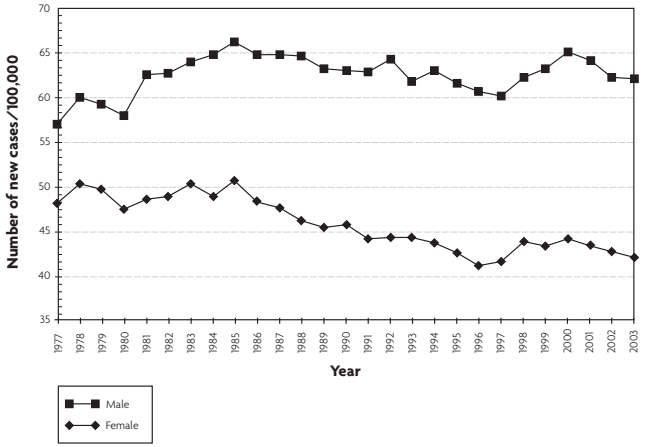
343. In Canada, fortification of food with folic acid was permitted in December 1996 and became mandatory in November 1998. Age-adjusted trends in CRC incidence in Canada[9] for 1977 to 2003 (see Figure 2) show non-significant increases of 1.7% per year after 1997 for men and 1.2% per year after 1996 for women (Canadian Cancer Society/National Cancer Institute of Canada, 2006). Since 2000, rates have reverted to the previous longer time trend showing a decline in CRC incidence for men and women.

7 The NHIS is conducted annually by the National Center for Health Statistics of the Centers for Disease Control and Prevention. It is a continuing, nationwide in-person survey of approximately 40,000 households in the civilian non-institutionalised population.

8 The term *endoscopy* refers to screening procedures that may have consisted of rigid procto-sigmoidoscopy, flexible sigmoidoscopy, or colonoscopy.

9 Incidence data were available for all the provinces and territories. Incidence rates are estimates for Quebec 2002 and 2003 and for Ontario 2003.

Figure 2: Colorectal cancer incidence rates, Canada 1977-2003 (per 100,000/year, age-standardised to the 1991 Canadian population)



344. As in the USA, the timing of the increase in CRC incidence differed for men and women; however the increase in Canada occurred earlier for women than for men, whereas the reverse was observed in the USA.

345. There are no nationally representative data available on blood folate concentrations in Canada before and after fortification. A retrospective study which analysed clinical samples obtained from Canadian women aged 65 years and older from Ontario and British Columbia reported a 64% increase in mean serum folate concentration: from 14.8nmol/L (6.5µg/L) before fortification (January 1996-December 1997) to 24.2nmol/L (10.7µg/L) post-fortification (January 1998-December 2000) (Ray *et al*, 2003). Analysis of clinical samples obtained from women aged 18-42 years in Ontario reported that mean red blood cell folate concentration increased by 41%, from 527nmol/L (232.7µg/L) pre-fortification to 741nmol/L (327µg/L) post-fortification (Ray *et al*, 2002). Caution should be applied in the interpretation of these results as they are based on clinical samples from 1-2 provinces. It is not known if the decline in CRC incidence after 2000 coincided with a decline in blood folate concentrations as there are no data on folate status in Canada after 2000.

346. Survey data on the use of colorectal cancer screening are available for only a few regions[10] which may not be representative of the Canadian population and there are no data on trends in cancer screening practices.

347. Examination of trends for the incidence of other cancers shows that after 1996 there was a significant decline in the incidence of lung cancer (1.6%) for men and a significant increase in the incidence of prostate cancer (3.4%); for women, there was a non-significant decline (1.0%) in Non-Hodgkin lymphoma after 1997.

Committee on Carcinogenicity

348. Evidence on the possible association between folic acid and cancer risk was referred to the Committee on Carcinogenicity (2006) for their consideration. The Committee agreed there were multiple plausible mechanisms by which folic acid could influence cancer risk. It was agreed that evidence from animal studies suggested timing of the folic acid intervention, i.e. before or after development of premalignant lesions, may be an important factor in cancer risk. It was observed, however, that: folic acid deficient diets were associated with a reduction in body weight (Song *et al*, 2000), which may also influence cancer risk; dose levels used in several of the animal studies (e.g. Wargovich *et al*, 1996; Reddy *et al*, 1996; Le Leu *et al*, 2000) were considerably higher than would be achieved by fortification; and a clear dose-response relationship was not apparent (Wargovich *et al*, 1996). The difficulties of extrapolating findings from animal studies to humans were noted.

349. With regard to the human studies, it was noted that a reduced cancer risk was suggested by epidemiological studies; however the presence of other micronutrients in multivitamin supplements was a complicating factor. It was agreed that preliminary findings reported in the abstract of the unpublished RCT by Cole *et al* (2005) suggest that folic acid may increase the development of colorectal adenomas in people with a previous history of this condition. It was noted that a number of factors might determine the susceptibility of individuals or groups to increased risk of cancer. The Committee concluded that it was uncertain if the possible association of high folic acid intakes with increased cancer risk outweighed the known and potential health benefits and that this balance might vary across individuals and populations by genetic characteristics and life stage.

10 British Columbia, Newfoundland and Labrador covered 100% of the population, Saskatchewan (covered 63% of the population) and Ontario (covered 27% of the population; Toronto not included).

Summary

350. Although evidence from prospective studies suggests a trend towards a protective effect of folate intake on colon cancer risk, some studies did not adjust for all likely confounding factors, e.g. dietary fibre intake, which has been shown to be correlated with folate intakes and CRC risk (Bingham *et al*, 2005).

351. Some animal models suggest a dual action of folic acid in cancer development: high intakes may suppress the development of early lesions in normal tissue but may increase the progression of established neoplasms.

352. The evidence for an association between folic acid and increased or reduced cancer risk in humans is equivocal. No RCTs designed to investigate the relationship between folic acid and cancer incidence have yet reported.

353. Time trends for CRC incidence in the USA and Canada show that mandatory fortification of foods with folic acid occurred at around the same time as non-significant increases in CRC incidence. If the increased rate was caused by folic acid fortification, the effect of folic acid on cancer progression would have to have been immediate. The increases in CRC incidence occurred at different times for men and women in both countries. In the USA, the timing of the increase in CRC incidence also varied for different age groups. The timing of changes in average blood folate concentration of the USA population was also not clearly consistent with changes in CRC incidence. The increases in CRC incidence rates cannot be readily explained by changes in screening practices for CRC detection. Time trends in national cancer incidence rates can be affected by various factors; fluctuations in rates over a few years are often observed but can rarely be attributed with confidence to any single factor.

354. Although the trend data do not prove an association, they raise concerns about a possible relationship between folic acid fortification and increased CRC progression. Further RCTs would be required to determine if folic acid is associated with increased risk of cancer in susceptible populations.

Folate and cognitive function

355. The relationship between folate status and cognitive function was previously considered by COMA (DH, 2000). No conclusions could be drawn at that time. Deficiencies of several B vitamins have been known to lead to neurological deterioration and cognitive decline, but it may be that moderately low or subclinical B vitamin status is associated with cognitive impairment in people aged 65 years and over (Selhub *et al*, 2000).

Plasma homocysteine and cognitive decline or dementia in people aged 65 years and over

356. In people aged 65 years and over, plasma tHcy concentrations have been shown to be positively associated with age, independently of vitamin status, and negatively associated with folate status, independently of age and other vitamin status (Selhub *et al*., 1993).

357. In cross-sectional and case-control studies of people aged 65 years and over, elevated plasma tHcy concentrations, and low folate and vitamin B12 status, have been associated with poor cognition (Riggs *et al*, 1996; Selhub *et al*, 2000; Duthie *et al*, 2002; Durga *et al*, 2006), dementia and Alzheimer's disease (Clarke *et al*, 1998; Quadri *et al*, 2004). Hyperhomocysteinemia has also been associated with Parkinson's disease in case-control studies (Kuhn *et al*, 1998).

Prospective epidemiological studies

358. Several prospective cohort studies have investigated the association between plasma tHcy concentrations, B vitamin status and cognitive decline or dementia. In the study by Clarke *et al* (1998), 43 patients with dementia of Alzheimer's type were followed-up for 3 years and radiological evidence of disease progression was determined each year. Patients with plasma tHcy concentrations > 11µmol/L showed a more rapid progression of the clinical syndrome of dementia than those below this cut-off. The association between blood concentrations of folate and vitamin B12 at the first visit and disease progression showed a similar trend, but this was not statistically significant.

359. In a study of 32 normal elderly subjects (aged 69-80 years) followed-up for 5 years (McCaddon *et al*, 2001), plasma tHcy concentration was found to predict cognitive scores (mini-mental state examination and Alzheimer's

disease assessment scale, cognitive component) independently of age, sex, education, renal function, serum folate and vitamin B12 concentrations, smoking and hypertension ($p<0.001$). However, the number of subjects in this study and in the study by Clarke *et al* (1998) were very small.

360. In a cohort of 370 elderly people (aged 75 years and over), low baseline serum concentrations of both folate and vitamin B12 were associated with twice the risk of Alzheimer's disease three years later (Wang *et al* 2001). Plasma tHcy concentrations were not measured in this study.

361. In a European multicentre study of 586 subjects (aged 75-80 years) followed-up for 5 years (Eussen *et al*, 2002), no associations were observed between mental health (mini-mental state examination and geriatric depression scale) and plasma vitamin B12 or folate concentrations. Plasma tHcy concentration was not measured in this study.

362. A subsample of the Framingham Study cohort in the USA (n=1092, aged 68-97 years and without dementia) was followed-up for a median of eight years. During this time, 111 subjects developed dementia, including 83 with Alzheimer's disease. Increased plasma tHcy concentrations were associated with an increased risk of dementia and Alzheimer's disease independently of age, sex, apolipoprotein E genotype, CVD risk factors other than plasma concentrations of tHcy, folate, vitamin B12 and vitamin B6 (Seshadri *et al*, 2002).

363. A follow-up of the Framingham Study cohort included 2096 subjects without dementia or stroke who were followed-up for 11 years and stratified into three age groups (40-49 years, 50-59 years, 60-82 years) (Elias *et al*, 2005). In subjects aged over 60 years (n=705) higher plasma tHcy concentrations were associated with lower multiple measures of cognitive performance independently of age, sex, CVD risk factors other than plasma concentrations of tHcy, folate, vitamin B12 and vitamin B6. In the 60-82 years group, plasma vitamin B12 concentrations, but not plasma folate or vitamin B6, showed a positive association with cognition. In subjects aged less than 60 years, no associations with cognitive performance were observed.

364. A study of subjects drawn from the Maastricht Aging Study (n=144; aged 30-80 years) and followed-up for 6 years, investigated the relationship between cognitive performance, plasma tHcy concentrations, serum vitamin B12

concentrations and folate concentrations (Teunissen *et al*, 2003). Word learning and delayed recall tests (though not cognitive speed and information processing) were negatively associated with plasma tHcy concentration independently of age, sex and education level. No significant associations with serum vitamin B12 and folate concentrations were observed.

365. A cohort of 180 healthy community-dwelling older adults (aged 65 years or over) in the USA, post-fortification, was followed-up for 2.3 years and assessed using psychometric measures (Garcia *et al*, 2004). Of the cognitive tests employed, Stroop scores (a measure of executive cognitive function) correlated significantly with plasma tHcy concentrations independently of age, sex, education, diabetes mellitus, hypertension, serum vitamin B12 concentrations and red blood cell folate concentrations.

366. Luchsinger *et al* (2004) followed up a cohort of 679 subjects in the USA, pre- and post-fortification, without dementia (aged 65 years or over) for an average of 4.7 years and determined cognitive function and dementia diagnosis. No association between plasma tHcy concentrations and either cognitive function or dementia was observed. Plasma concentrations or dietary intakes of B vitamins were not measured.

367. A cohort of 499 healthy community-dwelling older adults (aged 70-79 years) in the USA, pre-fortification, was followed-up for 7 years and assessed using five standardised cognitive performance tests (Kado *et al*, 2005). In age and sex-adjusted analyses, increasing quartiles of plasma tHcy concentrations and decreasing quartiles of plasma folate and vitamin B6 concentrations were associated with increased risk of cognitive decline. After adjustment for folate and plasma tHcy concentrations, however, only low plasma folate concentrations predicted cognitive decline.

368. A prospective study in the Netherlands (Mooijaart *et al*, 2005) assessed whether plasma concentrations of tHcy, folic acid, or vitamin B12 predicted cognitive decline in old age. Participants (n=599; aged 85 years and over) were followed-up for 4 years. Cognitive tests (including MMSE, Stroop test, letter digit coding test, word recall test) were administered at baseline and then annually for 4 years. Cross-sectional analyses found that higher serum concentrations of tHcy and lower serum concentrations of folic acid were significantly associated with cognitive impairment. There was no association

between serum concentrations of vitamin B12 and cognitive decline. In the longitudinal analyses, no association was observed between plasma tHcy, folic acid, or vitamin B12 and rate of cognitive decline.

369. A prospective study of 3718 elderly subjects in the USA (aged 65 years or over), followed-up for a median of 5.5 years, unexpectedly found that a high folate intake, especially from supplements, was associated with a faster rate of cognitive decline (Morris *et al*, 2005). The rate of cognitive decline among persons in the highest quintile of folate intake (median, 742µg/day) was twice that of those in the lowest quintile of intake (median, 186µg/day). These differences in the annual rates of cognitive decline between the first and fifth quintiles of intake were the equivalent of seven years of older age. The rate of cognitive decline was more pronounced in those taking folic acid supplements of more than 400µg/day (p=0.001 compared to nonusers). The effect was less pronounced with folate intake from food (p=0.04). In a multiple-adjusted model that included folate intake, the rate of decline for an average 80-year old, consuming a supplemental dose of 20µg/day of vitamin B12, was 25% lower than the rate of cognitive decline for a similar person consuming 2.4µg/day; vitamin B12 intake, with or without supplementation, was not significantly associated with cognitive decline overall.

370. There are a number of limitations to this study: dietary intakes were determined by food frequency questionnaire; in 86% of participants this information was collected before the introduction of the mandatory fortification of flour with folic acid in 1998; and there were no follow-up measures. Furthermore, no measures of folate or vitamin B12 status were reported and the extent of biochemical vitamin B12 deficiency was not determined.

371. In Italy, a cohort of 816 dementia-free subjects (aged 65 years and over) was followed-up for an average of 4 years and assessed for subsequent development of dementia and Alzheimer's disease (Ravaglia *et al*, 2005). Serum vitamin B12, folate and tHcy concentration were measured at baseline. Plasma tHcy concentrations (>15µmol/L) were associated with an increased risk of dementia and Alzheimer's disease independently of age, sex, education, apolipoprotein E genotype, cardiovascular disease hypertension, diabetes, smoking status, BMI and serum concentrations of creatinine, folate

and vitamin B12. Low serum folate concentrations (≤ 11.8nmol or 5.2µg/L) but not vitamin B12 concentrations, were also independently associated with an increased risk of dementia and Alzheimer's disease.

372. A study in the USA, pre-fortification, followed 321 men (aged 50-85 years) for an average of 3 years and examined the relationship between measures of cognitive function (mini-mental state examination and tests of working memory, recall, language and spatial copying) and plasma tHcy concentration and B vitamin (folate, vitamin B6 and vitamin B12) intakes and status (Tucker *et al*, 2005). Measures of spatial copying ability were significantly associated with plasma tHcy concentration (negatively) and with concentrations and intakes of the B vitamins (positively), but only folate (status and intakes) remained independently protective against a decline in spatial copying after adjustment for other B vitamins and plasma tHcy concentration.

373. A prospective study which combined data from the Health Professionals' Follow-up Study and the Nurses' Health Study cohorts examined the relationship between folate intake and Parkinson's disease (Chen *et al*, 2004). A total of 47,341 men (aged 40-75 years) and 88,716 women (aged 30-55 years) were followed-up for an average of 12.7 and 17.3 years respectively. During this time, 248 men and 167 women developed Parkinson's disease. Baseline intakes of folate, vitamin B12, and vitamin B6 were not associated with risk of Parkinson's disease.

Randomised controlled trials

374. A Cochrane review identified four small RCTs that examined the effect of folic acid supplementation (with or without vitamin B12) in preventing cognitive impairment or slowing its progress (Malouf *et al*, 2003). Insufficient evidence was found for any effect of folic acid in either older healthy women or in patients with mild to moderate cognitive decline and dementia.

375. A Cochrane review, which included two small trials, examined the effect of vitamin B12 administration (oral or intramuscular) in preventing cognitive impairment or slowing its progress (Malouf & Areosa Sastre, 2003). The review concluded that there was insufficient evidence of any effect of vitamin B12 therapy on cognitive function of older people with low serum vitamin B12 concentrations and dementia. The authors noted that large RCTs, of sufficient duration, were required to evaluate any effect of vitamin B12 on

cognitive function in older people with low serum vitamin B12 concentrations, with and without dementia.

376. Results from 3 placebo-controlled RCTs have been reported subsequent to the Cochrane reviews. A trial of 140 older subjects (average age 74-75 years; plasma MMA concentration of 0.40-2.00μmol/L), given intramuscular injections of either vitamin B12 (1mg) or control, every 4 weeks for 3 months (Hvas *et al*, 2004), reported no effect of vitamin B12 administration on cognitive function or symptoms of depression.

377. In a trial of 195 older subjects (average age 76 years), 7.2% had vitamin B12 deficiency and 11% had folate deficiency; 64% of men and 45% of women had plasma tHcy concentrations of 16μmol/L or higher. The intervention group (n=126) were supplemented daily with 500μg vitamin B12, 800μg folic acid and 3mg vitamin B6, while the control group (n=69) received placebo for 4 months (Lewerin *et al*, 2005). Although B vitamin status and plasma tHcy concentrations were normalised, no effect of B vitamin supplementation was observed on movement or cognitive function.

378. In another trial of 276 adults (aged 65 years and older), participants received either a daily dietary supplement containing 1mg of 5-methyltetrahydrofolate, 500μg vitamin B12 and 10mg of vitamin B6, or placebo for 2 years (McMahon *et al*, 2006). Although plasma tHcy concentration was significantly lower in the intervention group than the placebo group, there were no significant differences in cognitive function.

379. Clarke (2006) noted that the trial by McMahon *et al* (2006) lacked statistical power as it included too few participants and treatment duration was too short. These limitations also apply to the trials by Hvas *et al* (2004) and Lewerin *et al* (2005). Clarke (2006) also suggests that randomised evidence for the effects of 3-7 years of B-vitamin treatment on cognitive function may be available for 20,000-50,000 participants from a meta-analysis of the large homocysteine-lowering trials for the prevention of cardiovascular events (see section 7).

Summary

380. Observational studies suggest that higher plasma tHcy concentrations are associated with poorer cognitive function and risk of dementia in older

people. The results from studies investigating an association between B vitamin intakes and status and cognitive function are equivocal.

381. RCTs have, as yet, failed to demonstrate any effect of folic acid or vitamin B12 therapy on cognitive function in older people, which might be due to insufficient sample size and duration.

382. There are indications of possible benefit but, overall, the evidence for either beneficial or deleterious effects of folic acid or vitamin B12 therapy on cognitive function in older people is presently inconclusive.

Folate and depression

383. A number of case-control and cross-sectional studies have reported lower blood folate concentrations in patients with depression compared to patients with other mental disorders or healthy subjects (Alpert *et al*, 2000). These studies have a number of limitations including small sample size, inadequate control groups, and confounding with a number of other factors associated with depression. Two population-based cross-sectional studies which controlled for possible confounders did not observe an association between serum folate concentration and depression (Penninx *et al*, 2000; Lindeman *et al*, 2000). Another population-based cross-sectional study (Morris *et al*, 2003) observed an association between folate status and lifetime depression only in subjects who had recovered from depression during the previous year but not in subjects still suffering from depression.

384. One prospective study of 2,313 men (aged 42-60 years), followed up for an average of 13 years (Tolmunen *et al*, 2004), observed a higher risk of receiving a discharge diagnosis of depression (RR 3.01, 95% CI: 1.56-5.82, p=0.001) in those with an energy-adjusted folate intake below the median relative to those above the median. The mean energy-adjusted folate intake in this population was 256µg/day; the median intake was not provided.

385. A Cochrane review (Taylor *et al*, 2006) identified 3 RCTs which examined the effectiveness of folic acid in the treatment of depression. Two studies compared treatment with 500µg/day of folic acid for 10 weeks (Coppen & Bailey, 2000) or 15mg/day methyltetrahydrofolate for 6 months (Godfrey, 1990) to placebo in the context of continued use of antidepressant medication; one study compared treatment with 50mg/day

methyltetrahydrofolate for 8 weeks to 50mg/day of an antidepressant drug (Passeri *et al*, 1993). The review concluded that although there was some evidence that augmentation of antidepressant treatment with folic acid may improve outcome, the limited evidence meant that the size of any potential benefit was uncertain and may be clinically insignificant. The trial which compared use of methyltetrahydrofolate as an alternative to antidepressant medication identified no benefit but was underpowered to measure a moderate difference between treatment.

Summary

386. Overall, there is insufficient evidence from prospective studies and RCTs to suggest an association between folate and depression.

Folate and bone health

387. Two prospective cohort studies have observed an association between raised plasma tHcy concentrations and non-vertebral osteoporotic fractures in older people (McLean *et al*, 2004; van Meurs *et al*, 2004) independent of age and bone mineral density (van Meurs *et al*, 2004), as well as other potential risk factors. Interference of collagen cross-linking by plasma tHcy has been a suggested mechanism behind this association, and van Meurs *et al* (2004) speculated that raised plasma tHcy concentration interferes with the development of the microarchitecture of bone independently of the amount of mineral in the bone.

388. A prospective study in 1748 postmenopausal women (aged 45-58 years; followed-up for 5 years) observed an association between the MTHFR TT genotype and low bone mineral density and increased fracture incidence (Abrahamsen *et al*, 2003). Macdonald *et al* (2004), observed no association between the MTHFR genotype and bone mineral density in a cohort of 1241 women (aged 45-54 years) who were followed-up for an average of 6.6 years. There was also no association between B vitamin intake (including folate) and bone mineral density.

389. An RCT in 628 patients (aged 65 years or over) with stroke, examined the effect of folic acid and vitamin B12 supplementation on the occurrence of hip fractures (Sato *et al*, 2005). Patients received either placebo or a daily dose of 5mg folic acid and 1.5mg vitamin B12 for two years. At baseline all patients

had high plasma tHcy and low serum vitamin B12 concentrations. After two years, plasma tHcy concentrations decreased by 38% in the treatment group and increased by 31% in the placebo group. The treatment group had significantly fewer hip fractures (RR=0.20; 95% CI: 0.08-0.50) than the placebo group. The fall frequency and reduction in metacarpal bone mineral density was similar in both groups.

390. Van Meurs and Uitterlinden (2005) have urged caution in the interpretation of these results and suggested that their generalisability may be limited as the patients had an unusually high incidence of hip fracture (4.3% per year compared to 0.3% for women and 0.6% for men in the average Japanese population of the same age) and the trial had a low statistical power, with only 33 hip fractures during the study period. The mean plasma tHcy concentration of patients was also high (19.9μmol/L) and previous studies (McLean *et al*, 2004; van Meurs *et al*, 2004) suggest there is a threshold above which plasma tHcy concentration predicts increased fracture risk.

Summary

391. Although there are suggestions of possible benefit, overall, there is insufficient evidence for beneficial effects of folic acid on bone health. There is no evidence to suggest deleterious effects of folic acid on bone health.

8 The potential impact of mandatory fortification of flour with folic acid: benefits and risks

Modelling exercise

392. The effect of fortifying flour with different doses of folic acid on the total folate intake (taking account of current levels in fortified foods, supplement use, processing losses, overage) of different population age groups was investigated by modelling intake data from the NDNS series (Annex 2). Flour was considered the most appropriate vehicle for fortification because of its near universal and narrow variability of consumption in the population (COMA, 2000). The purpose of the modelling exercise was to explore the effect of mandatory folic acid fortification of flour on the:

- Average intakes of folic acid;
- proportion of the population with folate intakes below the RNI[11] ;
- risk of NTD-affected pregnancies;
- total numbers in the population who might be exposed to doses of folic acid above the UL per day set for folic acid[12] ;
- number of people aged 65 years and over, with low vitamin B12 status, who might be exposed to doses of folic acid above 1mg/day.

393. In the UK, a GL of 1mg/day for folic acid was set for adults (EVM, 2003). A GL was not set for children as there were no data reporting adverse effects of high levels of folic acid in children. ULs were set for children in the USA and Europe which were extrapolated from the UL for adults on the basis of body weight. As GLs were not set for children in the UK, ULs were used for the purpose of the modelling exercise in order to estimate the total proportion of the UK population (including children) who might be exposed to high intakes of folic acid.

11 RNI for adults & children: 100µg/d for 4-6y; 150µg/d for 7-10y; 200µg/d for 11y and above.

12 UL for adults (≥18y) (FNB, 1998; SCF, 2000): 1mg/d; UL for children (SCF, 2000): 300µg/d for 4-6y; 400µg/d for 7-10y; 600µg/d for 11-14y; 800µg/d for 15-17y.

394. The potential effects of mandatory fortification of flour with folic acid were assessed at four different levels: 100µg/100g flour, 200µg/100g flour, 300µg/100g flour and 450µg/100g flour. After processing losses this would result in actual levels of 75µg/100g flour, 150µg/100g flour, 225µg/100g flour and 338µg/100g flour respectively.

395. To enable a comparison to be made of the effects of mandatory fortification with/without folic acid intakes from voluntary fortification and with/without folic acid intakes from voluntary fortification and supplement consumption, the modelling exercise also considered the effect of mandatory fortification of flour with folic acid excluding the contribution of folic acid intakes from:

- fortified breakfast cereals and fortified fat spreads but including the contribution from supplements;
- fortified breakfast cereals, fortified fat spreads, and supplements.

Methods and assumptions

Folic acid intakes

396. Estimates of the contribution of folic acid intake from supplements, voluntarily fortified breakfast cereals, and fat spreads, are the mean of values including and excluding overage. This is to represent typical chronic folic acid consumption from these sources, which will vary according to when they are consumed (levels of folic acid will decrease over time and will be higher if consumed shortly after the manufacturing process).

397. Estimates of the contribution of folic acid intakes from voluntarily fortified foods are based on levels contained in these foods at time of publication.

NTD risk

398. Data to model the effects of folic acid fortification of flour on reducing NTD risk are limited. The reduction in NTD risk for women of childbearing age (‹50 years) was estimated using two different approaches, based on either maternal red cell folate concentrations (Daly *et al*, 1995; 1997) or maternal serum folate concentrations (Daly *et al*, 1995; Wald *et al*, 2001) in response to increasing levels of folate intake. Both approaches are based on the data from

the study by Daly *et al* (1995). These two methods were used to provide an estimated range of the number of NTD-affected pregnancies which could be prevented at the 4 different levels of folic acid fortification of flour. The lower estimate in the range is based on the analysis by Wald *et al* (2001) and the upper estimate is based on the analysis by Daly *et al* (1995; 1997). Estimates of NTD risk at different levels of fortification were based on the current estimate of 700-900 NTD-affected pregnancies in the UK (see paragraph 171). Further details of how NTD risk was estimated are provided in Annex 2.

399. A number of uncertainties in the data used to model the reduction in NTD risk (Daly *et al*, 1995; 1997) may over- or under-estimate the impact of fortification on NTD risk (see paragraph 104).

400. More importantly, the effect of folic acid on reducing NTD risk is dependent on background blood folate concentrations (Daly *et al*, 1997), so at a particular dose of folic acid the proportional reduction in risk will be less with higher initial blood folate concentrations. The study by Daly *et al* (1995), which estimated the dose-response relationship between red cell folate concentration and NTD risk, was carried out in an Irish population in the late 1980s who may have had lower blood folate concentrations than women in the UK in 2006; use of this data to estimate NTD risk reduction would tend, therefore, to overestimate the effect of fortification.

Low vitamin B12 status

401. To estimate the number of people people aged 65 years and over with low vitamin B12 status at risk of exceeding folic acid intakes of 1mg/day following mandatory fortification, it was assumed that the prevalence of low vitamin B12 status was 5-10% (Clarke *et al*, 2004).

402. Further details of the methods and assumptions used for the modelling exercise are provided in Annex 2.

Results of modelling exercise

403. The summary results obtained from the modelling exercise at each level of flour fortification are shown in Table 10 (including wholemeal flour) and Table 11 (excluding wholemeal flour). Results including/excluding foods voluntarily

fortified with folic acid (breakfast cereals and fat spreads) and supplements are also shown in Tables 10 and 11. More detailed results of the modelling exercise, by population age groups, are presented in Annex 2 (Tables 12-17).

Table 10: Effects on the UK population* of mandatory fortification of all flour with folic acid INCLUDING fortification of wholemeal flour

Fortification level of folic acid µg/100g flour (level in food after processing)	Average increase in folic acid intake (µg/day)[13]	Estimated mean total folate intakes (µg/day)	Estimated number (%) of people with intakes below RNI**[14]	Estimated number (%) exceeding the UL of folic acid/day**[15]	Estimated number aged 65y+ with low vitamin B12 status exceeding 1mg/d folic acid***	Estimated NTD pregnancies prevented per year[16] (% reduction in NTD risk)
Includes folate and folic acid from all sources (including estimates of overage)[17]						
0	0	302	13,261,000 (23%)	127,000 (0.2%)	900	0
100 (75)	57	359	5,876,000 (10%)	241,000 (0.4%)	1,700	47-99 (7-11%)
200 (150)	115	409	2,940,000 (5%)	460,000 (0.8%)	2,800	91-198 (13-22%)
300 (225)	172	474	1,518,000 (3%)	907,000 (1.5%)	3,300	126-285 (18-32%)
450 (338)	258	560	799,000 (1%)	2,535,000 (4.3%)	9,000	175-378 (25-42%)
Excluding folic acid from fortified breakfast cereals and fat spreads						
0	-74	232	21,257,000 (36%)	18,000 (0.03%)	800	-70 (-10%)
100 (75)	-17	290	9,043,000 (15%)	38,000 (0.06%)	800	-7 (-1%)
200 (150)	40	347	4,288,000 (7%)	52,000 (0.09%)	900	42-90 (6-10%)
300 (225)	98	404	2,195,000 (4%)	119,000 (0.2%)	900	84-189 (12-21%)
450 (338)	184	491	1,139,000 (2%)	660,000 (1.1%)	1,400	140-315 (20-35%)
Excluding folic acid from fortified breakfast cereals, fat spreads and supplements						
0	-85	217	22,404,000 (38%)	0	0	-91 (-13%)
100 (75)	-28	274	9,627,000 (16%)	0	0	-28 (-4%)
200 (150)	30	331	4,589,000 (8%)	0	0	28-54 (4-6%)
300 (225)	87	389	2,353,000 (4%)	59,000 (0.1%)	0	70-153 (10-17%)
450 (338)	173	475	1,224,000 (2%)	557,000 (0.9%)	0	126-288 (18-32%)

* Based on data from the NDNS which does not include pregnant or lactating women

** Figures rounded to the nearest 1,000

*** Figures rounded to the nearest 100

13 Across all population groups (Table 18, Appendix 1, Annex 2).

14 For each age group (DH, 1991).

15 For each age group: 4-6y, 300µg/d; 7-10y, 400µg/d; 11-14y, 600µg/d; 15-17y, 800µg/d; adults, 1mg/d (European Scientific Committee on Foods, 2001). The largest proportion of the numbers exceeding the UL are children 4-10y (see paragraphs 404, 409, 412, 415, 418)

16 See paragraph 398 which explains why there is a range for NTD prevention.

17 Mean of values for overage applied and not applied.

Table 11: Effects on the UK population* of mandatory fortification of flour with folic acid EXCLUDING fortification of wholemeal flour

Fortification level of folic acid µg/100g flour (level in food after processing)	Average increase in folic acid intake (µg/day)[18]	Estimated mean total folate intakes (µg/day)	Estimated number (%) of people with intakes below RNI**[19]	Estimated number (%) exceeding the UL of folic acid/day**[20]	Estimated number aged 65y+ with low vitamin B_{12} status exceeding 1mg/d folic acid***	Estimated NTD pregnancies prevented per year (% reduction in NTD risk) 0
Includes folate and folic acid from all sources (including estimates of overage)[21]						
100 (75)	0	302	13,261,000 (23%)	127,000 (0.2%)	900	0
200 (150)	51	353	6,417,000 (11%)	225,000 (0.4%)	1,700	42-93 (6-10%)
300 (225)	102	403	3,424,000 (6%)	404,000 (0.7%)	2,000	82-180 (12-20%)
450 (338)	152	454	1,888,000 (3%)	773,000 (1.3%)	2,500	114-261 (16-29%)
	228	530	1,235,000 (2%)	2,200,000 (3.7%)	6,300	163-369 (23-41%)
Excluding folic acid from fortified breakfast cereals and fat spreads						
0	-74	232	21,257,000 (36%)	18,000 (0.03%)	800	-70 (-10%)
100 (75)	-24	283	10,019,000 (17%)	38,000 (0.06%)	800	-14 (-2%)
200 (150)	27	334	5,146,000 (9%)	52,000 (0.09%)	900	35-63 (5-7%)
300 (225)	78	385	3,001,000 (5%)	115,000 (0.2%)	900	77-162 (11-18%)
450 (338)	154	461	1,636,000 (3%)	559,000 (1.0%)	900	126-279 (18-31%)
Excluding folic acid from fortified breakfast cereals, fat spreads and supplements						
0	-85	217	22,404,000 (38%)	0	0	-91 (-13%)
100 (75)	-34	267	10,645,000 (18%)	0	0	-35 (-5%)
200 (150)	17	318	5,400,000 (9%)	0	0	21-36 (3-4%)
300 (225)	68	369	3,308,000 (6%)	55,000 (0.1%)	0	63-126 (9-14%)
450 (338)	144	445	1,828,000 (3%)	470,000 (0.8%)	0	112-252 (16-28%)

* Based on data from the NDNS which does not include pregnant or lactating women

** Figures rounded to the nearest 1,000

*** Figures rounded to the nearest 100

18 Across all population groups (Table 18, Appendix 1, Annex 2).

19 For each age group (DH, 1991)

20 For each age group (European Scientific Committee on Foods, 2001); see footnote 15.

21 Mean of values for overage applied and not applied.

Effects of mandatory fortification of flour with folic acid including current levels of folic acid intake from voluntary fortification and supplements

Without mandatory fortification of flour with folic acid

404. It can be seen from Table 10 (line 1: fortification level, 0) that at current levels of folic acid intake in the UK (from voluntary fortification and supplements), without mandatory fortification of flour with folic acid:

- The average folate intake of the population is approximately 302µg/day;
- Approximately 23% of the population (13,261,000 people) have intakes below the RNI for folate;
- Approximately 0.2% of the population (127,000 people) are exceeding the UL/day set for folic acid intake; the largest proportion are children aged 4-6 years and 7-10 years (57% or 72,000 children) as the ULs/day are much lower for these age groups (300 and 400µg/day respectively) than for adults (1mg/day);
- Approximately 900 adults aged 65 years and over, with low vitamin B12 status, are exceeding folic acid intakes of 1mg/day.

Introduction of mandatory fortification of flour with folic acid

405. The first section of Tables 10 and 11 headed Including folate and folic acid from all sources, shows the effects of mandatory fortification of flour with folic acid at current levels of folic acid intake (from voluntarily fortified foods and supplements) including and excluding wholemeal flour respectively.

406. In the sections below, which describe the information presented in the tables, the figures are estimates of the effect of folic acid fortification of all flour; the figures in parentheses denote the effect of fortification if wholemeal flour is exempted.

Fortification with folic acid at 100µg/100g flour

407. The average folate intake of the population would increase by 57 (51) µg/day.

408. Approximately 47-99 (42-93) NTD-affected pregnancies would be prevented which represents a 7-11% (6-10%) reduction in risk. The average percentage of the population with intakes below the RNI for folate would decrease from 23% to 10% (11%).

409. Approximately 241,000 (225,000) people in the UK would exceed the UL/day for folic acid intakes, an increase of 90% (77%) or 114,000 (98,000) people. Sixty-two percent (65%) of individuals exceeding the UL/day would be children aged 4-10 years. Approximately 1700 (1700) people aged 65 years and over, with low vitamin B12 status, would be at risk of exceeding folic acid intakes of 1mg/day (an increase of 89% or 800 people).

Fortification with folic acid at 200µg/100g flour

410. The average folate intake of the population would increase by 115 (102) µg/day.

411. Approximately 91-198 (82-180) NTD-affected pregnancies would be prevented which represents a 13-22% (12-20%) reduction in risk. The average percentage of the population with intakes below the RNI would decrease from 23% to 5% (6%).

412. Approximately 460,000 (404,000) people in the UK would exceed the UL/day for folic acid intakes, an increase of 262% (218%) or 333,000 (277,000) people. Sixty-six percent (70%) of individuals exceeding the UL/day would be children aged 4-10 years. Approximately 2,800 (2,000) people aged 65 years and over, with low vitamin B12 status, would be at risk of exceeding folic acid intakes of 1mg/day, an increase of 211% (122%) or 1,900 (1,100) people.

Fortification with folic acid at 300µg/100g flour

413. The average folate intake of the population would increase by 172 (152) µg/day.

414. Approximately 126-285 (114-261) NTD affected pregnancies would be prevented, which represents an 18-32% (16-29%) reduction in risk. The average percentage of the population with intakes below the RNI would decrease from 23% to 3% (3%).

415. Approximately 907,000 (773,000) people would exceed the UL/day for folic acid intakes, an increase of 614% (509%) or 780,000 (646,000) people. Sixty-nine percent (71%) of individuals exceeding the UL/day would be children aged 4-10 years. Approximately 3,300 (2,500) people aged 65 years and over, with low vitamin B12 status, would be at risk of exceeding folic acid intakes of 1mg/day, an increase of 267% (177%) or 2,400 (1,600) people.

Fortification with folic acid at 450µg/100g flour

416. The average folate intake of the population would increase by 258 (228) µg/day.

417. Approximately 175-378 (163-369) NTD-affected pregnancies would be prevented which represents a 25-42% (23-41%) reduction in risk. The average percentage of the population with intakes below the RNI would decrease from 23% to 1% (2%).

418. Approximately 2,535,000 (2,200,000) people would exceed the UL/day for folic acid intakes, an increase of 1896% (1632%) or 2,408,000 (2,073,000) people. Fifty-six percent (58%) of individuals exceeding the UL/day would be children aged 4-10 years. Approximately 9,200 (6,400) people aged 65 years and over, with biochemical vitamin B12 deficiency, would be at risk of exceeding folic acid intakes of 1mg/day, an increase of 922% (611%) or 8,300 (5,500) people.

Effect of folic acid fortification on folate intakes of women of childbearing age

419. The effects of folic acid fortification on folate intakes of women of child bearing age (14-49 years) are detailed in tables 14-15, Annex 2. The average folate intake of women aged 14-49 years is approximately 281µg/day (Table 15, Annex 2). Women in two lowest quintiles of folate intake have average total folate intakes below the RNI of 200µg/day (132 and 195µg/day respectively) (Table 14, Annex 2).

420. The effect of folic acid fortification of flour by quintile of current folate intake on women aged 14-18 years is illustrated in Figure 4, Annex 2. It can be seen that folic acid fortification of flour would increase the total folate intakes across all quintiles, including those in the two lowest quintiles. Similar results were seen for women aged 19-49 years.

Effect of mandatory fortification *excluding* folic acid intakes from fortified breakfast cereals/fortified spreads and including folic acid intake from supplements

421. See second section of Tables 10 and 11, headed: Excluding folic acid from fortified breakfast cereals and fat spreads.

Without mandatory fortification of flour with folic acid

422. Without folic acid intakes from fortified breakfast cereals and fat spreads, it can be estimated that:

- The average folate intake of the population would decrease by 74µg/day.
- There would be approximately 70 more pregnancies affected by NTDs (10% increase in risk).
- The percentage of the population with intakes below the RNI for folate would increase from 23% to 36%.
- Approximately 18,000 people would exceed the UL/day for folic acid intake (decrease of 86% or 109,000 people).
- Approximately 800 adults aged 65 years and over, with low vitamin B12 status, would exceed folic acid intakes of 1mg/day (decrease of 11% or 100 people).

Introduction of mandatory fortification of flour[22] with folic acid

423. The introduction of mandatory fortification with folic acid at a level of 100µg/100g flour, without the contribution of folic acid intakes from fortified breakfast cereals/fortified spreads, would not be sufficient to overcome the decrease in folic acid intake and the subsequent increased risk of NTD-affected pregnancies.

424. Without folic acid intakes from fortified breakfast cereals/fat spreads, mandatory fortification with folic acid at a level of 200µg/100g flour would be required to reduce NTD risk by 6-10% (5-7%). At this level, average folic acid intakes would increase by 40 (27) µg/day and 7% (9%) of the population

22 Figures in parentheses denote the effect of fortification if wholemeal flour is exempted.

would have intakes below the RNI for folate. However, this would also result in approximately 52,000 (52,000) people exceeding the UL/day for folic acid intake (59% less than current levels). Compared to current levels, there would be no change in the number of people (including and excluding wholemeal flour) aged 65 years and over, with low vitamin B12 status, exceeding folic acid intakes of 1mg/day.

425. Mandatory fortification with folic acid at a level of 300µg/100g flour would reduce the risk of NTD-affected pregnancies by 12-21% (11-18%). Average folic acid intakes would increase by 98 (78) µg/day and the percentage of the population with intakes below the RNI for folate would be 4% (5%) . At this level, 119,000 (115,000) people would have intakes above the UL/day for folic acid which is 6% (9%) less than current levels. Compared to current levels, there would still be no change in the number of people (including and excluding wholemeal flour) aged 65 years and over, with low vitamin B12 status, exceeding folic acid intakes of 1mg/day.

426. With mandatory fortification at a level of 450µg/100g flour, the risk of NTD-affected pregnancies would be reduced by 20-35% (18-31%). Average folic acid intakes would increase by 184 (154) µg/day and the percentage of the population with intakes below the RNI for folate would be reduced to 2% (3%). However, the numbers with intakes above the UL/day for folic acid intake would be substantially higher than current levels, increasing by 420% (340%) or 539,000 (432,000) people. The number of people aged 65 years and over, with low vitamin B12 status, exceeding folic acid intakes of 1mg/day would also increase by 56% or 500 people (no change compared to current levels if wholemeal flour excluded).

Effect of mandatory fortification of flour with folic acid *excluding* the contribution of folic acid intakes from fortified breakfast cereals, fortified spreads, and supplements

427. See third section of Tables 10 and 11, headed: *Excluding folic acid from fortified breakfast cereals, fat spreads and supplements.*

Without mandatory fortification of flour

428. Without folic acid intakes from fortified breakfast cereals, fortified fat spreads, and supplements:

- The average folate intake of the population would decrease by 85µg/day.
- There would be approximately 91 more pregnancies affected by NTDs (13% increase in risk).
- The percentage of the population with intakes below the RNI for folate would increase from 23% to 38%.
- It is unlikely that anyone would exceed folic acid intakes above the UL/day.
- It is unlikely that any adults aged 65 years and over, with low vitamin B12 status, would exceed folic acid intakes of 1mg/day.

Introduction of mandatory fortification of flour[23] with folic acid

429. Mandatory fortification with folic acid at a level of 100µg/100g flour would not be sufficient to overcome the increased risk of NTD-affected pregnancies caused by removing the contribution of folic acid intakes from fortified breakfast cereals/fortified spreads and supplements.

430. Without folic acid intakes from fortified breakfast cereals/fat spreads and supplements, mandatory fortification with folic acid at a level of 200µg/100g flour would be required to reduce NTD risk by 4-6% (3-4%). At this level, average folic acid intakes would increase by 30 (17) µg/day and the percentage of the population with intakes below the RNI for folate would be 8% (9%). It is unlikely that anyone would exceed the UL/day for folic acid intake or any adults aged 65 years and over, with low vitamin B12 status, would exceed intakes of 1mg/day.

431. Without folic acid intakes from fortified breakfast cereals/fat spreads and supplements, mandatory fortification with folic acid at a level of 300µg/100g flour would reduce NTD risk by 10-17% (9-14%). Average folic acid intakes would increase by 87 (68) µg/day and the percentage of the population with

23 Figures in parentheses denote the effect of fortification if wholemeal flour is exempted.

intakes below the RNI for folate would be reduced to 4% (6%). Approximately 59,000 (55,000) people would have intakes above the UL/day for folic acid which is 54% (57%) less than current levels. It is unlikely that any adults aged 65 years and over, with low vitamin B12 status, would exceed folic acid intakes of 1mg/day.

432. Mandatory fortification with folic acid at a level of 450µg/100g flour would reduce the risk of NTD-affected pregnancies by 18-32% (16-28%). Average folic acid intakes would increase by 173 (144) µg/day and the percentage of the population with intakes below the RNI for folate would be reduced to 2% (3%). However, the numbers with intakes above the UL/day for folic acid intake would be substantially higher than current levels, increasing by 338% (270%) or 430,000 (343,000) people. It is unlikely that any adults aged 65 years and over, with low vitamin B12 status, would exceed folic acid intakes of 1mg/day.

Comparison of the effects of excluding folic acid intakes from fortified breakfast cereals and fat spreads only with excluding folic acid intakes from fortified breakfast cereals, fat spreads, and supplements

433. Including or excluding folic acid intakes from supplements would result in little difference in the number of people with intakes below the RNI for folate: 36% including supplements and 38% excluding supplements. This suggests that compared to fortified foods, supplements do not make an important contribution to helping people achieve the RNI for folate intake.

434. The increased risk of NTD-affected pregnancies is lower if folic acid intakes from supplements are included (10% increased risk/70 more NTD-affected pregnancies) than if they are excluded (13% increased risk/91 more NTD-affected pregnancies), indicating that supplements make an additional contribution to reducing NTD risk.

435. Approximately 127,000 people in the UK are currently exceeding the UL/day for folic acid. Out of this total, 14% can be attributed to supplement consumption (18,000 people) and 86% (109,000 people) to consumption of fortified foods. This suggests that folic acid intakes above the UL/day for folic acid are largely due to consumption of foods fortified with folic acid.

436. There would be approximately 800 adults aged 65 years and over, with low vitamin B12 status, exceeding folic acid intakes of 1mg/day if folic acid intakes from supplements are included and unlikely to be any if folic acid intakes from supplements are excluded. This suggests that folic acid intakes above 1mg/day in this age group are due to the consumption of supplements containing folic acid. However, it can be seen that fortified breakfast cereals and fat spreads contribute to folic acid intakes *just below* 1mg/day in this age group because when folic acid intake from all sources is included (first section, Tables 10 & 11), the introduction of mandatory fortification at a level of 100µg/100g flour (line 2, column 5) leads to 800 more adults aged 65 years and over, with low vitamin B12 status, exceeding folic acid intakes of 1mg/day; this increase can be attributed to fortified breakfast cereals and fortified spreads because when folic acid is excluded from these foods (but included from supplements) (second section, Tables 10 & 11) and mandatory fortification is introduced at 100µg/100g (line 2, column 5), there is no increase in the numbers exceeding 1mg/day.

Summary

437. At current levels of folic acid intake, including intakes from voluntary fortification and supplements, mandatory fortification of flour with increasing levels of folic acid would progressively: reduce NTD risk and the percentage of the population with intakes below the RNI for folate; increase the number of people with intakes above the UL/day for folic acid and the number of adults aged 65 years and over, with low vitamin B12 status, exceeding folic acid intakes of 1mg/day.

438. Including wholemeal flour in the fortification process confers little additional benefit in reducing the risk of NTD-affected pregnancies. Exempting wholemeal flour from fortification would reduce the numbers at risk of exceeding the UL/day for folic acid intakes.

439. Removing the contribution of folic acid intakes from fortified breakfast cereals and fortified fat spreads (but including intakes from supplements) to current intakes, would: increase the risk of NTD-affected pregnancies and the percentage of the population with intakes below the RNI for folate; decrease the percentage of the population with intakes above the UL/day for folic acid intake and the number of adults aged 65 years and over with low vitamin B12 status, with folic acid intakes above 1mg/day.

440. Removing the contribution of folic acid intakes from fortified breakfast cereals, fortified fat spreads and supplements to current intakes would further increase the number of NTD-affected pregnancies and the percentage of the population with intakes below the RNI for folate. However, it is unlikely that anyone would exceed the UL/day for folic acid intake and it is unlikely that any adults aged 65 years and over with low vitamin B12 status, would exceed folic acid intakes of 1mg/day.

441. Without the contribution of folic acid intakes from fortified breakfast cereals and fortified spreads, or from fortified breakfast cereals, fortified spreads *and* supplements, mandatory fortification of flour with folic acid at 200µg/100g flour would be the lowest level required to overcome the increased risk of NTD-affected pregnancies. At doses of 200µg/100g and above, the additional folic acid from flour would exceed the amount lost by excluding folic acid intake from voluntary sources. NTD risk would be progressively reduced with increasing levels of fortification.

442. At all levels of mandatory fortification (100-450µg/100g flour), without the contribution of folic acid intakes from fortified breakfast cereals and fortified spreads, or from fortified breakfast cereals, fortified spreads *and* supplements, the proportion of the population with intakes below the RNI for folate would be lower than current levels. With increasing levels of folic acid fortification, the proportion of the population with intakes below the RNI for folate intake would be progressively reduced.

443. After excluding the contribution of folic acid intakes from fortified breakfast cereals and fortified fat spreads (but including intakes from supplements), mandatory fortification with folic acid at doses of 100-300µg/100g, would result in 38,000-119,000 people exceeding the UL/day for folic acid intake; this would, however, still be less than the numbers exceeding the UL/day at current voluntary fortification levels, without mandatory fortification. Only fortification at 450µg/100g flour would result in the number of people with intakes above the UL/day exceeding current levels.

444. After excluding the contribution of folic acid intakes from fortified breakfast cereals, fortified fat spreads *and* supplements, it is unlikely that mandatory fortification with folic acid at doses up to 200µg/100g flour, would expose any individuals to intakes above the UL/day for folic acid. With fortification

at 300µg/100g flour, the percentage of the population exceeding the UL/day for folic acid would still be 54% (57%) lower than current levels. At fortification levels above this amount, the number of people with folic acid intakes above the UL/day would exceed current levels.

445. Without the contribution of folic acid intakes from fortified breakfast cereals and fortified fat spreads (but including intakes from supplements), it is unlikely that mandatory fortification with folic acid at doses up to 300µg/100g flour would increase the number of adults aged 65 years and over, with low vitamin B12 status, exceeding intakes of 1mg/day, compared to current levels.

446. Without the contribution of folic acid intakes from fortified breakfast cereals, fortified fat spreads *and* supplements, at all levels of mandatory fortification (100-450µg/100g flour), it is unlikely that any adults aged 65 years and over, with low vitamin B12 status, would exceed intakes of 1mg/day.

447. Current intakes of folic acid above the UL/day for folic acid intake are largely due to consumption of foods voluntarily fortified with folic acid.

448. Present consumption patterns of supplements and foods voluntarily fortified with folic acid contribute to the highly variable intakes of folic acid, resulting in large sections of the population with intakes below the RNI for folate and considerable numbers of people with intakes of folic acid above the UL/day.

449. Results from the modelling exercise suggest that, without the contribution of folic acid from voluntary sources, mandatory fortification of flour with folic acid would confer a more even distribution of folic acid intakes across the population compared to current voluntary fortification and supplement use. The redistribution of folic acid intakes would mean that women at greatest risk of NTD-affected pregnancies, i.e. those with the lowest folate intakes, would be reached through mandatory fortification.

450. If no foods were voluntarily fortified, the option that appears to provide the optimum balance between benefits and possible risks is mandatory fortification at a level of 300µg of folic acid per 100g flour (excluding wholemeal flour). At this level it is estimated that: 77-162 NTD-affected pregnancies/year could be prevented (11-18% risk reduction); the proportion

of the population with intakes below the RNI would be reduced from 23% to 5%; the number of people with folic acid intakes exceeding the UL/day would be reduced by 12,000; and there would be no change in the number of adults aged 65 years and over, with low vitamin B12 status, exceeding intakes of 1mg/day.

Comparison of results from the modelling exercise with COMA estimates of the impact of mandatory fortification of flour with folic acid

451. COMA considered the effect of increasing folic acid intake through fortification of flour (DH, 2000) at slightly different levels of folic acid (140, 200, 240, 280, 420µg/100g flour). In the current modelling exercise, the additional increase in daily average intakes of folic acid at various levels of fortification are lower than those estimated by COMA (DH, 2000). For example, at folic acid doses of 200µg/100g flour (including wholemeal), COMA estimated an increase of 167µg/day in the average folic acid intake of women aged 16-45 years, compared to the current estimate of 100µg/day for women aged 14-49 years (see Table 15, Annex 2). This is because COMA did not take processing losses into account and also overestimated the percentage of flour in food products.

452. COMA concluded that folic acid fortification of *all* flour at a dose of 240µg/100g flour would have a significant effect in preventing NTD-affected pregnancies without resulting in unacceptably high intakes in any group of the population. It was estimated that fortification at this level would increase the folic acid intake of women aged 16-45 years by 201µg/d and reduce the risk of NTD-affected pregnancies by 41%. Results from the current modelling suggest that this is equivalent to a fortification level of 450µg/100g flour (excluding wholemeal flour) which would increase folic acid intake of women aged 14-49 years by 202µg/d (Table 15, Annex 2) and reduce NTD-affected pregnancies by 23-41%.

Comparison of results from the modelling exercise with the experience in the USA following mandatory fortification

453. It has been estimated that folic acid fortification in the USA has resulted in additional intakes of approximately 215-240µg/d of folic acid (Quinlivan & Gregory, 2003), which has resulted in a 19% decline in NTD-affected births and a 27% decline in NTD affected pregnancies. Data from the modelling exercise suggest that to achieve an increase in folic acid intake equivalent to that observed in the USA, would require fortification of flour at levels between 300-450µg/100g, which would result in additional folic acid intakes of 172-258µg/d.

454. Pfeiffer *et al* (2005) compared data from NHANES 1999-2000 and NHANES III carried out in 1988-1994. Median serum folate concentrations increased by 158% from 12.5nmol/L (5.52µg/L) in NHANES III to 32.2nmol/L (14.2µg/L) in NHANES 1999-2000 and median red blood cell folate concentrations increased from 392nmol/L (173µg/L) to 635nmol/L (280µg/L), an increase of 62%. The prevalence of low serum folate concentrations (defined as 6.8nmol/L or 3µg/L) decreased from 16% in NHANES III to 0.5% in NHANES 1999-2000 for the US population and from 20% to 0.8% in women of childbearing age (12-49 years). The prevalence of low red blood cell folate concentrations (defined as <317nmol/L or 140µg/L) decreased from 31% in NHANES III to 3% in NHANES 1999-200 for the USA population and from 38% to 5% in women of childbearing age.

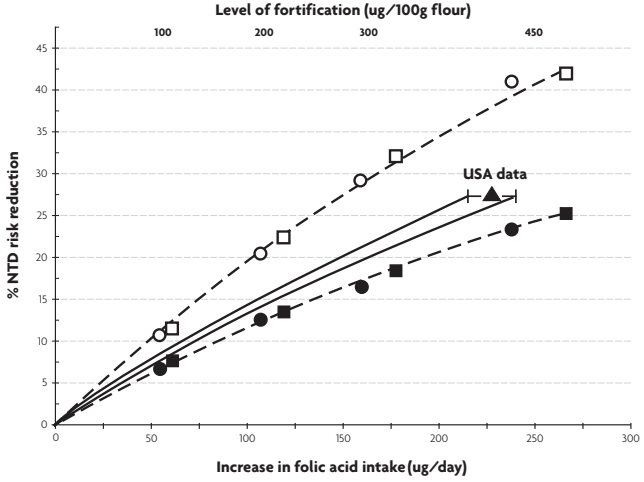
455. The impact of folic acid on NTD reduction is affected by background folate blood concentrations (see paragraph 400). The 2001 NDNS of adults aged 19-64 years (Ruston *et al*, 2004) shows that in the UK median serum folate concentrations for men and women were 18.8nmol/L (8.3µg/L) and 21nmol/L (9.3µg/L) respectively; median red cell folate concentrations were 694nmol/L (306.4µg/L) for men and 685nmol/L (302.4µg/L) for women. These data suggest that blood folate concentrations may be higher in the UK than they were in the USA pre-fortification. However, caution should be applied when comparing blood folate concentrations across different laboratories and countries as there is considerable uncertainty about the reliability of analytical methods and considerable inter-assay and inter-laboratory variation (Gunter *et al*, 1996; Puwastein *et al*, 2005). Different assay

methodologies were used to assess folate status in the USA and UK; application of a correction factor to adjust for known assay-bias between the different methods suggests that current values in the UK are of a similar magnitude to those in the USA prior to mandatory fortification (C Bates; personal communication, 2006).

456. Figure 3, compares the NTD risk reduction estimated from the modelling exercise with the decrease in NTD risk observed in the USA following mandatory fortification. It can be seen that the change in NTD rates in the USA falls within the range of values for NTD risk reduction estimated by the modelling.

Figure 3: Modelling estimates of the effect of four levels of fortification on NTD risk including and excluding wholemeal flour

The upper estimates (□ including wholemeal, ○ excluding wholemeal) are based on red cell folate concentrations (Daly *et al*, 1995; Daly *et al*, 1997) and the lower estimates (■ including wholemeal, ● excluding wholemeal) are based on plasma folate concentrations (Daly *et al*, 1995; Wald *et al*, 2001). The change observed in the USA following fortification is also shown (▲) together with limits based on uncertainty in the actual level of fortification achieved (Quinlivan & Gregory III, 2003). The USA data are extrapolated to lower levels of fortification.



457. There are no data available from the USA on whether the risk of vitamin B12 deficiency being undetected has increased post fortification. Data from hospital discharge surveys, compiled by the CDC and the National Center for

Health Statistics, provide an indication of clinical disease associated with vitamin B12 deficiency. The number of listed discharge diagnoses from short-stay hospitals for pernicious anaemia, for adults aged 65 years and above, was 25,000 in 1995 (Graves & Gillum, 1997), 28,000 in 1999 (Popovic, 2001), and 18,000 in 2003 (Kozak *et al*, 2006).

458. In the same years, no discharge diagnoses were listed for subacute combined degeneration of the spinal cord. A minimum sample size of 30 is one of the criteria used to define reliability of estimates and the number of reports for subacute combined degeneration of the spinal cord has remained too small to allow a reliable estimate (J Wright; personal communication, 2006). The data indicate that the number of diagnoses of subacute combined degeneration of the spinal cord have not increased post-fortification. This suggests that mandatory fortification has not lead to a delay in the detection of vitamin B12 deficiency by masking the diagnosis of pernicious anaemia.

459. Comparison of data from the NHANES 1999-2000 and NHANES III (1988-1994) show that the proportion of people over 60 years with serum concentrations of vitamin B12 below 185pmol/L has decreased from 13% pre-fortification to 7% post-fortification (Pfeiffer *et al*, 2005). The prevalence of people over 60 years with serum vitamin B12 concentrations below 148pmol/L was slightly lower (3%) pre-fortification compared to post-fortification (5%).

Summary

460. Results from the current modelling exercise suggest that the daily average intakes of folic acid following mandatory fortification would be lower than those estimated by COMA (DH, 2000).

461. Mandatory fortification with folic acid at doses between 300-450µg/100g flour would be required to achieve an increase in folic acid intakes equivalent to those in the USA following the introduction of mandatory fortification.

462. National data from short-stay hospital discharge surveys in the USA suggest that cases of subacute combined degeneration of the spinal cord have not increased following mandatory fortification.

9 Overall summary and conclusions

463. Following the COMA report, *Folic Acid and the Prevention of Disease* (DH, 2000), this report considered: UK dietary intakes of folate and other B vitamins involved in folate metabolism; trends in the incidence of neural tube defect (NTD) affected pregnancies in the UK and in countries where mandatory fortification has been introduced; the possible effects of mandatory fortification of flour with folic acid on reducing the risk of NTD-affected pregnancies in women of childbearing age; the possible effects on people aged 65 years and over with low vitamin B12 status; and other potential health benefits and risks to the UK population of mandatory fortification of flour with folic acid.

464. *Folate* is a generic term for a family of B-group vitamins. There are large numbers of naturally occurring folates but methyl- and formyl-tetrahydropteroylpolyglutamates are the main forms found in foods. Folic acid (pteroylmonoglutamic acid) is a synthetic form used in supplements and food fortification.

465. Folates are metabolised following absorption to 5-methyl tetrahydrofolate, which is usually the only form found in plasma. Oral doses of folic acid (in excess of about 260µg) can lead to the appearance of unmetabolised folic acid in the blood. This has raised concerns regarding high intakes of folic acid.

466. In the USA and Europe, the recommended tolerable upper intake level (UL) for folic acid from fortified foods and supplements is 1mg/day[24] for adults. The UL represents the highest level of a nutrient that is likely to pose no risk of adverse health effects for most individuals in the population. In the UK, the evidence for adverse effects of folic acid was considered insufficient to confidently establish a safe upper level (SUL)[25]. Instead, a Guidance Level (GL) of 1mg/day of folic acid was recommended for adults. The GL is based on limited data and represents an *approximate* indication of intakes that would not be expected to cause adverse effects. By setting a GL, the EVM recognised that the data on adverse effects of folic acid were not sufficiently robust to set an SUL. The UL/GL set for folic acid were based on concerns

24 The UL of 1mg/d was extrapolated back from a lowest observed adverse effect level of 5mg/d and includes a safety factor of 5.

25 The SUL represents the amount of a nutrient that can be consumed daily over a lifetime without significant risk to health and is based on adequate available evidence.

relating to vitamin B12 deficiency. The effects of long-term exposure to folic acid intakes above 1mg/day on other health outcomes are unknown.

467. There are no data to suggest that high intakes of folic acid have any adverse effects on children. The critical endpoints relating to vitamin B12 deficiency, which were used as the basis to set a UL for adults, have not been reported for children. ULs set for children in the USA and Europe were, therefore, extrapolated from the UL for adults on the basis of body weight. As there were no data reporting adverse effects in children, GLs were not set for children in UK.

468. Data from the British National Diet and Nutrition Survey (NDNS) series indicate that average daily folate intakes are above the reference nutrient intake (RNI[26]) for all age groups. Red cell folate concentrations less than 350nmol/L (154µg/L) were reported in 8% of women aged 19-24 years and 4% of those aged 25-34 years indicating an increased risk of marginal folate status (defined as red cell folate concentration of 230-345nmol/L or 101-152µg/L). Evidence of marginal folate status was reported in 21% of free-living and 19% of institutionalised people aged 65 years and over; folate deficiency (red cell folate concentration ‹230nmol/L or 101µg/L) was reported in 8% of free-living and 16% of institutionalised people aged 65 years and over. However, caution should be applied in the interpretation of these values as different assays were used to measure folate status across different surveys in the NDNS series. Values for folate status and normal ranges are dependent on the assay method used which makes it difficult to make direct comparisons between different surveys/studies and it may not be appropriate to apply cutoffs for adequacy obtained from one assay method to measurements obtained by a different assay method.

469. There is also evidence of widespread marginal vitamin B2 (riboflavin) status (EGRAC ›1.3), particularly in girls, women, and people aged 65 years and over. Intakes of vitamin B6 appear to be adequate, however there is evidence of marginal vitamin B6 status in the population, especially in people aged 65 and over. There is no evidence to suggest inadequate vitamin B12 intakes in the general population. Low vitamin B12 status (defined as serum vitamin B12 ‹118pmol/L) was found in 6% of free-living and 9% of institutionalised people aged 65 years and over.

26 The RNI does not cover the recommendation that all women who could become pregnant should take an additional 400 µg/day prior to conception until the 12th week of pregnancy.

470. Evidence from RCTs has clearly proven the protective effect of folates in reducing NTDs. Other factors such as previous history of an NTD-affected pregnancy, inherited variations in genes controlling folate metabolism, maternal weight, and diabetes also influence NTD risk.

471. The current recommendation is for all women who could become pregnant to take 400µg of folic acid per day as a medicinal or food supplement prior to conception and until the twelfth week of pregnancy. Women with a previous pregnancy affected by NTD, who wish to conceive, are advised to take a daily supplement of 5mg folic acid (DH, 1992). These amounts are intended to supplement the RNI. Data on the uptake of this recommendation are incomplete. Although supplementation with folic acid prior to conception has been advised since 1992, about half of all pregnancies are unplanned, which limits the value of recommendations for pre-conceptional supplementation. Even amongst women planning their pregnancy, only about half take folic acid supplements or modify their diet to increase folate intake. Younger mothers and those from the most socioeconomically deprived areas are the least likely to take any action.

472. Evidence from studies which have examined the efficacy of European Union policies recommending women to consume folic acid supplements to reduce NTD-affected pregnancies have shown no effect on the number of NTD-affected pregnancies. In England and Wales, the recommendation in 1992 for women to consume folic acid supplements pre- and peri-conceptionally had no detectable impact.

473. In the UK, the incidence of NTD-affected pregnancies has shown a steady decline from the 1960s until the beginning of the 1990s, which may partly be due to an increase in folic acid intake from supplements and fortified breakfast cereals. No change was observed in the incidence of NTD-affected pregnancies in England and Wales during the 1990s. In Scotland and Northern Ireland, the incidence of NTD-affected pregnancies remained fairly constant throughout the 1990s although there may have been a downward trend from the late 1990s onwards in the numbers of NTD-affected births in Scotland.

474. Improved systems are required for ascertainment of NTD-affected births and terminations as the available data are insecure, mainly due to under-reporting. In 2003, after adjusting for underreporting, there were

approximately 630-850 NTD-affected pregnancies in England and Wales. There were approximately 49 NTD-affected pregnancies in Scotland and 11 NTD-affected births in Northern Ireland, in 2003. The extent of under-reporting in the NTD-affected pregnancy data from Scotland and Northern Ireland is not known. It can be estimated that for the UK as a whole, there were 700-900 NTD-affected pregnancies in 2003. Approximately 70% of NTD-affected pregnancies in England and Wales in 2003 and 50% of NTD-affected pregnancies in Scotland were terminated therapeutically.

475. In countries where mandatory fortification with folic acid has been introduced, there have been reductions of 27% to over 50% in the incidence of NTD-affected pregnancies. At current levels of folic acid intake (including intake from voluntarily fortified foods and supplements), it is estimated from a modelling exercise (summarised in Tables 10 & 11, pages 98-99) that mandatory fortification of all flour (including wholemeal) with folic acid at levels of 100, 200, 300, 450µg/100g flour would reduce the number of pregnancies affected by NTDs in the UK by 47-99 (7-11%), 91-198 (13-22%), 126-285 (18-32%), and 175-378 (25-42%), respectively. If wholemeal flour were exempted from mandatory fortification the number of NTD-affected pregnancies would be reduced by 42-93 (6-10%), 82-180 (12-20%), 114-261 (16-29%), and 163-369 (23-41%) respectively.

476. The reduction in NTD risk may be closer to the lower estimates in Tables 10 and 11. This is because of uncertainties in the data used to predict reduction in NTD risk with mandatory fortification.

477. The modelling may also overestimate the beneficial effects of mandatory fortification of flour with folic acid in reducing NTD-affected pregnancies as it does not take account of the other factors which may influence NTD risk (see paragraph 470).

478. An increased risk of clinical vitamin B12 deficiency is characterised by low blood concentrations of vitamin B12 or elevated concentrations of its related metabolites. The biochemical measures used to assess vitamin B12 status are of limited reliability and there are no agreed criteria to define deficiency. Estimates of the prevalence of low vitamin B12 status have ranged between 5-20% depending on the biochemical markers and criteria used to define deficiency. A combined analysis of three studies using two biochemical markers estimated the prevalence of low vitamin B12 status as 5% in people aged 65-74 years and 10% in people over 75 years (Clarke *et al*, 2004).

479. Clinical evidence of vitamin B12 deficiency presents as anaemia or peripheral neuropathy, which may be reversible. In some cases, clinical vitamin B12 deficiency leads to the irreversible and serious condition of subacute combined degeneration of the spinal cord. The incidence and prevalence of clinical disease caused by vitamin B12 deficiency are uncertain and the extent to which low vitamin B12 status progresses to clinical disease is not known. This means that it is not possible to predict the number of people with low vitamin B12 status that might develop clinically evident reversible disease such as anaemia or peripheral neuropathy, or rare irreversible disease.

480. As high doses of folic acid can correct the anaemia associated with clinical vitamin B12 deficiency, there are concerns that folic acid fortification of flour may lead to a delay in the diagnosis of vitamin B12 deficiency and allow the neuropathy to progress. Folic acid intakes of 1mg/day or less are not associated with masking anaemia. Assuming the prevalence of low vitamin B12 status in people aged 65 years and over to be 5-10%, it can be estimated that 900 adults aged 65 years or over in the UK, with low vitamin B12 status, are currently at risk of consuming more than 1mg/day of folic acid from fortified foods and/or supplements. At current levels of folic acid intake (including intake from voluntary fortification and supplements), mandatory fortification of all flour (including wholemeal flour) with folic acid at doses of 100, 200, 300, and 450µg/day could increase average numbers at risk of consuming over 1mg/day of folic acid to approximately 1700, 2800, 3300, and 9000 respectively (1700, 2000, 2500, 6300, respectively, if wholemeal flour were exempted). The proportion that might develop neurological disease is unknown.

481. No data are available on trends in the clinical incidence of vitamin B12 deficiency, nor for any related neurological damage, from countries that have introduced mandatory fortification. Although no procedures to monitor clinical vitamin B12 deficiency were implemented following the introduction of mandatory fortification, data from national hospital discharge surveys in the USA suggest that the number of cases of subacute combined degeneration of the spinal cord has not increased post-fortification. Evidence from the USA, also indicates that the proportion of people over 60 years with low serum concentrations of vitamin B12 (defined as <185pmol/L) has decreased from 13% pre-fortification to 7% post-fortification.

482. Fortification of flour with vitamin B12 to improve the status in people aged 65 years and over would require doses in excess of 400 times the RNI for vitamin B12 (1.5µg/d) to correct vitamin B12 deficiency caused by malabsorption. This is not a feasible option as the effect of population exposure to such high doses of vitamin B12 is not known.

483. Evidence from the NDNS shows that people aged 65 years and over may also be at greater risk of folate deficiency than other population groups. The modelling exercise suggests that fortification of flour with folic acid would have beneficial effects in reducing folate deficiency in older people and would also benefit other population age groups in achieving the RNI for folate. Evidence from the USA has shown improvements in the folate status of the population following mandatory fortification: the prevalence of low red blood cell folate concentrations (317nmol/L; 140µg/L) decreased from 31% before fortification to 3% after fortification and the prevalence of low serum folate concentrations (≤ 6.8nmol/L; 3.0µg/L) decreased from 16% to 0.5%.

484. Typical folic acid intakes in the USA following mandatory fortification appear to have increased by more than twice the amount originally intended. This is probably due to overage (the practice of adding nutrients to foods above the stated level, to allow for losses during manufacture and storage).

485. Other proposed benefits of the mandatory fortification of flour with folic acid include a reduced risk of cardiovascular disease, certain cancers, bone disease and slower age-related cognitive decline. Evidence for these proposed benefits comes mainly from observations that could be confounded by associated factors, such as other food constituents or other B group vitamins. There is currently insufficient evidence from RCTs examining chronic disease risk to either support or advise against mandatory fortification of flour with folic acid on these grounds.

486. Some evidence from animal models suggests a dual action of folic acid on cancer development i.e., high intakes of folic acid may inhibit tumour development in normal tissue but may promote the progression of premalignant lesions. The doses of folic acid used in the animal studies were considerably higher than the amounts of folic acid likely to be consumed by humans if mandatory fortification is introduced.

487. No RCTs designed to examine an association between folic acid and cancer incidence in humans have yet reported. Preliminary results (abstract) from an unpublished RCT (Cole *et al*, 2005) suggest folic acid intakes above 1mg/day may promote the progression of existing premalignant colorectal lesions. Results from the HOPE (HOPE 2 investigators, 2006) and NORVIT (Bonaa *et al*, 2006) trials, which examined the effect of folic acid supplementation on cardiovascular outcomes, did not show an effect of folic acid supplementation (2.5mg/day and 800µg/day of folic acid respectively) on colorectal cancer (CRC) incidence, however both trials were underpowered to address this question. Long term RCTs designed to examine an association between folic acid and CRC risk in susceptible populations are required.

488. Overall time trends for CRC incidence in the USA and Canada show that mandatory fortification of foods with folic acid in 1996 occurred at around the same time as a temporary non-significant increase in rates which interrupted a more long term trend for a decline in CRC incidence. Incidence rates continued to decline after 1998. If the increases in CRC incidence occurred as a result of folic acid fortification then the effect of folic acid on cancer development would have to have been immediate. It is not clear why the increase in CRC incidence occurred at different times for men and women and different age groups. There are also inconsistencies relating to the timing of the change in rates with changes in population folate status in the USA. The increased CRC incidence is not readily explained by improved screening practices for CRC detection. Although the trend data do not prove a link, they raise concerns about a possible association between folic acid fortification and CRC development in people with established pre-cancerous lesions.

489. There is currently insufficient evidence for an adequate risk assessment of folic acid and cancer risk or the intake levels which might be associated with risk. The UL/GL of 1mg/day set for folic acid intake was based on concerns relating to vitamin B12. Cancer risk was not considered in setting the UL/GL. A substantial increase in current average population intakes of folic acid and the numbers consuming more than the UL/day for folic acid should therefore be avoided.

490. The main dietary sources of folic acid are supplements and foods which are fortified on a voluntary basis. Foods voluntarily fortified with folic acid

include a number of breakfast cereals and some brands of reduced/low-fat spreads. Consumption of these foods and supplements contribute to a large proportion of total folate intake for some people.

491. Folic acid intake from voluntarily fortified foods and supplements is currently placing approximately 127,000[27] people in the UK at risk of consuming intakes above the UL/day for folic acid. As levels of folic acid in voluntarily fortified foods are presently uncontrolled, the number of people with intakes above the UL/day could be higher if folic acid levels in these foods are increased in the future.

492. Although the modelling exercise estimated the total number of people currently exceeding the UL/day for folic acid intake, the largest proportion are children. This is because the UL/day for children is much lower than that for adults. It is not known if high intakes of folic acid are associated with any adverse effects in children (see paragraph 467).

493. At current levels of voluntary food fortification and supplement use, the introduction of mandatory fortification of all flour with folic acid at levels of 100, 200, 300, and 450µg/100g flour, would increase the number of people exceeding the UL/day for folic acid from 127,000 to 241,000, 460,000, 907,000, and 2,535,000 respectively. Without the contribution of folic acid from voluntarily fortified foods, mandatory fortification with folic acid at levels of 100, 200, and 300µg/100g flour would reduce the numbers exceeding the UL/day for folic acid from 127,000 to 38,000, 52,000, and 119,000 respectively; at 450µg/100g flour the numbers exceeding the UL/day would increase from 127,000 to 666,000. Without voluntary fortification of foods or consumption of folic acid supplements, mandatory fortification at 100, 200, and 300µg/100g would further reduce the numbers at risk of exceeding the UL/day to 0, 0, and 59,000 respectively; fortification at 450µg/100g would result in an increase from 127,00 to 557,000.

494. Although voluntary fortification of foods with folic acid may contribute to reducing NTD risk, these foods are more expensive and are not usually consumed by all sections of the population. Replacement of voluntary fortification of certain foods with mandatory fortification of flour with folic acid would lead to a redistribution of folic acid intakes within the population and would be the most effective way to reach those sections of the

27 The largest proportion of the numbers exceeding the UL/day for folic acid intake are children aged 4-10 years as the UL/day for this age group is much lower than that for adults.

population with the lowest folate intakes: younger women from the most socioeconomically deprived areas, who do not follow advice regarding pre-conceptional supplementation with folic acid or routinely consume supplements or foods with the highest levels of voluntary fortification.

495. Without the contribution of folic acid from voluntarily fortified foods, mandatory fortification would reduce risks of intakes exceeding the UL per day for folic acid relative to the current practice of voluntary fortification. This is because voluntary fortification of foods with folic acid and inappropriate supplement use are harder to quantify and control and, unlike flour, their consumption is very variable.

496. If no foods were voluntarily fortified, and assuming overage is controlled, the optimal level for mandatory fortification of flour with folic acid would be 300µg/100g flour. This represents the level that would be effective in reducing NTD risk without increasing the number of people with intakes of folic acid above the UL/day. It is also unlikely that there would be an increase in the number of adults aged 65 years and over, with low vitamin B12 status, exceeding folic acid intakes of 1mg/day. Exempting wholemeal flour from fortification would have little effect on NTD risk but would further reduce the numbers with intakes of folic acid above the UL/day. At this level of fortification (not including wholemeal flour and if no foods are voluntarily fortified), the average total folate intake of the population would be 385µg/day compared to current average folate intakes of 302µg/day.

497. At fortification levels above 300µg/100g flour there would be further reductions in NTD risk, however the number of people with folic acid intakes above the UL/day and the number of adults aged 65 years and over, with low vitamin B12 status, exceeding intakes of 1mg/day, would be higher than current levels.

10 Recommendations

498. As previously recommended by COMA (DH, 2000), all women who could become pregnant should take 400µg/day folic acid as a medicinal or food supplement prior to conception and until the twelfth week of pregnancy. Women with a history of a previous NTD-affected pregnancy are advised to take 5mg/day of folic acid prior to conception and until the twelfth week of pregnancy[28].

499. Individual long-term intakes of folic acid from fortified foods and supplements above the GL/UL per day[29] for folic acid should be avoided. A proportion of the UK population is currently exceeding the GL/UL per day due to consumption of foods fortified with folic acid on a voluntary basis and supplement use. The current risk posed by voluntary fortification of food with folic acid and supplement use in contributing to intakes above the GL/UL per day for folic acid needs to be addressed.

500. Mandatory fortification of flour with folic acid would improve the folate status of women most at risk of NTD-affected pregnancies. It would also improve the folate status of other population groups in the UK. However, mandatory fortification, combined with the current practice of voluntary fortification of foods with folic acid and inappropriate supplement use, would substantially increase the numbers in the population consuming levels of folic acid above the GL/UL per day.

501. Mandatory fortification should only be introduced in the UK if it is accompanied by:

- Action to reduce folic acid intakes from voluntarily fortified foods to ensure that the numbers of people with intakes above the GL/UL per day do not exceed current levels and there is no substantial increase in mean intakes or in the folate status of the UK population[30];

- Measures for careful monitoring of emerging evidence on the effects of long-term exposure to intakes of folic acid above the GL/UL per day and the postulated adverse effects, including neurological damage, CVD, and cancer.

28 Diabetes UK recommends that all women with diabetes planning a pregnancy should also take a 5mg dose of folic acid daily.

29 Although a GL of 1mg/d of folic acid was set for adults in the UK (EVM, 2003), the EVM did not set GLs for children. ULs were set for children in the USA (FNB, 1998) and Europe (SCF, 2000) on the basis of body weight.

30 This recommendation is supported by the Committee on Carcinogenicity.

502. The introduction of mandatory fortification will require:

- The establishment of a new baseline for folic acid intakes and blood folate concentrations prior to fortification to ensure that mandatory fortification does not lead to substantial increases in folic acid intake or folate status and so that trends can be monitored in future surveillance programmes;

- The adoption of a sufficiently robust common standard analytical method for measurement of folate status at baseline and in all future surveillance studies;

- The establishment of suitable reference ranges to predict folate adequacy and deficiency.

503. If mandatory fortification is introduced, the evidence on benefits and postulated adverse effects should be reviewed after a period of five years.

504. Mandatory fortification of flour with folic acid, accompanied by action to reduce folic acid intake from voluntarily fortified foods, would lead to a redistribution of folic acid intakes of the population. This could provide the most secure method of balancing the benefits and possible risks to the UK population as, relative to current practice, it would reduce exposure to intakes of folic acid above the GL/UL and increase the intake of low consumers. Careful consideration would need to be given to the issue of overage.

505. Clear guidance is needed on the use of folic acid containing supplements by the general population.

506. If mandatory fortification is introduced in the UK, all women who could become pregnant and those with a history of a previous NTD-affected pregnancy should continue to supplement their diet with 400µg/day and 5mg/day of folic acid respectively prior to conception and until the twelfth week of pregnancy.

507. There are a number of uncertainties regarding the GL/UL per day set for folic acid which is based on limited data and is related to concerns regarding

vitamin B12 deficiency. Further research is required on safe upper levels of folic acid intake in relation to other postulated risks, such as cancer.

508. More reliable diagnostic indices to identify vitamin B12 deficiency should be developed. The development of a clinical strategy to manage issues related to vitamin B12 is necessary irrespective of a decision on future mandatory fortification of flour with folic acid.

509. The prevalence of poor vitamin B2 (riboflavin) status in the UK population needs to be addressed.

References

Abrahamsen B, Madsen JS, Tofteng CL, Stilgren L, Bladbjerg EM, Kristensen SR, Brixen K & Mosekilde L. A common methylenetetrahydrofolate reductase (C677T) polymorphism is associated with low bone mineral density and increased fracture incidence after menopause: longitudinal data from the Danish osteoporosis prevention study. *J Bone Miner Res.* 2003; **18**:723-729.

Abramsky L, Botting B, Chapple J & Stone D. Has advice on periconceptional folate supplementation reduced neural-tube defects? *Lancet*. 1999; **354**:998-999.

Alberg AJ, Selhub J, Shah KV, Viscidi RP, Comstock GW & Helzlsouer KJ. The risk of cervical cancer in relation to serum concentrations of folate, vitamin B12, and homocysteine. *Cancer Epidemiol Biomarkers Prev*. 2000; **9**:761-764.

Alpert JE, Mischoulon D, Nierenberg AA, Fava M. Nutrition and depression: focus on folate. *Nutrition*. 2000; **16**:544-6.

Andrès E, Affenberger S, Vinzio S, Kurtz JE, Noel E, Kaltenbach G, Maloisel F, Schlienger JL, Blickle JF. Food-cobalamin malabsorption in elderly patients: clinical manifestations and treatment. *Am J Med.* 2005; **118**:1154-9.

Andrès E, Loukili NH, Noel E, Kaltenbach G, Ben Abdelgheni M, Perrin AE, Noblet-Dick M, Maloisel F, Schlienger J & Blicklé J. Vitamin B12 (cobalamin) deficiency in elderly patients. *CMAJ.* 2004; **171**:251-259.

Andrès E, Perrin AE, Demangeat C, Kurtz JE, Vinzio S, Grunenberger F, Goichot B, Schlienger JL. The syndrome of food-cobalamin malabsorption revisited in a department of internal medicine. A monocentric cohort study of 80 patients. *Eur J Intern Med.* 2003; **14**: 221-226.

Appel LJ, Miller ER 3rd, Jee SH, Stolzenberg-Solomon R, Lin PH, Erlinger T, Nadeau MR & Selhub J. Effect of dietary patterns on serum homocysteine: results of a randomized, controlled feeding study. *Circulation.* 2000; **102**:852-857.

Baik HW & Russell RM. Vitamin B12 deficiency in the elderly. *Annu Rev Nutr*. 1999; **19**:357-377.

Baker F, Picton D, Blackwood S, Hunt J, Erskine M, Dyas M, Ashby M, Siva A & Morris MJ. Blinded comparison of folic acid and placebo in patients with ischemic heart disease: an outcome trial. *Circulation*. 2002; **106**:3642 (abstr).

Banerjee A, Quirke P. Experimental models of colorectal cancer. *Dis Colon Rectum*. 1998; **41**: 490-505.

Bates CJ, Pentieva KD, Prentice A, Mansoor, MA, Finch S. Plasma pyridoxal phosphate and pyridoxic acid and their relationship to plasma homcysteine in a representative sample of British men and women aged 65 years and over. *Brit J Nutr*. 1999; **81**:191-201.

Bates CJ, Schneede J, Mishra G, Prentice A & Mansoor MA. Relationship between methylmalonic acid, homocysteine, vitamin B12 intake and status and socio-economic indices, in a subset of participants in the British National Diet and Nutrition Survey of people aged 65 y and over. *Eur J Clin Nutr*. 2003; **57**:349-357.

Becerra JE, Khoury MJ, Cordero JF, Erickson JD, Diabetes mellitus during pregnancy and the risks for specific birth defects: a population-based case-control study. *Pediatrics*. 1990; **85**:1-9.

Berg MJ, Fincham RW, Ebert BE, Schottelius DD. Phenytoin pharmacokinetics: before and after folic acid administration. *Epilepsia*. 1992; **33**:712-720.

Berry RJ, Kihlberg R & Devine O. Impact of misclassification of in vitro fertilisation in studies of folic acid and twinning: modelling using population based Swedish vital records. *BMJ*. 2005; **330**:815.

Bingham SA, Norat T, Moskal A, Ferrari P, Slimani N, Clavel-Chapelon F, Kesse E, Nieters A, Boeing H, Tjonneland A, Overvad K, Martinez C, Dorronsoro M, Gonzalez CA, Ardanaz E, Navarro C, Quiros JR, Key TJ, Day NE, Trichopoulou A, Naska A, Krogh V, Tumino R, Palli D, Panico S, Vineis P, Bueno-de-Mesquita HB, Ocke MC, Peeters PH, Berglund G, Hallmans G, Lund E, Skeie G, Kaaks R & Riboli E. Is the association with fiber from foods in colorectal cancer

confounded by folate intake? *Cancer Epidemiol Biomarkers Prev*. 2005; **14**:1552-1556.

Blackmore S., Pfeiffer C, Hamilton MS, Lee A. Recoveries of folate species from serum pools sent to participants of the UK NEQAS Haematinics Scheme in February and March 2004. *Clin Chim Acta*. 2005; **355**:S4599.

Blake M, Herrick K & Kelly Y. *Health Survey for England 2002: Maternal and Infant Health*. London. TSO, 2003.

Bland JM. Taking folate in pregnancy and the risk of maternal breast cancer. *BMJ*. 2005; **330**:600.

Bonaa KH, Njolstad I, Ueland PM, Schirmer H, Tverdal A, Steigen T, Wang H, Nordrehaug JE, Arnesen E, Rasmussen K; NORVIT Trial Investigators. Homocysteine lowering and cardiovascular events after acute myocardial infarction. *N Engl J Med*. 2006; **354**:1578-88.

Bostom AG & Garber C. Endpoints for homocysteine-lowering trials. *Lancet*. 2000; **355**:511-512.

Boushey CJ, Beresford SA, Omenn GS, Motulsky A. A quantitative assessment of plasma homocysteine as a risk factor for vascular disease. Probable benefits of increasing folic acid intakes. *JAMA*. 1995; **274**:1049-57.

Botting B. The impact of more complete data from Wales on the National Congenital Anomaly System. *Health Statistics Quarterly*. 2000; **5**:7-9.

Botting B. Trends in neural tube defects. *Health Statistics Quarterly*. 2001; **10**:5-12.

Botto LD, Moore CA, Khoury MJ, & Erickson JD. Neural-tube defects. *N.Engl.J Med*. 1999; **341**:1509-1519.

Botto LD & Yang Q. 5,10-Methylenetetrahydrofolate reductase gene variants and congenital abnormalities: a HuGE review. *Am J Epidemiol*. 2000; **151**:862-877.

Botto LD, Lisi A, Robert-Gnansia E, Erickson JD, Vollset SE, Mastroiacovo P, Botting B, Cocchi G, de Vigan C, de Walle H, Feijoo M, Irgens LM, McDonnell B, Merlob P, Ritvanen A, Scarano G, Siffel C, Metneki J, Stoll C, Smithells R & Goujard J. International retrospective cohort study of neural tube defects in relation to folic acid recommendations: are the recommendations working? *BMJ.* 2005; **330**:571.

Boyd PA, Armstrong B, Dolk H, Botting B, Pattenden S, Abramsky L, Rankin J, Vrijheid M, Wellesley D. Congenital anomaly surveillance in England – ascertainment deficiencies in the national system. *BMJ.* 2005; **330**:27-29.

Breen N, Wagener DK, Brown ML, Davis WW, Ballard-Barbash R. Progress in cancer screening over a decade: results of cancer screening from the 1987, 1992, and 1998 National Health Interview Surveys. *J Natl Cancer Inst.* 2001; **93**(22):1704-13.

Brody LC, Conley M, Cox C, Kirke PN, McKeever MP, Mills JL, Molloy AM, O'leary VB, Parle-McDermott A, Scott JM, & Swanson DA. A polymorphism, R653Q, in the trifunctional enzyme methylenetetrahydrofolate dehydrogenase/ methenyltetrahydrofolate cyclohydrolase/ formyltetrahydrofolate synthetase is a maternal genetic risk factor for neural tube defects: report of the Birth Defects Research Group. *Am J Hum Genet.* 2002; **71**:1207-1215.

Brown RD, Jun R, Hughes W, Watman R, Arnold B, Kronenberg H. Red cell folate assays: some answers to current problems with radioassay variability. *Pathology.* 1990; **22**(2):82-7.

Busby A, Abramsky L, Dolk H & Armstrong B. Preventing neural tube defects in Europe: population based study. *BMJ.* 2005; **330**:574-575.

Butterworth CE Jr & Tamura T. Folic acid safety and toxicity: a brief review. *Am J Clin Nutr.* 1989; **50**:353-8.

B-Vitamin Treatment Trialists' Collaboration. Homocysteine-lowering trials for prevention of cardiovascular events: A review of the design and power of the large randomized trials. *Am Heart J.* 2006; **151**:282-7.

Canadian Cancer Society/National Cancer Institute of Canada: *Canadian Cancer Statistics 2006,* Toronto, Canada, 2006. April 2006, ISSN 0835-2976. Available on the internet at http://www.cancer.ca and http://www.ncic.cancer.ca

Cappuccio FP, Bell R, Perry IJ, Gilg J, Ueland PM, Refsum H, Sagnella GA, Jeffery S & Cook DG. Homocysteine levels in men and women of different ethnic and cultural background living in England. *Atherosclerosis.* 2002; **164**:95-102.

Carmel R. Cobalamin, the stomach, and aging. *Am J Clin Nutr.* 1997; **66**:750-759.

Carmel R. Current concepts in cobalamin deficiency. *Annu Rev Med.* 2000; **51**:357-75.

Casas JP, Bautista LE, Smeeth L, Sharma P & Hingorani AD. Homocysteine and stroke: evidence on a causal link from mendelian randomisation. *Lancet.* 2005; **365**:224-232.

Centers for Disease Control and Prevention. Spina bifida and anencephaly before and after folic acid mandate--United States, 1995-1996 and 1999-2000. *Morb Mortal Wkly Rep.* 2004; **53**:362-5.

Chambers JC, Ueland PM, Obeid OA, Wrigley J, Refsum H & Kooner JS. Improved vascular endothelial function after oral B vitamins: An effect mediated through reduced concentrations of free plasma homocysteine. *Circulation.* 2000; **102**:2479-2483.

Charles D, Ness AR, Campbell D, Davey Smith G & Hall MH. Taking folate in pregnancy and risk of maternal breast cancer. *BMJ.* 2004; **329**:1375-1376.

Chasan-Taber L, Selhub J, Rosenberg IH, Malinow MR, Terry P, Tishler PV, Willett W, Hennekens CH & Stampfer MJ. A prospective study of folate and vitamin B6 and risk of myocardial infarction in US physicians. *J Am Coll Nutr.* 1996; **15**:136-143.

Chen H, Zhang SM, Schwarzschild MA, Hernan MA, Logroscino G, Willett WC & Ascherio A. Folate intake and risk of Parkinson's disease. *Am J Epidemiol.* 2004; **160**:368-375.

Choumenkovitch SF, Selhub J, Wilson PW, Rader JI, Rosenberg IH, Jacques PF. Folic acid intake from fortification in United States exceeds predictions. *J Nutr.* 2002; **132**:2792-2798.

Clarke R, Smith AD, Jobst KA, Refsum H, Sutton L & Ueland PM. Folate, vitamin B12, and serum total homocysteine levels in confirmed Alzheimer's disease. *Arch Neurol.* 1998; **55**:1449-55.

Clarke R, Refsum H, Birks J, Evans JG, Johnston C, Sherliker P, Ueland PM, Schneede J, McPartlin J, Nexo E & Scott JM. Screening for vitamin B-12 and folate deficiency in older persons. *Am J Clin Nutr.* 2003; **77**:1241-1247.

Clarke R, Grimley Evans J, Schneede J, Nexo E, Bates C, Fletcher A, Prentice A, Johnston C, Ueland PM, Refsum H, Sherliker P, Birks J, Whitlock G, Breeze E & Scott JM. Vitamin B12 and folate deficiency in later life. *Age Ageing.* 2004; **33**:34-41.

Clarke R. Vitamin B12, folic acid, and the prevention of dementia. *N Engl J Med.* 2006; **354**:2817-9.

Cole BF, Baron JA, Sandler RS, et al. A randomized trial of folic acid to prevent colorectal adenomas [abstract]. *Proc Am Assoc Cancer Res.* 2005; **46**: 4399.

Cooney CA, Dave AA, & Wolff GL. Maternal methyl supplements in mice affect epigenetic variation and DNA methylation of offspring. *J Nutr.* 2002; **132**(8 Suppl):2393S-2400S.

Coppen A, Bailey J. Enhancement of the antidepressant action of fluoxetine by folic acid: a randomised, placebo controlled trial. *J Affect Disord.* 2000; **60**(2):121-130.

Cress RD, Morris C, Ellison GL, Goodman MT. Secular changes in colorectal cancer incidence by subsite, stage at diagnosis, and race/ethnicity, 1992-2001. *Cancer.* 2006; **107**(5):1142-52.

Cuskelly GJ, McNulty H & Scott JM. Effect of increasing dietary folate on red-cell folate: implications for prevention of neural tube defects. *Lancet.* 1996; **347**:657-9.

Czeizel AE, Metneki J & Dudas I.The higher rate of multiple births after periconceptional multivitamin supplementation: an analysis of causes. *Acta Genet Med Gemellol (Roma).* 1994; **43**:175-184.

Dacie JV, Lewis SM. *Practical Haematology*. 8th edition. Edinburgh: Churchill Livingstone, 1995.

Daly S, Kirke PN, Molloy AM, Weir DG & Scott JM. Folate levels and neural tube defects, implications for prevention. *JAMA*. 1995; **274**:1696-1702.

Daly S, Mills J L, Molloy A M, Conley M, Lee Y L, Kirke, P N Weir D G Scott J M. Minimum effective dose of folic acid for food fortification to prevent neural-tube defects. *Lancet*. 1997; **350**:1666-1669.

Danesh J, Lewington S. Plasma homocysteine and coronary heart disease: systematic review of published epidemiological studies. *J Cardiovasc Risk*. 1998; **5**:229-32.

de Bree A, Verschuren WM, Blom HJ & Kromhout D. Lifestyle factors and plasma homocysteine concentrations in a general population sample. *Am J Epidemiol*. 2001; **154**:150-154.

De Marco P, Calevomg, Moroni A, Arata L, Merello E, Cama A, Finnell RH, Andreussi L, & Capra V. Polymorphisms in genes involved in folate metabolism as risk factors for NTDs. *Eur J Pediatr Surg*. 2001; **11**:Suppl 1, S14-S17.

De Marco P, Calevo MG, Moroni A, Merello E, Raso A, Finnell RH, Zhu H, Andreussi L, Cama A, & Capra V. Reduced folate carrier polymorphism (80A->G) and neural tube defects. *Eur J Hum Genet*. 2003; **11**:245-252.

De Marco P, Merello E, Calevo MG, Mascelli S, Raso A, Cama A, & Capra V. Evaluation of a methylenetetrahydrofolate-dehydrogenase 1958G›A polymorphism for neural tube defect risk. *J Hum Genet*. 2006; **51**:98-103.

de Wals P, Rusen ID, Lee NS, Morin P & Niyonsenga T. Trend in prevalence of neural tube defects in Quebec. *Birth Defects Res*. 2003; **67**:919-923.

Department of Environment, Food and Rural Affairs. *Expenditure and Food Survey London*: TSO, 2005.

Department of Health. *Dietary reference values for food energy and nutrients in the UK*. Report on health and social subjects 41. *London*: HMSO, 1991.

Department of Health. *Folic acid and the prevention of neural tube defects*. Report from an Expert Advisory Group. 1992. (Available from DH, PO Box 410, Wetherby LS23 7LN).

Department of Health. *Nutritional aspects of the development of cancer*. Report on health and social subjects 48. London: TSO, 1998.

Department of Health. *Folic acid and the prevention of disease*. Report on health and social subjects 50. London: TSO, 2000.

Department of Health. *Scientific Review of the Welfare Food Scheme. Report on health and social subjects 51*. London: TSO, 2002.

Dietrich M, Brown CJ, Block G. The effect of folate fortification of cereal-grain products on blood folate status, dietary folate intake, and dietary folate sources among adult non-supplement users in the United States. *J Am Coll Nutr*. 2005; **24**(4):266-74.

Doshi SN, McDowell IF, Moat SJ, Lang D, Newcombe RG, Kredan MB, Lewis MJ & Goodfellow J. Folate improves endothelial function in coronary artery disease: an effect mediated by reduction of intracellular superoxide? *Arteriosclerosis Thromb Vascular Biol*. 2001; **21**:1196-1202.

Doshi SN, McDowell IF, Moat SJ, Payne N, Durrant HJ, Lewis MJ & Goodfellow J. Folic acid improves endothelial function in coronary artery disease via mechanisms largely independent of homocysteine lowering. *Circulation*. 2002; **105**, 22-26.

Durga J, van Boxtel MP, Schouten EG, Bots ML, Kok FJ, Verhoef P. Folate and the methylenetetrahydrofolate reductase 677C-->T mutation correlate with cognitive performance. *Neurobiol Aging*. 2006; **27**:334-43.

Dusitanond P, Eikelboom JW, Hankey GJ, Thom J, Gilmore G, Loh K, Yi Q, Klijn CJ, Langton P, van Bockxmeer FM, Baker R & Jamrozik K. Homocysteine-lowering treatment with folic acid, cobalamin, and pyridoxine does not reduce blood markers of inflammation, endothelial dysfunction, or hypercoagulability in patients with previous transient ischemic attack or stroke: a randomized substudy of the VITATOPS trial. *Stroke*. 2005; **36**:144-146.

Duthie SJ, Whalley LJ, Collins AR, Leaper S, Berger K & Deary IJ. Homocysteine, B vitamin status, and cognitive function in the elderly. *Am J Clin Nutr*. 2002; **75**:908-913.

Edwards BK, Brown ML, Wingo PA, Howe HL, Ward E, Ries LA, Schrag D, Jamison PM, Jemal A, Wu XC, Friedman C, Harlan L, Warren J, Anderson, RN, Pickle LW. Annual report to the nation on the status of cancer, 1975-2002, featuring population-based trends in cancer treatment. *J Natl Cancer Inst*. 2005; **97**(11): 1407-27

Elias MF, Sullivan LM, D'Agostino RB, Elias PK, Jacques PF, Selhub J, Seshadri S, Au R, Beiser A, Wolf PA. Homocysteine and Cognitive Performance in the Framingham Offspring Study: Age Is Important. *Am J Epidemiol*. 2005; **162**:1-10.

Engbersen AM, Franken DG, Boers GH, Stevens EM, Trijbels FJ & Blom HJ. Thermolabile 5,10-methylenetetrahydrofolate reductase as a cause of mild hyperhomocysteinemia. *Am J Hum Genet*. 1995; **56**:142-150.

Ericson A, Kallen B & Aberg A. Use of multivitamins and folic acid in early pregnancy and multiple births in Sweden. *Twin Res*. 2001; **4**:63-66.

European Scientific Committee on Food. Opinion of the Scientific Committee on Food on the Tolerable Upper Intake Level of Folate 2000. Online at http://ec.europa.eu/comm/food/fs/sc/scf/out80e_en.pdf

Eussen SJ, de Groot LC, Clarke R, Schneede J, Ueland PM, Hoefnagels WH & van Staveren WA.Oral cyanocobalamin supplementation in older people with vitamin B12 deficiency: a dose-finding trial. *Arch Intern Med*. 2005; **165**:1167-1172.

Eussen SJ, Ferry M, Hininger I, Haller J, Matthys C & Dirren H. Five year changes in mental health and associations with vitamin B12/folate status of elderly Europeans. *J Nutr Health Aging*. 2002; **6**:43-50.

Expert Group on Vitamins and Minerals. *Safe Upper Levels for Vitamins and Minerals*. London: Food Standards Agency, 2003.

Feigelson HS, Jonas CR, Robertson AS, McCullough ML, Thun MJ & Calle EE. Alcohol, folate, methionine, and risk of incident breast cancer in the American Cancer Society Cancer Prevention Study II Nutrition Cohort. *Cancer Epidemiol Biomarkers Prev*. 2003; **12**:161-164.

Finch S, Doyle W, Lowe C, Bates CJ, Prentice A, Smithers G & Clarke PC. National Diet and Nutrition Survey: people aged 65 years and over. Volume 1: report of the diet and nutrition survey. London: TSO, 1998.

Flood A, Caprario L, Chaterjee N, Lacey JV Jr, Schairer C & Schatzkin A. Folate, methionine, alcohol, and colorectal cancer in a prospective study of women in the United States. *Cancer Causes Control*. 2002; **13**:551-561.

Folsom AR, Nieto FJ, McGovern PG, Tsai MY, Malinow MR, Eckfeldt JH, Hess DL & Davis CE. Prospective study of coronary heart disease incidence in relation to fasting total homocysteine, related genetic polymorphisms, and B vitamins: the atherosclerosis risk in communities (ARIC) study. *Circulation*. 1998; **98**: 204-210

Food and Nutrition Board, Institute of Medicine. *Dietary Reference Intakes: Thiamin, Riboflavin, Niacin, Vitamin B6,* Folate, *Vitamin B12, Pantothenic Acid,* Biotin, and Choline. Washington, DC: National Academy Press, 1998.

Food Standards Agency (2002a). Folic acid and the prevention of disease. http://www.food.gov.uk/multimedia/pdfs/FSA02_05_02Annex345.pdf

Food Standards Agency (2002b). Agency advice to health ministers on folic acid and the prevention of disease.
http://www.food.gov.uk/multimedia/pdfs/folicacid_disease_annexa.pdf

Food Standards Agency (2002c). *Nutrient Analysis of Breakfast Cereals*. Unpublished.

Ford ES, Byers TE & Giles WH. Serum folate and chronic disease risk: findings from a cohort of United States adults. *Int J Epidemiol*. 1998; **27**: 592-598.

Frame PS, Berg AO, Woolf S. U.S. Preventive Services Task Force: highlights of the 1996 report. *Am Fam Physician*. 1997; **55**:567-76,581-2.

French AE, Grant R, Weitzman S, Ray JG, Vermeulen MJ, Sung L, Greenberg M, Koren G. Folic acid food fortification is associated with a decline in neuroblastoma. *Clin Pharmacol Ther*. 2003; **74**:288-94.

Friso S, Choi SW, Girelli D, Mason JB, Dolnikowski GG, Bagley PJ, Olivieri O, Jacques PF, Rosenberg IH, Corrocher R, & Selhub J. (2002). A common mutation in the 5,10-methylenetetrahydrofolate reductase gene affects genomic DNA methylation through an interaction with folate status. *Proc Natl Acad Sci*. USA. 2002; **99**:5606-5611.

Fung TT, Hunter DJ, Spiegelman D, Colditz GA, Speizer FE & Willett WC. Vitamins and carotenoids intake and the risk of basal cell carcinoma of the skin in women (United States). *Cancer Causes Control*. 2002; **13**:221-230.

Fung TT, Spiegelman D, Egan KM, Giovannucci E, Hunter DJ & Willett WC. Vitamin and carotenoid intake and risk of squamous cell carcinoma of the skin. *Int J Cancer*. 2003; **103**:110-115.

Ganji V & Kafai MR. Demographic, health, lifestyle, and blood vitamin determinants of serum total homocysteine concentrations in the third National Health and Nutrition Examination Survey, 1988-1994. *Am J Clin Nutr*. 2003; **77**:826-833.

Ganji V & Kafai MR. Trends in serum folate, RBC folate, and circulating total homocysteine concentrations in the United States: analysis of data from National Health and Nutrition Examination Surveys, 1988-1994, 1999-2000, and 2001-2002. *J Nutr*. 2006; **136**(1):153-8.

Garcia A, Haron Y, Pulman K, Hua L, Freedman M. Increases in homocysteine are related to worsening of stroop scores in healthy elderly persons: a prospective follow-up study. *J Gerontol A Biol Sci Med Sci*. 2004; **59**:1323-1327.

Giovannucci E, Rimm EB, Ascherio A, Stampfer MJ, Colditz GA & Willett WC. Alcohol, low-methionine--low-folate diets, and risk of colon cancer in men. *J Natl Cancer Inst*. 1995; **87**:265-273.

Giovannucci E, Stampfer MJ, Colditz GA, Hunter DJ, Fuchs C, Rosner BA, Speizer FE & Willett WC. Multivitamin use, folate, and colon cancer in women in the Nurses' Health Study. *Ann Intern Med*. 1998; **129**:517-524.

Glatzle D, Korner WF, Christeller S & Wiss O. Method for the detection of biochemical riboflavin deficiency. *Int J Vit Nutr Res*. 1970; **40**:166-183.

Glynn SA, Albanes D, Pietinen P, Brown CC, Rautalahti M, Tangrea JA, Gunter EW, Barrett MJ, Virtamo J & Taylor PR. Colorectal cancer and folate status: a nested case-control study among male smokers. *Cancer Epidemiol Biomarkers Prev*. 1996; **5**:487-494.

Godfrey PS, Toone BK, Carney MW, Flynn TG, Bottiglieri T, Laundy M, Chanarin I, Reynolds EH. Enhancement of recovery from psychiatric illness by methylfolate. *Lancet*. 1990; **13**:336:953-4.

Graves EJ, Gillum BS. Detailed diagnoses and procedures, National Hospital Discharge Survey, 1995. *Vital Health Stat 13*. 1997; (130):1-146.

Gregory J, Lowe S, Bates CJ, Prentice A, Jackson LV, Smithers G, Wenlock R & Farron M. National Diet and Nutrition Survey: young people aged 4 to 18 years. Volume 1: report of the diet and nutrition survey. London: TSO, 2000.

Gregory JF 3rd. Bioavailability of Folate. *Eur J Clin Nutr*. 1997; **51**(1 Suppl): S54:59.

Gregory JR, Collins DL, Davies PSW, Hughes JM & Clarke PC. National Diet and Nutrition Survey: children aged 1 ½ to 4 ½ years. Volume 1: report of the diet and nutrition survey. London: HMSO, 1995.

Gregory R, Foster K, Tyler H & Wiseman M. The Dietary and Nutritional Survey of British Adults: aged 16-64 years. London: HMSO, 1990.

Gunter E, Bowman BA, Caudill SP, Twite DB, Adams MJ and Sampson EJ. Results of an international round robin for serum and whole blood folate. *Clin Chem*. 1996; **42**: 1689-1694.

Hackam DG, Peterson JC & Spence JD. What level of plasma homocyst(e)ine should be treated? Effects of vitamin therapy on progression of carotid atherosclerosis in patients with homocyst(e)ine levels above and below 14 micromol/L. *Am J Hypertens*. 2000; **13**:105-110.

Haggarty P, McCallum H, McBain H, Andrews K, Duthie S, McNeill G, Templeton A, Haites N, Campbell D, Bhattacharya S. Effect of B vitamins and genetics on success of in-vitro fertilisation: prospective cohort study. *Lancet*. 2006; **367**:1513-9.

Halstead C. *Alcohol and folate interaction: clinical implication*. In: Bailey LB ed. Folate in health and disease. New York, NY: Marcel Dekker, 1995:313-328.

Hamlyn B, Brooker S, Oleinikova K & Wands S. Infant Feeding 2000. *A survey conducted on behalf of DH, the Scottish Executive, the National Assembly for Wales and DHth, Social Services and Public Safety in Northern Ireland*. London: TSO, 2002.

Harnack L, Jacobs DR Jr, Nicodemus K, Lazovich D, Anderson K & Folsom AR. Relationship of folate, vitamin B-6, vitamin B-12, and methionine intake to incidence of colorectal cancers. *Nutr Cancer*. 2002; **43**:152-158.

Hathcock JN, Troendle GJ. Oral cobalamin for treatment of pernicious anemia? *JAMA*. 1991; **265**:96-7.

He K, Merchant A, Rimm EB, Rosner BA, Stampfer MJ, Willett WC & Ascherio A. Folate, vitamin B6, and B12 intakes in relation to risk of stroke among men. *Stroke*. 2004; **35**:169-174.

Henderson L, Gregory J, Irving K & Swann G. The National Diet & Nutrition Survey: adults aged 19 to 64 years. London: TSO, 2003.

Herrmann W, Obeid R, Schorr H, Geisel J. Functional vitamin B12 deficiency and determination of holotranscobalamin in populations at risk. *Clin Chem Lab Med.* 2003; **41**:1478-88.

Herrmann W, Schorr H, Obeid R & Geisel J. Vitamin B-12 status, particularly holotranscobalamin II and methylmalonic acid concentrations, and hyperhomocysteinemia in vegetarians. *Am J Clin Nutr.* 2003; **78**:131-136.

Herzlich B, Herbert V. Depletion of serum holotranscobalamin II. An early sign of negative vitamin B12 balance. *Lab Invest.* 1988; **58**:332-7.

Hin H, Clarke R, Sherliker P, Atoyebi W, Emmens K, Birks J, Schneede J, Ueland PM, Nexo E, Scott J, Molloy A, Donaghy M, Frost C, Evans JG. Clinical relevance of low serum vitamin B12 concentrations in older people: the Banbury B12 study. *Age Ageing.* 2006; **35**:416-22.

Hirsch S, de la Maza P, Barrera G, Gattas V, Petermann M & Bunout D. The Chilean flour folic acid fortification program reduces serum homocysteine levels and masks vitamin B-12 deficiency in elderly people. *J Nutr.* 2002a; **132**:289-291.

Hirsch S, de la Maza P, Yanez P, Glasinovic A, Petermann M, Barrera G, Gattas V, Escobar E & Bunout D. Hyperhomocysteinemia and endothelial function in young subjects: effects of vitamin supplementation. *Clin Cardiol.* 2002b; **25**:495-501.

Homocysteine Studies Collaboration. Homocysteine and risk of ischemic heart disease and stroke: a meta- analysis. *JAMA* 2002; **288**:2015-2022.

Honein MA, Paulozzi LJ, Mathews TJ, Erickson JD, Wong LY. Impact of folic acid fortification of the US food supply on the occurrence of neural tube defects. *JAMA.* 2001; **285**:2981-6.

HOPE 2 Investigators. Homocysteine lowering with folic acid and B vitamins in vascular disease. *N Engl J Med.* 2006; **354**(15):1567-77.

Hubner RA, Muir KR, Liu JF, Sellick GS, Logan RFAm Grainge M, Armitage N, Chau I, Houlston RS, and the United Kingdom Colorectal Adenoma

Prevention Consortium. Folate metabolism polymorphisms influence risk of colorectal adenoma recurrence. *Cancer Epidemiol Biomarkers Prev*. 2006; **15**(9):1607-1613.

Hultberg B, Andersson A, Sterner G. Plasma homocysteine in renal failure. *Clin Nephrol*. 1993; **40**:230-5.

Hultdin J, van Guelpen B, Bergh A, Hallmans G & Stattin P. Plasma folate, vitamin B12 and homocysteine and prostate risk: a prospective study. *In J Cancer*. 2005; **113**:819-824.

Hung J, Beilby JP, Knuiman MW & Divitini M. Folate and vitamin B-12 and risk of fatal cardiovascular disease: cohort study from Busselton, Western Australia. *BMJ*. 2003; **326**:131-136.

Husemoen LL, Thomsen TF, Fenger M & Jorgensen AT. Effect of lifestyle factors on plasma total homocysteine concentrations in relation to MTHFR(C677T) genotype. *Eur J Clin Nutr*. 2004; **58**:1142-1150.

Hustad S, Ueland PM, Vollset SE, Zhang Y, Bjorke-Monsen AL & Schneede J. Riboflavin as a determinant of plasma total homocysteine: effect modification by the methylenetetrahydrofolate reductase C677T polymorphism. *Clin Chem*. 2000; **46**:1065–1071.

Hvas AM, Juul S, Gerdes LU, Nexo E. The marker of cobalamin deficiency, plasma methylmalonic acid, correlates to plasma creatinine. *J Intern Med*. 2000; **247**:507-12.

Hvas AM, Ellegaard J, Nexo E. Vitamin B12 treatment normalizes metabolic markers but has limited clinical effect: a randomized placebo-controlled study. *Clin Chem*. 2001; **47**:1396-404.

Hvas AM, Juul S, Lauritzen L, Nexo E & Ellegaard J.No effect of vitamin B-12 treatment on cognitive function and depression: a randomized placebo controlled study. *J Affect Disord*. 2004; **81**:269-273.

Hvas AM & Nexo E. Holotranscobalamin – a first choice assay for diagnosing early vitamin B deficiency? *J Intern Med*. 2005; **257**:289-298.

Imai K, Matsuyama S, Miyake S, Suga K, Nakachi K. Natural cytotoxic activity of peripheral-blood lymphocytes and cancer incidence: an 11-year follow-up study of a general population. *Lancet*. 2000; **356**:1795-9.

Jacobs EJ, Connell CJ, Chao A, McCullough ML, Rodriguez C, Thun MJ & Calle EE. Multivitamin use and colorectal cancer incidence in a US cohort: does timing matter? *Am J Epidemiol*. 2003; **158**:621-628.

Jacques PF, Kalmbach R, Bagley PJ, Russo GT, Rogers G, Wilson PW, Rosenberg IH & Selhub J. The relationship between riboflavin and plasma total homocysteine in the Framingham Offspring cohort is influenced by folate status and the C677T transition in the methylenetetrahydrofolate reductase gene. *J Nutr*. 2002; **132**:283-288.

Johnson MA, Hawthorne NA, Brackett WR, Fischer JG, Gunter EW, Allen RH & Stabler SP. Hyperhomocysteinemia and vitamin B-12 deficiency in elderly using Title IIIc nutrition services. *Am J Clin Nutr*. 2003; **77**:211-220.

Jones PA, Baylin SB. The fundamental role of epigenetic events in cancer. *Nat Rev Genet*. 2002; **3**:415-28

Joosten E, van den Berg A, Riezler R, Naurath HJ, Lindenbaum J, Stabler SP & Allen RH. Metabolic evidence that deficiencies of vitamin B-12 (cobalamin), folate, and vitamin B-6 occur commonly in elderly people. *Am J Clin Nutr*. 1993; **58**:468-476.

Kado DM, Karlamangla AS, Huang MH, Troen A, Rowe JW, Selhub J, Seeman TE. Homocysteine versus the vitamins folate, B6, and B12 as predictors of cognitive function and decline in older high-functioning adults: MacArthur Studies of Successful Aging. *Am J Med*. 2005; **118**:161-167.

Kato I, Dnistrian AM, Schwartz M, Toniolo P, Koenig K, Shore RE, Akhmedkhanov A, Zeleniuch-Jacquotte A & Riboli E. Serum folate, homocysteine and colorectal cancer risk in women: a nested case-control study. *Br J Cancer*. 1999; **79**:1917-1922.

Kelemen LE, Sellers TA, Vierkant RA, Harnack L & Cerhan JR. Association of folate and alcohol with risk of ovarian cancer in a prospective study of postmenopausal women. *Cancer Causes Control.* 2004; **15**:1085-1093.

Kelly, J McPartlin, M Goggins, DG Weir & JM Scott. Unmetabolized folic acid in serum: acute studies in subjects consuming fortified food and supplements *Am J Clin Nutr.* 1997; **65**:1790-1795.

Kim YI. Role of folate in colon cancer development and progression. *J Nutr.* 2003; **133** Suppl: 3731S-3739S.

Kim YI. Will mandatory folic acid fortification prevent or promote cancer? *Am J Clin Nutr.* 2004; **80**:1123-8.

Kinzler WL, Ananth CV & Vintzileos AM. Medical and economic effects of twin gestations. *J Soc Gynecol Investig.* 2000; **7**:321-327.

Kirke PN, Daly LE & Elwood JH. A randomised trial of low dose folic acid to prevent neural tube defects. The Irish Vitamin Study Group. *Arch Dis Child.* 1992, **67**:1442-1446.

Klerk M, Verhoef P, Clarke R, Blom HJ, Kok FJ & Schouten, EG and the MTHFR studies collaboration group. MTHFR 677 C T polymorphism and risk of coronary heart disease. *JAMA.* 2002; **288**: 2023-2031.

Konings EJ, Goldbohm RA, Brants HA, Saris WH & van den Brandt PA. Intake of dietary folate vitamers and risk of colorectal carcinoma: results from The Netherlands Cohort Study. *Cancer.* 2002; **95**:1421-1433.

Kozak LJ, Lees KA, DeFrances CJ. National Hospital Discharge Survey: 2003 annual summary with detailed diagnosis and procedure data. *Vital Health Stat 13.* 2006; **160**:1-206.

Kuhn W, Roebroek R, Blom H, van Oppenraaij D, Przuntek H, Kretschmer A, Buttner T, Woitalla D, Muller T. Elevated plasma levels of homocysteine in Parkinson's disease. *Eur Neurol.* 1998; **40**:225-227.

Lange H, Suryapranata H, De Luca G, Borner C, Dille J, Kallmayer K, Pasalary MN, Scherer E & Dambrink JHE. Folate therapy and in-stent restenosis after coronary stenting. *New Engl J Med*. 2004; **350**:2673-2681.

Larsson SC, Giovannucci E & Wolk A. Dietary folate intake and incidence of ovarian cancer: the Swedish Mammography Cohort. *J Natl Cancer Inst*. 2004; **96**:396-402.

Larsson SC, Giovannucci E & Wolk A. A prospective study of dietary folate intake and risk of colorectal cancer: modification by caffeine intake and cigarette smoking. *Cancer Epidemiol Biomarkers Prev*. 2005; **14**:740-743.

Le Leu RK, Young GP, McIntosh GH. Folate deficiency reduces the development of colorectal cancer in rats. *Carcinogenesis*. 2000; **21**:2261-5.

Levin B, Bond JH. Colorectal cancer screening: recommendations of the U.S. Preventive Services Task Force. *Gastroenterology*. 1996; **111**:1381-4.

Lewerin C, Matousek M, Steen G, Johansson B, Steen B & Nilsson-Ehle H. Significant correlations of plasma homocysteine and serum methylmalonic acid with movement and cognitive performance in elderly subjects but no improvement from short-term vitamin therapy: a placebo-controlled randomized study. *Am J Clin Nutr*. 2005; **81**:1155-1162.

Lewis SJ, Ebrahim S, Davey Smith G. A meta-analysis of the MTHFR 677C→T polymorphism and coronary heart disease: does the totality of the evidence support a causal role for homocysteine and the preventive potential of folate? *BMJ*. 2005; **331**:1053-1056.

Li Z, Gindler J, Wang H, Berry RJ, Li S, Correa A, Zheng JC, Erickson JD & Wang Y. Folic acid supplements during early pregnancy and likelihood of multiple births: a population-based cohort study. *Lancet*. 2003; **361**:380-384.

Lindenbaum J, Healton EB, Savage DG, Brust JC, Garrett TJ, Podell ER, Marcell PD, Stabler SP & Allen RH. Neuropsychiatric disorders caused by cobalamin deficiency in the absence of anaemia or macrocytosis. *N Engl J Med*. 1988; **318**:1720-1728.

Lindenbaum J, Savage DG, Stabler SP & Allen RH. Diagnosis of cobalamin deficiency: II. Relative sensitivities of serum cobalamin, methylmalonic acid, and total homocysteine concentrations. *Am J Hematol*. 1990; **34**:99-107.

Lindeman RD, Romero LJ, Koehler KM, Liang HC, LaRue A, Baumgartner RN, Garry PJ. Serum vitamin B12, C and folate concentrations in the New Mexico elder health survey: correlations with cognitive and affective functions. *J Am Coll Nutr*. 2000; **19**:68-76.

Little J, Sharp L, Duthie S & Narayanan S. Colon cancer and genetic variation in folate metabolism: the clinical bottom line. *J Nutr*. 2003; **133**(suppl):3758S-3766S.

Lloyd-Wright Z, Hvas AM, Moller J, Sanders TA & Nexo E. Holotranscobalamin as an indicator of dietary vitamin B12 deficiency. *Clin Chem*. 2003; **49**:2076-2078.

Liu S, West R, Randell E, Longerich L, O'Connor KS, Scott H, Crowley M, Lam A, Prabhakaran V & McCourt C. A comprehensive evaluation of food fortification with folic acid for the primary prevention of neural tube defects. *BioMedCentral Pregnancy and Childbirth*. 2004; **4**:20.

Lopez-Camelo JS, Orioli IM, da Graca Dutra M, Nazer-Herrara J, Rivera N, Ojeda ME, Canessa A, Wettig E, Fontannaz AM, Mellado C, Castilla EE. Reduction of birth prevalence rates of neural tube defects after folic acid fortification in Chile. *Am J Med Genet A*. 2005; **135**(2):120-5.

Luchsinger JA, Tang MX, Shea S, Miller J, Green R & Mayeux R. Plasma homocysteine levels and risk of Alzheimer disease. *Neurology*. 2004; **62**:1972-1976.

Macdonald HM, McGuigan FE, Fraser WD, New SA, Ralston SH & Reid DM. Methylenetetrahydrofolate reductase polymorphism interacts with riboflavin intake to influence bone mineral density. *Bone* 2004; **35**:957-964.

Malouf R, Areosa Sastre A. Vitamin B12 for cognition. (Cochrane Review). In: *The Cochrane Library*, Issue 3. 2003. Chichester, UK: John Wiley & Sons, Ltd.

Malouf M, Grimley EJ, Areosa SA. Folic acid with or without vitamin B12 for cognition and dementia. In: *The Cochrane Library*, Issue 4. 2003. Chichester, UK: John Wiley & Sons, Ltd.

Mangoni AA, Sherwood RA, Swift CG & Jackson SH. Folic acid enhances endothelial function and reduces blood pressure in smokers: a randomized controlled trial. *J Intern Med.* 2002; **252**:497-503.

Martinez ME, Giovannucci E, Jiang R, Henning SM, Jacobs ET, Thompson P, Smith-Warner SA, Alberts DS. Folate fortification, plasma folate, homocysteine and colorectal adenoma recurrence. *Int J Cancer.* 2006; **119**:1440-1446.

McCaddon A, Hudson P, Davies G, Hughes A, Williams JH, Wilkinson C. Homocysteine and cognitive decline in healthy elderly. *Dement Geriatr Cogn Disord.* 2001; **12**:309-313.

McGown EL, Lewis CM, Dong MH, Sauberlich HE. Results with commercial radioassay kits compared with microbiological assay of folate in serum and whole-blood. *Clin Chem.* 1978; **24**(12):2186-91.

McKinley MC, McNulty H, McPartlin J, Strain JJ & Scott JM. Effect of riboflavin supplementation on plasma homocysteine in elderly people with low riboflavin status. *Eur J Clin Nutr.* 2002; **56**:850-856.

McKinley MC, McNulty H, McPartlin J, Strain JJ, Pentieva K, Ward M, Weir DG & Scott JM. Low-dose vitamin B-6 lowers fasting plasma homocysteine levels in healthy elderly persons who are folate and riboflavin replete. *Am J Clin Nutr.* 2001; **73**, 759–764.

McLean RR, Jacques PF, Selhub J, Tucker KL, Samelson EJ, Broe KE, Hannan MT, Cupples LA & Kiel DP. Homocysteine as a predictive factor for hip fracture in older persons. *N Engl J Med.* 2004; **350**:2042-2049.

McMahon JA, Green TJ, Skeaff CM, Knight RG, Mann JI, Williams SM. A controlled trial of homocysteine lowering and cognitive performance. *NEJM.* 2006; **354**:2764-72.

McNulty H, McKinley MC, Wilson B, McPartlin J, Strain JJ, Weir DG & Scott JM. Impaired functioning of thermolabile methylenetetrahydrofolate reductase is dependent on riboflavin status: implications for riboflavin requirements. Am *J Clin Nutr*. 2002; **76**: 436-441.

Mennen LI, de Courcy GP, Guilland JC, Ducros V, Bertrais S, Nicolas JP, Maurel M, Zarebska M, Favier A, Franchisseur C, Hercberg S & Galan P. Homocysteine, cardiovascular disease risk factors, and habitual diet in the French Supplementation with Antioxidant Vitamins and Minerals Study. *Am J Clin Nutr* 2002; **76**:1279-1289.

Michaud DS, Pietinen P, Taylor PR, Virtanen M, Virtamo J & Albanes D. Intakes of fruits and vegetables, carotenoids and vitamins A, E, C in relation to the risk of bladder cancer in the ATBC cohort study. *Br J Cancer*. 2002; **87**:960-5.

Miller JW, Garrod MG, Rockwood AL, Kushnir MM, Allen LH, Haan MN, Green R. Measurement of total vitamin B12 and holotranscobalamin, singly and in combination, in screening for metabolic vitamin B12 deficiency. *Clin Chem*. 2006; **52**:278-85.

Mills JL, Von Kohorn I, Conley MR, Zeller JA, Cox C, Williamson RE & Dufour DR. Low vitamin B-12 concentrations in patients without anaemia: the effect of folic acid fortification of grain. *Am J Clin Nutr*. 2003; **77**:1474-1477.

Mills JL & Signore C. Neural tube defect rates before and after food fortification with folic acid. *Birth Defects Res A Clin Mol Teratol*. 2004; **70**:844-5.

Moat SJ, Ashfield-Watt PA, Powers HJ, Newcombe RG & McDowell IF. Effect of riboflavin status on the homocysteine-lowering effect of folate in relation to the MTHFR (C677T) genotype. *Clin Chem*. 2003; **49**:295-302.

Mojtabai R. Body mass index and serum folate in childbearing age women. *Eur J Epidemiol*. 2004; **19**:1029-1036.

Mooijaart SP, Gussekloo J, Frölich M, Jolles J, Stott DJ, Westendorp RGJ, de Craen AJM. Homocysteine, vitamin B-12, and folic acid and the risk of

cognitive decline in old age: the Leiden 85-Plus Study. *Am J Clin Nutr*. 2005; **82**:866-71.

Morris JK & Wald NJ. Quantifying the decline in the birth prevalence of neural tube defects in England and Wales. *J Med Screen*. 1999; **6**:182-185.

Morris MS, Fava M, Jacques PF, Selhub J, Rosenberg IH. Depression and folate status in the US Population. *Psychother Psychosom*. 2003; **72**:80-7.

Morris MC, Evans DA, Bienias JL, Tangney CC, Hebert LE, Scherr PA & Schneider JA. Dietary folate and vitamin B12 intake and cognitive decline among community-dwelling older persons. *Arch Neurol*. 2005; **62**:641-645.

Morrison HI, Schaubel D, Desmeules M & Wigle DT. Serum folate and risk of fatal coronary heart disease. *JAMA*. 1996; **275**: 1893-1896.

MRC Vitamin Study Research Group. Prevention of neural tube defects: results of the Medical Research Council Vitamin Study. *Lancet*. 1991; **338**:131-137.

Munoz-Moran E, Dieguez-Lucena JL, Fernandez-Arcas N, Peran-Mesa S. & Reyes-Engel A. Genetic selection and folate intake during pregnancy. *Lancet*. 1998; **352**:1120-1121.

Murphy M, Seagroatt V, Hey K, O'Donnell M, Godden M, Jones N & Botting B. Neural tube defects 1974-94 – down but not out. *Arch Dis Child Foetal Neonatal Ed*. 1996, **75**:F133-4.

Murphy M, Whiteman D, Stone D, Botting B, Schorah C & Wild J. Dietary folate and the prevalence of neural tube defects in the British Isles: the past two decades. *Br J Obstetrics Gynaecol*. 2000; **107**:885-889.

National Cancer Institute. *Cancer Trends Progress Report – 2005 Update*. DHSS, Betheseda, MD. December 2005, http://progressreport.cancer.gov

Nurk E, Tell GS, Vollset SE, Nygard O, Refsum H, Nilsen RM & Ueland PM. Changes in lifestyle and plasma total homocysteine: the Hordaland Homocysteine Study. *Am J Clin Nutr*. 2004; **79**:812-819.

Office for National Statistics (2004). The Health of Children and Young People: Congenital anomalies. www.statistics.gov.uk/children/

O'Grady HL, Leahy A, McCormick PH, Fitzgerald P, Kelly CK & Bouchier-Hayes DJ. Oral folic acid improves endothelial dysfunction in cigarette smokers. *J Surg Res.* 2002; *106*:342-345.

Olthof MR, van Vliet T, Boelsma E, Verhoef P. Low dose betaine supplementation leads to immediate and long term lowering of plasma homocysteine in healthy men and women. *J Nutr.* 2003; 133, **12**:4135-8.

Ou CY, Stevenson RE, Brown VK, Schwartz CE, Allen WP, Khoury MJ, Rozen R, Oakley GP Jr, Adams MJ Jr. 5,10 Methylenetetrahydrofolate reductase genetic polymorphism as a risk factor for neural tube defects. *Am J Med Genet.* 1996; **63**:610-4.

Persad VL, Van den Hof MC, Dube JM & Zimmer P. Incidence of open neural tube defects in Nova Scotia after folic acid fortification. *CMAJ.* 2002; **167**:241-245.

Passeri M, Cucinotta D, Abate G, Senin U, Ventura A, Stramba Badiale M, Diana R, La Greca P, Le Grazie C. Oral 5'-methyltetrahydrofolic acid in senile organic mental disorders with depression: results of a double-blind multicenter study. *Aging (Milano).* 1993; **5**:63-71.

Penninx BW, Guralnik JM, Ferrucci L, Fried LP, Allen RH, Stabler SP. Vitamin B(12) deficiency and depression in physically disabled older women: epidemiologic evidence from the Women's Health and Aging Study. *Am J Psychiatry.* 2000; **157**:715-21.

Pfeiffer CM, Fazili Z, McCoy L, Zhang M, Gunter EW. Determination of folate vitamers in human serum by stable-isotope-dilution tandem mass spectrometry and comparison with radioassay and microbiologic assay. *Clin Chem.* 2004; **50**:423-32.

Pfeiffer CM, Caudill SP, Gunter EW, Osterloh J, Sampson EJ. Biochemical indicators of B vitamin status in the US population after folic acid fortification: results from the National Health and Nutrition Examination Survey 1999-2000. *Am J Clin Nutr.* 2005; **82**:442-50.

Popovic JR. 1999 National Hospital Discharge Survey: annual summary with detailed diagnosis and procedure data. *Vital Health Stat 13.* 2001; (151):i-v, 1-206.

Pullin CH, Ashfield-Watt PA, Burr ML, Clark ZE, Lewis MJ, Moat SJ, Newcombe RG, Powers HJ, Whiting JM & McDowell IF. Optimization of dietary folate or low-dose folic acid supplements lower homocysteine but do not enhance endothelial function in healthy adults, irrespective of the methylenetetrahydrofolate reductase (C677T) genotype. *J Am Coll Cardiol.* 2001; **38**:1799-1805.

Puwastein P, Pinprapai N, Judprasong K and Tamura T. International interlaboratory analyses of food folate. *J Food Comp Analysis.* 2005 **18**: 387-397.

Quadri P, Fragiacomo C, Pezzati R, Zanda E, Forloni G, Tettamanti M & Lucca U. Homocysteine, folate, and vitamin B-12 in mild cognitive impairment, Alzheimer disease, and vascular dementia. *Am J Clin Nutr.* 2004; **80**:114-122.

Quinlivan EP, McPartlin J, McNulty H, Ward M, Strain JJ, Weir DG & Scott JM. Importance of both folic acid and vitamin B12 in reduction of risk of vascular disease. *Lancet.* 2002, **359**: 227-228.

Quinlivan EP & Gregory JF III. Effect of food fortification on folic acid intake in the United States. *Am J Clin Nutr.* 2003; **77**:221-225.

Rader JI, Weaver CM & Angyal G. Total folate in enriched cereal-grain products in the United States following fortification. *Food Chem.* 2000; **70**:275-289.

Ravaglia G, Forti P, Maioli F, Martelli M, Servadei L, Brunetti N, Porcellini E & Licastro F. Homocysteine and folate as risk factors for dementia and Alzheimer disease. *Am J Clin Nutr.* 2005; **82**:636-643.

Ray JG, Meier C, Vermeulen MJ, Boss S, Wyatt PR & Cole DE. Association of neural tube defects and folic acid food fortification in Canada. *Lancet.* 2002; **360**:2047-2048.

Ray JG, Vermeulen MJ, Langman LJ, Boss SC & Cole DE. Persistence of vitamin B12 insufficiency among elderly women after folic acid food fortification. *Clin Biochem.* 2003; **36**:387-391.

Ray JG, Wyatt PR, Vermeulen MJ, Meier C & Cole DE. Greater maternal weight and the ongoing risk of neural tube defects after folic acid flour fortification. *Obstet Gynecol.* 2005a; **105**:261-265.

Ray JG, Langman LJ, Mamdani MM, Cole DE. Absence of effect of folic acid flour fortification on anticonvulsant drug levels. *Am J Med.* 2005b; **118**:444-445.

Reddy BS, Wang CX, Aliaga C, Rao CV, Lubert RA, Steele VE, & Kelloff GJ. Potential chemopreventative activity of perillyl alcohol and enhancement of experimental colon carcinogenesis by folic acid and genestein. *Proc Am Assoc Cancer Res.* 1996; **37**:A1849.

Relton CL, Wilding CS, Pearce MS, Laffling AJ, Jonas PA, Lynch SA, Tawn EJ, & Burn J. Gene-gene interaction in folate-related genes and risk of neural tube defects in a UK population. *J Med Genet.* 2004; **41**:256-260.

Reyes-Engel A, Munoz E, Gaitan MJ, Fabre E, Gallo M, Dieguez JL, Ruiz M, & Morell M. Implications on human fertility of the 677C-->T and 1298A-->C polymorphisms of the MTHFR gene: consequences of a possible genetic selection. *Mol Hum Reprod.* 2002; **8**:952-957.

Reynolds EH. Benefits and risks of folic acid to the nervous system. *J Neurol Neurosurg Psychiatry.* 2002; **72**:567-571.

Riggs KM, Spiro A, Tucker K & Rush D. Relations of vitamin B-12, vitamin B-6, folate, and homocysteine to cognitive performance in the Normative Aging Study. *Am J Clin Nutr.* 1996; **63**:306–314.

Rimm EB, Willett WC, Hu FB, Sampson L, Colditz GA, Manson JE, Hennekens C & Stampfer MJ Folate and vitamin B6 from diet and supplements in relation to risk of coronary heart disease among women. *JAMA.* 1998; **279**: 359-364.

Robien K. Folate during antifolate chemotherapy: what we know... and do not know. *Nutr Clin Pract.* 2005; **20**:411-22.

Ruston D, Henderson L, Gregory J, Bates CJ, Prentice A, Birch M, Swan G & Farron M. The National Diet and Nutrition Survey: adults aged 19 to 64 years. Volume 4: Nutritional Status (anthropometry and blood analytes), blood pressure and physical activity, London: TSO, 2004.

Rogers AE, Nauss KM. Rodent models for carcinoma of the colon. *Dig Dis Sci.* 1985; **30**:87S-102S.

Sanjoaquin MA, Allen N, Couto E, Roddam AW & Key TJ Folate intake and colorectal cancer risk: A meta-analytical approach. *Int J Cancer.* 2005; **113**:825–828.

Sato Y, Honda Y, Iwamoto J, Kanoko T & Satoh K. Effect of folate and mecobalamin on hip fractures in patients with stroke: a randomized controlled trial. *JAMA.* 2005; **293**:1082-1088.

Sauberlich HE, Skala JH, Dowdy RP. *Laboratory tests for the assessment of nutritional status.* CRC Press. Cleveland, Ohio: 1974.

Savage DG & Lindenbaum J. Neurological complications of acquired cobalamin deficiency: clinical aspects. In: *Bailliere's clinical haematology*. London: Bailliere Tindall, 1995; **8**:657-678.

Schnyder G, Roffi M, Pin R, Flammer Y, Lange H, Eberli FR, Meier B, Turi ZG & Hess OM. Decreased rate of coronary restenosis after lowering of plasma homocysteine levels. *N Engl J Med.* 2001; **345**:1593-1600.

Schnyder G, Roffi M, Flammer Y, Pin R, & Hess OM. Effect of homocysteine-lowering therapy with folic acid, vitami B12 and vitamin B6 on clinical outcome after percutaneous coronary intervention. *JAMA.* 2002; **288**:973-979.

Schroder H, Clausen N, Ostergard E, Pressler T. Folic acid supplements in vitamin tablets: a determinant of hematological drug tolerance in maintenance therapy of childhood acute lymphoblastic leukemia. *Pediatr HemtolOncol.* 1986; **3**:241-7.

Scientific Advisory Committee on Nutrition. A framework for the Evaluation of Evidence. 2002 http://www.sacn.gov.uk/pdfs/sacn_02_02a.pdf.

Sellers TA, Kushi LH, Cerhan JR, Vierkant RA, Gapstur SM, Vachon CM & Olson JE, Therneau TM, Folsom AR. Dietary folate intake, alcohol, and risk of breast cancer in a prospective study of postmenopausal women. *Epidemiology*. 2001; **12**:420-428.

Sellers TA, Grabrick DM, Vierkant RA, Harnack L, Olson JE, Vachon CM & Cerhan JR. Does folate intake decrease risk of postmenopausal breast cancer among women with a family history? *Cancer Causes Control*. 2004; **15**:113-120.

Selhub J, Miller JW. The pathogenesis of homocysteinemia: interruption of the coordinate regulation by S-adenosylmethionine of the remethylation and transsulfuration of homocysteine. *Am J Clin Nutr*. 1992; **55**:131-8.

Selhub J, Jacques PF, Wilson PW, Rush D & Rosenberg IH. Vitamin status and intake as primary determinants of homocysteinemia in an elderly population. *JAMA*. 1993; **270**:2693-2698.

Selhub J, Bagley LC, Miller J & Rosenberg IH. B vitamins, homocysteine, and neurocognitive function in the elderly. *Am J Clin Nutr*. 2000; **71**(suppl): 614S–620S

Seshadri S, Beiser A, Selhub J, Jacques PF, Rosenberg IH, D'Agostino RB, Wilson PW & Wolf PA. Plasma homocysteine as a risk factor for dementia and Alzheimer's disease. *N Engl J Med*. 2002; **346**:476-483.

Shane B, Tamura T, Stokstad EL. Folate assay: a comparison of radioassay and microbiological methods. Clin Chim Acta. 1980; 100(1):13-9.

Sharp L & Little J. Polymorphisms in Genes Involved in Folate Metabolism and Colorectal Neoplasia: A HuGE Review. *Am J Epidemiol*. 2004; **159**:423-443.

Shaw GM, Velie EM & Schaffer D. Risk of neural tube defect-affected pregnancies among obese women. *JAMA*. 1996; **275**:1093-1096.

Shaw GM, Carmichael SL, Nelson V, Selvin S & Schaffer DM. Food fortification with folic acid and twinning among California infants. *Am J Med Genet.* 2003; **119A**:137-140.

Shrubsole MJ, Gao YT, Cai Q, Shu XO, Dai Q, Hebert JR, Jin F & Zheng W. MTHFR polymorphisms, dietary folate intake, and breast cancer risk: results from the Shanghai Breast Cancer Study. *Cancer Epidemiol Biomarkers Prev.* 2004; **13**:190-6.

Signore C, Mills JL, Cox C, Trumble AC. Effects of folic acid fortification on twin gestation rates. *Obstet Gynecol.* 2005; **105**:757-62.

Skinner HG, Michaud DS, Giovannucci EL, Rimm EB, Stampfer MJ, Willett WC, Colditz GA & Fuchs CS. A prospective study of folate intake and the risk of pancreatic cancer in men and women. *Am J Epidemiol.* 2004; **160**:248-258.

Solomon LR. Cobalamin-responsive disorders in the ambulatory care setting: unreliability of cobalamin, methylmalonic acid, and homocysteine testing. *Blood.* 2005; **105**:978-85.

Song J, Medline A, Mason JB, Gallinger S, Kim YI. Effects of dietary folate on intestinal tumorigenesis in the apcMin mouse. *Cancer Res.* 2000; **60**:5434-40.

Spence JD, Bang H, Chambless LE, Stampfer MJ. Vitamin Intervention For Stroke Prevention trial: an efficacy analysis. *Stroke.* 2005; **36**:2404-9

Steenge GR, Verhoef P & Katan MB. Betaine supplementation lowers plasma homocysteine in healthy men and women. *J Nutr.* 2003; **133**:1291-1295.

Stern LL, Mason JB, Selhub J, & Choi SW. Genomic DNA hypomethylation, a characteristic of most cancers, is present in peripheral leukocytes of individuals who are homozygous for the C677T polymorphism in the methylenetetrahydrofolate reductase gene. *Cancer Epidemiol. Biomarkers Prev.* 2000; **9**:849-853.

Stolzenberg-Solomon RZ, Pietinen P, Barrett MJ, Taylor PR, Virtamo J & Albanes D. Dietary and other methyl-group availability factors and pancreatic cancer risk in a cohort of male smokers. *Am J Epidemiol.* 2001; **153**:680-687.

Strandhagen E, Landaas S, Thelle DS. Folic acid supplement decreases the homocysteine increasing effect of filtered coffee. A randomised placebo-controlled study. *Eur J Clin Nutr*. 2003; **57**(11):1411-7.

Su LJ & Arab L. Nutritional status of folate and colon cancer risk: evidence from NHANES I epidemiologic follow-up study. *Ann Epidemiol*. 2001; **11**:65-72.

Sweeney MR, McPartlin J, Weir DG, Daly L, Scott JM. Postprandial serum folic acid response to multiple doses of folic acid in fortified bread. *Br J Nutr*. 2006; **95**:145-51.

Taylor MJ, Carney S, Geddes J, Goodwin G. Folate for depressive disorders (Cochrane Review). In: *The Cochrane Library*, Issue 3. 2006. Chichester, UK: John Wiley & Sons, Ltd.

Teunissen CE, Blom AH, Van Boxtel MP, Bosma H, de Bruijn C, Jolles J, Wauters BA, Steinbusch HW & de Vente J. Homocysteine: a marker for cognitive performance? A longitudinal follow-up study. *J Nutr Health Aging*. 2003; **7**:153-159.

Terry P, Jain M, Miller AB, Howe GR & Rohan TE. Dietary intake of folic acid and colorectal cancer risk in a cohort of women. *Int J Cancer*. 2002; **97**:864-867.

Thurnham DI, Migasena P, Vudhivai N & Supawan V. Riboflavin supplementation in a resettlement village in north-east Thailand. *Br J Nutr*. 1972, **28**:91-97.

Thurnham DI & Rathakette P. Incubation of NAD(P)H2:glutathione oxidoreductase (EC 1.6.4.2) with flavin adenine dinucleotide for maximal stimulation in the measurement of riboflavin status. *Br J Nutr*. 1982, **48**:459-466.

Till U, Rohl P, Jentsch A, Till H, Muller A, Bellstedt K, Plonne D, Fink HS, Vollandt R, Sliwka U, Herrmann FH, Petermann H & Riezler R. Decrease of carotid intima-media thickness in patients at risk to cerebral ischemia after supplementation with folic acid, Vitamins B6 and B12. *Atherosclerosis* 2005, **181**:131-135.

Title LM, Cummings PM, Giddens K, Genest JJ, Jr & Nassar BA. Effect of folic acid and antioxidant vitamins on endothelial dysfunction in patients with coronary artery disease. *J Am Coll Cardiol*. 2000, **36**, 758-765.

Tolmunen T, Hintikka J, Ruusunen A, Voutilainen S, Tanskanen A, Valkonen VP, Viinamaki H, Kaplan GA & Salonen JT. Dietary folate and the risk of depression in Finnish middle-aged men. A prospective follow-up study. *Psychother Psychosom*. 2004, **73**:334-339.

Tomeo CA, Colditz GA, Willett WC, Giovannucci E, Platz E, Rockhill B, Dart H, Hunter DJ. Harvard Report on Cancer Prevention. Volume 3: prevention of colon cancer in the United States. *Cancer Causes Control*. 1999; **10**(3):167-80.

Toole JF, Malinow MR, Chambless LE, Spence JD, Pettigrew LC, Howard VJ, Sides EG, Wang CH & Stampfer M. Lowering homocysteine in patients with ischemic stroke to prevent recurrent stroke, myocardial infarction, and death: the Vitamin Intervention for Stroke Prevention (VISP) randomized controlled trial. *JAMA*. 2004; **291**:565-575.

Troen AM, Mitchell B, Sorensen B, Wener MH, Johnston A, Wood B, Selhub J, McTiernan A, Yasui Y, Oral E, Potter JD, Ulrich CM. Unmetabolized folic acid in plasma is associated with reduced natural killer cell cytotoxicity among postmenopausal women. *J Nutr*. 2006; **136**:189-94.

Tucker KL, Qiao N, Scott T, Rosenberg I & Spiro A 3rd. High homocysteine and low B vitamins predict cognitive decline in aging men: the Veterans Affairs Normative Ageing Study. *Am J Clin Nutr*. 2005; **82**:627-635.

Ulrich CM, Potter JD. Folate supplementation: too much of a good thing? *Cancer Epidemiol Biomarkers Prev*. 2006; **15**:189-93.

van Dam RM, Huang Z, Giovannucci E, Rimm EB, Hunter DJ, Colditz GA, Stampfer MJ & Willett WC. Diet and basal cell carcinoma of the skin in a prospective cohort of men. *Am J Clin Nutr*. 2000, **71**:135-41.

van den Berg H, Finglas PM, Bates C. FLAIR intercomparisons on serum and red cell folate. *Int J Vitam Nutr Res.* 1994; **64**(4):288-93.

van Dijk RA, Rauwerda JA, Steyn M, Twisk JW & Stehouwer CD. Long-term homocysteine-lowering treatment with folic acid plus pyridoxine is associated with decreased blood pressure but not with improved brachial artery endothelium-dependent vasodilation or carotid artery stiffness: a 2-year, randomized, placebo-controlled trial. *Arterioscler Thromb Vasc Biol.* 2001; **21**:2072-2079.

Van Guelpen B, Hultdin J, Johansson I, Hallmans G, Stenling R, Riboli E, Winkvist A, Palmqvist R. Low folate levels may protect against colorectal cancer. *Gut.* 2006; **55**(10):1461-6.

van Oort FV, Melse-Boonstra A, Brouwer IA, Clarke R, West CE, Katan MB & Verhoef P. Folic acid and reduction of plasma homocysteine concentrations in older adults: a dose-response study. *Am J Clin Nutr.* 2003; **77**:1318-1323.

van Meurs JB, Dhonukshe-Rutten RA, Pluijm SM, van der Klift M, de Jonge R, Lindemans J, de Groot LC, Hofman A, Witteman JC, van Leeuwen JP, Breteler MM, Lips P, Pols HA & Uitterlinden AG. Homocysteine levels and the risk of osteoporotic fracture. *N Engl J Med.* 2004; **350**:2033-2041.

van Meurs JB & Uitterlinden AG. Homocysteine and fracture prevention. *JAMA.* 2005; **293**:1121-1122.

Verhoef P, Pasman WJ, Van Vliet T, Urgert R & Katan MB. Contribution of caffeine to the homocysteine-raising effect of coffee: a randomized controlled trial in humans. *Am J Clin Nutr.* 2002; **76**:1244-1248.

Vermeulen EG, Stehouwer CD, Twisk JW, van den Berg M, de Jong SC, Mackaay AJ, van Campen CM, Visser FC, Jakobs CA, Bulterjis EJ & Rauwerda JA. Effect of homocysteine-lowering treatment with folic acid plus vitamin B6 on progression of subclinical atherosclerosis: a randomized, placebo-controlled trial. *Lancet.* 2000; **355**:517-522.

Victor M, Lear AA. Subacute combined degeneration of the spinal cord; current concepts of the disease process; value of serum vitamin B12; determinations in clarifying some of the common clinical problems. *Am J Med*. 1956; **20**(6):896-911.

Voorrips LE, Goldbohm RA, Brants HA, van Poppel GA, Sturmans F, Hermus RJ & van den Brandt PA. A prospective cohort study on antioxidant and folate intake and male lung cancer risk. *Cancer Epidemiol Biomarkers Prev*. 2000; **9**:357-65.

Voutilainen S, Rissanen TH, Virtanen J, Lakka TA & Salonen JT. Kuopio Ischemic Heart Disease Risk Factor Study Group. Low dietary folate intake is associated with an excess incidence of acute coronary events. *Circulation*. 2001; **103**:2674-2680.

Voutilainen S, Virtanen JK, Rissanen TH, Alfthan G, Laukkanen J, Nyyssonen K, Mursu J, Valkonen VP, Tuomainen TP, Kaplan GA & Salonen JT. Serum folate and homocysteine and the incidence of acute coronary events: the Kuopio Ischaemic Heart Disease Risk Factor Study. *Am J Clin Nutr*. 2004; **80**:317-323.

Wald NJ, Law MR, Morris JK, Wald DS. Quantifying the effect of folic acid. *Lancet*. 2001; **358**:2069-73.

Wald DS, Law M & Morris JK. Homocysteine and cardiovascular disease: evidence on causality from a meta-analysis. *BMJ*. 2002; **325**:1202-1206.

Wang HX, Wahlin A, Basun H, Fastbom J, Winblad B & Fratiglioni L. Vitamin B12 and folate in relation to the development of Alzheimer's disease. *Neurology*. 2001; **56**:1188-1194.

Waller DK, Tita AT & Annegers JF. Rates of twinning before and after fortification of foods in the US with folic acid, Texas, 1996 to 1998. *Paediatr Perinat Epidemiol*. 2003; **17**:378-383.

Ward M, McNulty H, McPartlin J, Strain JJ, Weir DG & Scott JM. Plasma homocysteine, a risk factor for cardiovascular disease is lowered by physiological doses of folic acid. *Q J Med*. 1997; **90**:519-524.

Ward M, Strain JJ, McPartlin J, Scott JM & McNulty H. Plasma homocysteine is a reliable funtional indicator of folate status. *Proc Nutr Soc*. 2002; **61**:93A.

Wargovich MJ, Chen CD, Jimenez A, Steele VE, Velasco M, Stephens LC, Price R, Gray K, Kelloff GJ.Aberrant crypts as a biomarker for colon cancer: evaluation of potential chemopreventive agents in the rat. *Cancer Epidemiol Biomarkers Prev*. 1996; **5**:355-60.

Waterland RA & Jirtle RL. Transposable elements: Targets for early nutritional effects on epigenetic gene regulation. *Mol Cell Biol*. 2003; **23**:5293-5300.

Watkins ML, Scanlon KS, Mulinare J & Khoury MJ. Is maternal obesity a risk factor for anencephaly and spina bifida? *Epidemiology*. 1996; **7**:507-512.

Wei EK, Giovannucci E, Wu K, Rosner B, Fuchs CS, Willett WC & Colditz GA. Comparison of risk factors for colon and rectal cancer. *Int J Cancer*. 2004; **108**:433-442.

Weinstein SJ, Hartman TJ, Stolzenberg-Solomon R, Pietinen P, Barrett MJ, Taylor PR, Virtamo J & Albanes D. Null association between prostate cancer and serum folate, vitamin B6, vitamin B12, and homocysteine. *Cancer Epidemiol Biomarkers Prev*. 2003; **12**:1271-1272.

Werler MM, Louik C, Shapiro S & Mitchell AA. Pre-pregnant weight in relation to risk of neural tube defects. *JAMA*. 1996; **275**:1089-1092.

Werler MM, Cragan JD, Wasserman CR, Shaw GM, Erickson JD & Mitchell AA. Multivitamin supplementation and multiple births. *Am J Med Genet*. 1997; **71**:93-96.

Wharton B, Booth I. Fortification of flour with folic acid. *BMJ*. 2001; **323**:1198-99.

Widlansky ME, Gokce N, Keaney JF Jr & Vita JA. The clinical implications of endothelial dysfunction. *J Am Coll Cardiol*. 2003; **42**:1149-1160.

Willems FF, Aengevaeren WR, Boers GH, Blom HJ & Verheugt FW. Coronary endothelial function in hyperhomocysteinemia: improvement after treatment with folic acid and cobalamin in patients with coronary artery disease. *J Am Coll Cardiol*. 2002; **40**:766-772.

Williams G. Disorders of glucose homeostasis. In: Warrrell DA, Cox TM, Firth JD (eds): *Oxford Textbook of Medicine*. Oxford: Oxford University Press 2003, 4th edition, pp 317-360.

Wolff GL., Kodell RL, Moore SR, & Cooney CA. Maternal epigenetics and methyl supplements affect agouti gene expression in Avy/a mice. *FASEB J*. 1998; **12**:949-957.

Woltman HW, The nervous systems in pernicious anaemia: an analysis of one hundred and fifty cases. *American Journal of Medical Sciences*. 1919; **157**:400-409.

Wu K, Helzlsouer KJ, Comstock GW, Hoffman SC, Nadeau MR & Selhub J. A prospective study on folate, B12, and pyridoxal 5'-phosphate (B6) and breast cancer. *Cancer Epidemiol Biomarkers Prev*. 1999; **8**:209-217.

Yang Q, Botto LD, Erickson JD, Berry RJ, Sambell C, Johansen H, Friedman JM. Improvement in stroke mortality in Canada and the United States, 1990 to 2002. *Circulation*. 2006; **113**:1335-43.

Yuan JM, Stram DO, Arakawa K, Lee HP & Yu MC. Dietary cryptoxanthin and reduced risk of lung cancer: the Singapore Chinese Health Study. *Cancer Epidemiol Biomarkers Prev*. 2003; **12**:890-898.

Zhang S, Hunter DJ, Hankinson SE, Giovannucci EL, Rosner BA, Colditz GA, Speizer FE & Willett WC. A prospective study of folate intake and the risk of breast cancer. *JAMA*. 1999; **281**:1632-1637.

Zhang SM, Hunter DJ, Rosner BA, Giovannucci EL, Colditz GA, Speizer FE & Willett WC. Intakes of fruits, vegetables, and related nutrients and the risk of non-Hodgkin's lymphoma among women. *Cancer Epidemiol Biomarkers Prev*. 2000; **9**:477-85.

Zhang SM, Willett WC, Selhub J, Hunter DJ, Giovannucci EL, Holmes MD, Colditz GA & Hankinson SE. Plasma folate, vitamin B6, vitamin B12, homocysteine, and risk of breast cancer. *J Natl Cancer Inst*. 2003; **95**:373-380.

Zhu H., Wicker NJ, Shaw GM, Lammer EJ, Hendricks K, Suarez L, Canfield M, & Finnell RH. (2003). Homocysteine remethylation enzyme polymorphisms and increased risks for neural tube defects. *Mol Genet Metab*. 2003; **78**:216-221.

Annex 1

SACN working procedures

Meetings

1. The SACN folate sub group was established in 2004 and met twice during this year. The group met once in 2005 and again in February 2006.

Comments received in response to the draft SACN report on Folate and Disease Prevention

2. The draft report was placed on the SACN website on 23 November 2005. Interested parties were invited to submit comments on the draft report by 18 January 2006.

3. Responses were received from the following organisations and individuals:

    1. Association for Spina Bifida & Hydrocephalus
    2. British Dietetic Association
    3. British Nutrition Foundation
    4. Co-operative Group
    5. Diabetes UK
    6. Folic Acid Action
    7. Food and Drink Federation
    8. Hennock Industries
    9. Institute of Food Research
    10. McDaddon, Andrew
    11. MRC Human Nutrition Research
    12. National Public Health Service for Wales
    13. NHS Wales
    14. Nichols, John
    15. Rollins School of Public Health of Emory University, USA
    16. Rowett Research Institute
    17. Royal Free Hampstead, NHS
    18. Society for Research into Hydrocephalus and Spina Bifida

19. St Patrick's Centre for Community Health
20. Sydney West Area Health Service, Australia
21. The Federation of Bakers
22. The Incorporated National Association of British and Irish Millers Limited
23. The Nutrition Society
24. The University of Sheffield
25. Thrower, Jan
26. University of Newcastle, Australia
27. University of Western Ontario, USA
28. University of Oxford
29. Vos, Eddie
30. Wilson, J.D
31. Wolfson Institute of Preventive Medicine
32. University of Ulster

4. Thirty two responses were received to the draft report. The responses can be viewed, in full, on the SACN website (www.sacn.gov.uk).

5. The majority of respondents welcomed the report as a comprehensive and significant review of information available on folic acid and disease prevention.

6. The Agency also consulted informally through meetings with various stakeholder groups, including the Association for Spina Bifida and Hydrocephalus, millers, bakers and related industries and consumer representatives.

Annex 2

Potential impact of mandatory folic acid fortification of flour on folate intakes

1. The aim of this analysis is to detail current folate (natural folate and folic acid) intake of the population using National Diet and Nutrition Survey (NDNS) data and to model the effect of fortification of flour with folic acid on:

   - Total folate intake, taking into account existing voluntarily fortified foods, supplement use, processing losses and overages.
   - The proportion of the total population with folate intakes below the Recommended Nutrient Intake (RNI)[1].
   - The risk of neural tube defect (NTD) affected pregnancies;
   - The total number of people who would be exposed to doses of folic acid above the upper level (UL)[2] of folic acid.
   - The number of people aged 65 years and over with low vitamin B12 status: who might be exposed to folic acid intakes above 1mg/day.

Dietary methodology

Current intakes

2. The most recent consumption data available for each age group of the British population (aged 4 years and above) was obtained from the NDNS series (Table 18, Appendix 1). It was assumed that children aged 0-3 years do not consume flour and would therefore not be affected by fortification.

3. The amount of flour consumed per day, and the percentage food energy obtained from flour was calculated for each population group, based on estimates of the percentage of flour in NDNS food groups described in Appendix 2 (Table 19). The percentage of food energy derived from bread was also calculated.

1 RNI for folate is 200µg/day for adults and children above 11 years of age; 100µg/day and 150µg/day for children aged 4-6 and 7-10 years respectively (Department of Health, 1991).

2 UL for folic acid is 1mg/day for adults aged 18 years and above; 300µg/day, 400µg/day, 600µg/day and 800µg for children aged 4-6 years, 7-10 years, 11-14 years and 15-17 years respectively (European Scientific Committee on Food, 2000).

4. Data on the folate content of food, including natural folate and foods voluntarily fortified with folic acid, were taken from the nutrient databank contemporaneous with each NDNS.

5. Currently many breakfast cereals and a few brands of fat spreads are voluntarily fortified with folic acid. Along with supplements, these fortified foods make a considerable contribution to the total folate intake of some individuals (see results section Table 13).

6. For the purpose of the modelling exercise, a number of assumptions were made in relation to food products contributing to current folate intake and factors which would affect fortification of flour with folic acid. These assumptions are shown in Appendix 2 (Table 20).

7. Folic acid added to food decays during the manufacturing process and in certain environments over time. To compensate for these losses, food manufacturers add more than the declared amount to products during the fortification process, a practice known as overage. This is so the product fulfils the claim for content at the point of sale and throughout its shelf life. If consumed shortly after manufacture, levels of folic acid could be significantly greater than declared on the label.

8. The following overages for voluntarily fortified products were assumed: 32% for fortified breakfast cereals, 20% for fortified fat spreads and 30% for supplements. Appendix 2 (Table 20) sets out the basis for these assumptions. For calculations where the contribution of folic acid from fortified breakfast cereals, fat spreads and supplements was included, the mean of values for overage included and overage excluded was used, to represent typical average levels of folic acid consumed over time.

9. Adjustments were made to account for known changes in voluntary fortification procedures since the NDNS series was carried out (most recent fieldwork in 2000-2001). The model takes the following considerations into account (Appendix 2, Table 20):

    - Voluntarily fortified breakfast cereals currently contain levels of folic acid that differ from the data held in the NDNS nutrient databanks. Label data on breakfast cereals were therefore taken

from a recently conducted analytical survey on breakfast cereals (FSA, 2002) (after checking that voluntary fortification levels were consistent with current levels in 2006).

- Voluntarily fortified fat spreads are estimated to account for one third of consumption of reduced and low fat spreads.
- Overages are used by manufacturers in voluntary fortification of breakfast cereals, fat spreads and supplements.
- The majority of bread is no longer fortified with folic acid, so natural folate levels for a group of fortified breads in the NDNS databank were adjusted downwards to represent current natural folate levels in bread.

10. These data on food composition were used together with the NDNS consumption data to estimate average folate intakes for each age/sex group with a breakdown of constituents (natural folate and folic acid from fortified breakfast cereals, fat spreads and supplements) contributing to total folate intake.

Potential intake post-fortification

11. The potential impact of mandatory flour fortification, was estimated by identifying the NDNS food groups contributing to flour consumption and estimating the amount of flour in each food group based on calculations used in the COMA report (Department of Health, 2000). As these values overestimated the percentage of flour in bread, they were adjusted to represent more recent recipes and are shown in appendix 2 (Table 19).

12. A processing loss of 25% folic acid in all flour containing food groups was assumed (Appendix 2, Table 20).

13. Total folate intake was assessed for four different levels of fortification: 100µg/100g flour, 200µg/100g flour, 300µg/100g flour, 450µg/100g flour. After processing losses this would be equivalent to 75µg/100g, 150µg/100g, 225µg/100g and 338µg/100g of flour in food respectively for each level of fortification.

14. The analysis was carried out with the five dichotomous variables listed below, resulting in 32 different scenarios for each age group at each level of fortification:

    - Overages included/excluded.
    - Supplements included/excluded.
    - Fortified breakfast cereals included/excluded.
    - Fortified fat spreads consumed by one third of low and reduced fat spread consumers included/fortified fat spread consumption excluded.
    - Wholemeal flour included/excluded from fortification.

Effect on current intake & the number of people below RNI for folate

15. The effect of mandatory fortification on reducing the number of people with intakes below the RNI was estimated.

16. For women of childbearing age mean folate intakes were divided into quintiles of folate intake (5 equally sized parts in ascending order) for three population age groups: 14-18, 19-34, and 35-49 years and broken down into food sources (natural folate and folic acid from fortified breakfast cereals, fat spreads and supplements) contributing to total folate intake. The mean intakes were combined with the mean additional folic acid consumed by each quintile as a result of each level of flour fortification to illustrate the increase in total folate intake as a result of fortification. Results for females aged 14-18 years are illustrated graphically in Figure 4. Similar results were seen for females aged 19-34 and 35-49 years.

NTD-affected pregnancy risk

17. Percent NTD risk associated with each level of mandatory fortification was calculated for women of childbearing age (14-49 years). Two different approaches were used due to uncertainties in the available data.

18. The first approach is based on linear interpolation between observations of the change in NTD risk with changing red cell folate concentration and the effect of increasing folic acid intake on red cell folate concentration (Daly *et al*, 1995; Daly *et al*, 1997). It was estimated that increasing folic acid intake by 100µg/day, 200µg/day and 400µg/day would reduce

NTDs by 22%, 41% and 47% respectively. These estimates provided 3 points to use as a basis for interpolation.

19. The second approach to estimate NTD risk reduction is based on serum folate concentrations, using data from the same observational study by Daly *et al* (1995; 1997), but with further statistical analysis (Wald *et al*, 2001). Reduction in NTD risk was based on increasing serum folate concentration in women aged 20-35 years. Serum folate concentrations increased by 0.94ng/ml for every 0.1mg/day increase in folic acid intake. Every doubling of serum folate concentration was estimated to halve the risk of an NTD.

20. The estimates of NTD-affected pregnancies prevented as a result of mandatory fortification was based on the current estimate of 700-900 NTD-affected pregnancies a year in the UK (see main report). A range in NTD risk reduction was then calculated using both the Daly and Wald methods (Daly *et al*, 1995; 1997; & Wald *et al*, 2001) for each level of fortification. The Wald method provided a lower value and so was used with the lower estimate of 700 NTD-affected pregnancies a year to calculate the lower estimate of NTD risk reduction. The Daly method provided a higher value and was used with the upper estimate of 900 NTD-affected pregnancies a year to calculate the upper estimate of NTD risk reduction resulting in a range in NTD risk reduction.

21. The effect of mandatory fortification on NTD risk was estimated at: current levels of total folate intake from all sources (including voluntary fortification and supplements); excluding the contribution of folic acid from fortified breakfast cereals and fat spreads; and excluding the contribution of folic acid from fortified breakfast cereals, fat spreads and supplements.

22. Excluding the contribution of folic acid from fortified breakfast cereals and fat spreads only and excluding the contribution of folic acid from fortified breakfast cereals, fat spreads and supplements resulted in a net decrease in mean folic acid intake and consequently an increased NTD risk, both for current intake levels and at a fortification dose of 100µg/100g flour. The Wald equation, which estimates the changes in NTD risk (Wald *et al*, 2001, Daly *et al*, 1995; 1997), was used to extrapolate

for decreased intake. The method used by Daly *et al* (1995) could not be used as it only models the decrease in NTD risk for 3 discrete levels of increased folic acid exposure. At a fortification dose of 200µg/100g flour and above the additional folic acid from flour fortification exceeded the folic acid lost through the exclusion of fortified breakfast cereals and fat spreads only and the exclusion of fortified breakfast cereals, fat spreads and supplements.

Tolerable Upper Intake Levels

23. In the USA and Europe, the tolerable upper intake level (UL) for folic acid is 1mg/day for adults (see paragraphs 86-89 of main report). In the UK no Safe Upper Level has been set for folic acid due to insufficient data. A guidance level (GL) has been set at 1mg/day for supplemental folic acid for adults (Expert Group on Vitamins and Minerals, 2003) (see paragraph 90 of main report). ULs were used in the modelling exercise as no GLs have been set for children. The ULs set for children in the USA and Europe were extrapolated from the UL for adults on the basis of relative body weight. The modelling exercise used the ULs for children set by SCF (European Scientific Committee on Food, 2000) because the age bands used by SCF correspond most closely to those used in the NDNS series.

Adults aged 65 years and over

24. The number of adults aged 65 years and over with low vitamin B12 status exceeding 1mg/day folic acid was estimated. The prevalence of low vitamin B12 status was assumed to be 5% for adults aged 65-74 years and 10% for adults aged 75 years and over (Clarke *et al*, 2004).

25. The fieldwork for the NDNS survey for adults aged 65 years and over (Finch *et al*, 1998) was carried out over ten years ago. The summary report of the 2000/1 NDNS of adults aged 19 to 64 years (Hoare *et al*, 2004) shows an increase in supplement use from 17% of women and 9% of men in 1986/87 to 40% of women and 29% of men in 2000/1. Assuming that supplement intake has increased to a similar extent for adults aged 65 years and over means that supplement use will be considerably higher than it was in 1994/5 when the data for adults aged 65 years and over were collected. In order to obtain a more accurate reflection of current folate intakes, the number of adults aged 65 years and over with low

vitamin B12 status, exceeding folic acid intakes of 1mg/day was also estimated using intake data for adults aged 50-64 years, from the more recent survey of adults (NDNS 2002/3, field work carried out in 2000/1). Although mean folate intakes were greater using the data for adults aged 50-64 years, fewer individuals had folic acid intakes above 1mg/day compared with data for adults aged 65 years and over; these data are therefore not presented in the results of the modelling exercise.

Results[3]

Flour and bread consumption

Table 12: Flour and bread consumption by population groups

Age (yrs) and sex group	Flour consumed per day* (g)	% Food energy from flour	% Food energy from bread	% Flour consumed as bread
4-6 males and females (NDNS 2000) (n=355)	59	14	10	63
7-10 males and females (NDNS 2000) (n=482)	73	15	11	63
11-13 males and females (NDNS 2000) (n=360)	74	14	10	65
14-18 males (NDNS 2000) (n=235)	93	14	12	70
14-18 females (NDNS 2000) (n=267)	68	15	13	72
19-34 females(NDNS 2003) (n=289)	66	15	14	76
35-49 females (NDNS 2003) (n=379)	67	15	13	74
19-34 males(NDNS 2003) (n=221)	93	15	14	76
35-49 males (NDNS 2003) (n=303)	100	16	14	76
50-64 males and females (NDNS 2003) (n=532)	84	16	14	73
65-79 free living (FL) males and females (NDNS 1998) (n=828)	82	18	14	70
80+ free living (FL) males and females (NDNS 1998) (n=447)	75	18	14	67
65-79 institutional (Inst) males and females (NDNS 1998) (n=121)	87	16	12	64
80+ institutional (Inst) males and females (NDNS 1998) (n=291)	75	16	11	60
Mean (n=5110)	**80**	**16**	**13**	**72**

*From food groups containing flour illustrated in Appendix 2 (Table 19).

26. Table 12 shows flour and bread consumption across different population groups in the British population (NDNS 1998, 2000, 2003). COMA considered that flour would be an appropriate staple food for use in fortification, because of its near universal consumption and relatively narrow variability of consumption in the population (Department of Health, 2000). This is supported by the data in Table 12, which shows marginal variation in the amount of flour consumed per day and percentage food energy consumed as flour across the population groups.

3 Mean values and percentages are weighted to account for differences in sizes of population groups.

Current intakes of total folate

Table 13: Mean contribution of sources to current total folate intakes for different population groups. Each source as a percentage of mean total folate intakes illustrated in brackets.

Age (yrs) and population group	Natural folate (µg/day) (% of total folate)	Folic acid from fortified breakfast cereals (µg/day) (% of total folate)	Folic acid from fortified fat spreads (µg/day) (% of total folate)	Folic acid from supplements (µg/day) (% of total folate)	Folic acid from fortified breakfast cereals, fat spreads and supplements (µg/day) (% of total folate)	Total folate (µg/day)
4-6 children	133 (64%)	52 (25%)	20 (10%)	2 (1%)	74 (36%)	207
7-10 children	151 (63%)	59 (25%)	26 (11%)	2 (1%)	87 (37%)	238
11-13 children	166 (65%)	60 (24%)	26 (10%)	3 (1%)	89 (35%)	255
14-18 males	228 (67%)	77 (23%)	32 (9%)	4 (1%)	113 (33%)	341
14-18 females	178 (73%)	40 (16%)	22 (9%)	5 (2%)	67 (27%)	245
19-34 females	198 (73%)	32 (12%)	21 (8%)	19 (7%)	72 (27%)	270
35-49 females	218 (72%)	35 (12%)	21 (7%)	28 (9%)	84 (28%)	302
19-34 males	291 (73%)	40 (10%)	41 (10%)	24 (6%)	105 (27%)	396
35-49 males	296 (75%)	46 (12%)	40 (10%)	14 (4%)	100 (25%)	396
50-64 adults	265 (75%)	42 (12%)	28 (8%)	20 (6%)	90 (25%)	355
65-79 adults (FL)	206 (70%)	41 (14%)	37 (13%)	11 (4%)	89 (30%)	295
80+ adults (FL)	173 (66%)	36 (14%)	32 (12%)	21 (8%)	89 (34%)	262
65-79 adults (Inst)	192 (74%)	32 (12%)	36 (14%)	1 (0%)	69 (26%)	261
80+ adults (Inst)	172 (71%)	34 (14%)	32 (13%)	3 (1%)	69 (29%)	241
Mean	228 (72%)	43 (14%)	30 (9%)	16 (5%)	89 (28%)	317

27. Table 13 illustrates current folate intakes across different UK population groups and the contribution of different sources to total folate intake. It can be seen that:

- An average person consumes the majority of folate in their diet in the form of natural folate (72%). The remaining 28% is consumed as folic acid from fortified breakfast cereals (14%); fortified fat spreads (9%) and supplements (5%).
- Children aged 4-13 years and males aged 14-18 years obtain a higher proportion of their total folate from fortified breakfast cereals (25%) and a lower proportion from supplements (1%).
- The contribution of supplements to total folate intake is highest in women aged 35-49 years (9%).

- The contribution of fortified fat spreads to total folate intake is highest in adults aged 65 years and over (13%).
- Mean total folate intakes are above the RNI for each population group and mean total folic acid intakes are below the UL set for each age group.

Women of childbearing age

Current intakes of total folate

Table 14: Contribution of fortified breakfast cereals, fat spreads and supplements to total folate intake for women of childbearing age

Female age group (yrs)	Quintile	Total folate (µg/day)	Natural folate (µg/day) (% of total folate)	Folic acid from fortified breakfast cereals (µg/day) (% of total folate)	Folic acid from fortified spreads (µg/day) (% of total folate)	Folic acid from supplements (µg/day) (% of total folate)	Folic acid from fortified breakfast cereals, fat spreads and supplements (µg/day) (% of total folate)
14-18	1st	118	109 (92%)	10 (8%)	0	0	10 (8%)
	2nd	168	153 (91%)	15 (9%)	0	0	15 (9%)
	3rd	203	174 (86%)	29 (14%)	0	0	29 (14%)
	4th	282	215 (76%)	43 (15%)	20 (7%)	5 (2%)	68 (24%)
	5th	488	238 (49%)	123 (25%)	100 (20%)	27 (6%)	250 (51%)
19-34	1st	125	120 (96%)	5 (4%)	0	0	5 (4%)
	2nd	188	169 (89%)	19 (10%)	0	1 (1%)	20 (11%)
	3rd	242	208 (86%)	31 (13%)	0	3 (1%)	34 (14%)
	4th	319	242 (76%)	52 (16%)	21 (7%)	5 (2%)	78 (24%)
	5th	512	250 (49%)	80 (16%)	83 (16%)	98 (19%)	262 (51%)
35-49	1st	143	136 (95%)	6 (4%)	0	1 (1%)	7 (5%)
	2nd	209	191 (91%)	17 (8%)	0	1 (1%)	18 (9%)
	3rd	265	226 (85%)	38 (14%)	0	1 (1%)	39 (15%)
	4th	359	256 (71%)	70 (19%)	22 (6%)	11 (3%)	103 (29%)
	5th	591	279 (47%)	74 (13%)	95 (16%)	144 (24%)	313 (53%)
Weighted Mean of 14-49	1st	132	126 (95%)	6 (5%)	0	0	6 (5%)
	2nd	195	177 (91%)	18 (9%)	0	1 (1%)	19 (10%)
	3rd	247	212 (86%)	34 (14%)	0	2 (1%)	36 (15%)
	4th	332	245 (74%)	59 (18%)	21 (6%)	8 (2%)	88 (27%)
	5th	543	261 (48%)	83 (15%)	90 (17%)	109 (20%)	282 (52%)

28. Table 14 illustrates current folate intakes for women of childbearing age by quintiles. It can be seen that:

    - Women in the lowest quintiles have on average, total folate intakes below the RNI of 200µg/day (132µg/day folic acid for the lowest quintile and 195µg/day folic acid for the second lowest quintile).
    - Natural folate provides the majority of folate in the diet for the lowest quintiles (95% for the lowest quintile, 91% for the second lowest quintile).
    - Folic acid from supplements and fortified fat spreads make very little or no contribution to the total folate intake of the lower quintiles. The main source of folic acid for this group is fortified breakfast cereals (5% of total folate for the lowest quintile and 9% of total folate for the second lowest quintile).
    - In the highest quintile the majority of folate in the diet is consumed in the form of folic acid (52%).

Effect of flour fortification on total folate intakes

Table 15: Potential effect of fortification of flour with folic acid on folate intakes of women of childbearing age
(figures excluding wholemeal in brackets).

			Including folic acid from all soures[4]			Excluding folic acid from fortified breakfast cereals and fat spreads (including supplements)			Excluding folic acid from fortified breakfast cereals, fat spreads and supplements		
Age group (years)	Fortification level of folic acid (µg/100g of flour)	Average additional folic acid from flour (µg/day)	Mean total folate intakes (µg/day)	% below RNI of 200µg/day	Range of % reduction in NTD risk[5]	Mean total folate intakes (µg/day)	% below RNI of 200µg/day	Range of % reduction in NTD risk	Mean total folate intakes (µg/day)	% below RNI of 200µg/day	Range of % reduction in NTD risk
14-18	0	-	245	50	-	184	69	-11	178	71	-12
	100	51 (48)	296 (293)	24 (25)	7-11 (6-11)	235 (232)	35 (37)	-2	229 (226)	35 (37)	-3
	200	102 (96)	347 (341)	12 (14)	13-22 (12-21)	286 (280)	19 (20)	6-9 (5-7)	280 (274)	19 (20)	5-8 (4-6)
	300	153 (143)	398 (389)	8 (9)	18-32 (17-30)	337 (328)	10 (12)	12-20 (11-18)	331 (322)	10 (12)	11-19 (10-17)
	450	229 (215)	474 (460)	5 (7)	25-42 (24-41)	413 (399)	6 (8)	20-35 (19-32)	407 (393)	6 (8)	19-34 (18-31)
19-34	0	-	270	35	-	217	50	-9	198	53	-13
	100	49 (45)	319 (315)	20 (21)	6-11 (6-10)	267 (262)	27 (28)	0 (-1)	247 (243)	29 (30)	-3 (-4)
	200	98 (90)	369 (360)	12	13-22 (12-20)	316 (307)	14 (16)	7-11 (6-9)	296 (288)	15 (17)	4-7 (4-5)
	300	148 (135)	418 (405)	4 (6)	18-31 (16-29)	365 (352)	7 (9)	13-22 (12-20)	345 (333)	8 (10)	11-18 (10-16)
	450	221 (202)	492 (472)	3 (4)	25-42 (23-41)	439 (420)	4 (6)	21-36 (19-34)	419 (400)	5 (7)	19-33 (18-30)
35-49	0	-	302	28	-	246	41	-9	218	44	-15
	100	50 (44)	354 (347)	12 (15)	7-11 (6-10)	297 (290)	19 (22)	-1	268 (262)	20 (23)	-5 (-6)
	200	101 (88)	404 (391)	6 (9)	13-22 (11-19)	347 (334)	8 (11)	6-10 (6-9)	319 (306)	9 (12)	3-4 (2-3)
	300	151 (131)	455 (435)	4 (5)	18-32 (16-28)	398 (378)	4 (6)	13-21 (12-20)	369 (349)	4 (6)	9-15 (8-13)
	450	227 (198)	531 (501)	2 (3)	25-42 (23-41)	473 (444)	3 (4)	20-36 (19-34)	445 (415)	3 (4)	18-31 (16-28)
Mean 14-49	0	-	281	34	-	226	48	-10	204	51	-13
	100	50 (46)	331 (326)	17 (20)	7-11 (6-10)	276 (270)	25 (27)	-1	254 (249)	26 (28)	-4
	200	100 (91)	381 (364)	9 (11)	13-22 (12-20)	326 (315)	12 (14)	6-10 (6-8)	304 (294)	13 (15)	4-6 (3-5)
	300	150 (134)	432 (416)	4 (6)	18-32 (16-29)	376 (360)	6 (8)	13-21 (12-20)	354 (339)	7 (9)	10-17 (9-15)
	450	225 (202)	507 (483)	3 (4)	25-42 (23-41)	451 (428)	4 (5)	20-36 (19-33)	429 (406)	4 (6)	19-33 (17-23)

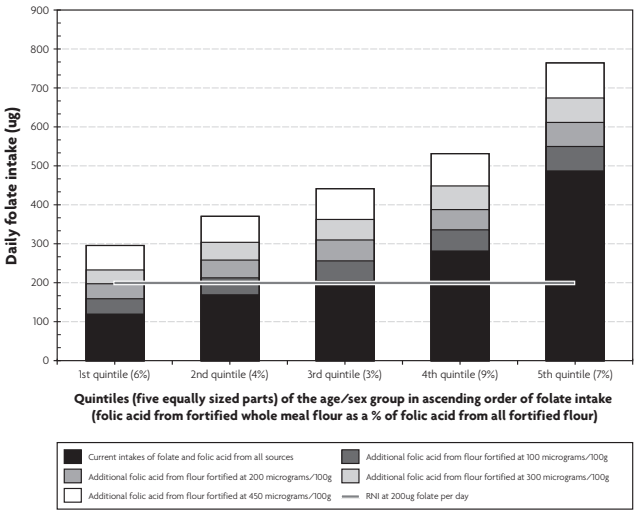
4 Figures including contribution of folic acid from fortified breakfast cereals, fat spreads and supplements are taken as the mean of values for overage included and overages excluded.

5 Percent reduction in NTD-affected pregnancy risk using both the Wald, lower figure (Wald et al, 2001, Daly et al, 1995, Daly et al 1997) and Daly, higher figure (Daly et al, 1995, Daly et al, 1997) approaches of analysis. The reduction is expected to lie between these figures.

29. Table 15 shows the effect of the four different levels of fortification on the total folate intakes of women of childbearing age. Comparing current folate intakes with intakes post-fortification shows that at each level of fortification the number of individuals with folate intakes below the RNI is progressively reduced, indicating that women with poor folate intakes would be reached through flour fortification.

30. Table 15 also shows that the risk of NTD-affected pregnancies is progressively reduced as the dose of folic acid in flour fortification is increased. Risk reduction for young women (14-18 years) was similar to risk reduction for women in the older age groups (19-34 and 35-49 years) for each dose, indicating that fortification of flour with folic acid would reduce risk of NTD-affected pregnancies consistently across age groups.

Figure 4: Total folate intakes for women aged 14-18 years at each level of fortification by quintile of current folate intake (including folic acid from voluntary fortification and supplements).



31. As an example, figure 4 illustrates total folate intakes for women aged 14-18 years (similar results were seen for women aged 19-49 years) and shows that folic acid fortification of flour increased the total daily folate intakes of women in all quintiles including the two lowest quintiles. This indicates that women at greatest risk of dietary related NTD-affected pregnancies would be reached through mandatory flour fortification.

Adults aged 65 years and over

Table 16: Potential effect of fortification of flour on folate intakes of free-living & institutional adults and above 65 yrs of age (figures excluding wholemeal in brackets).

			Includes folate and folic acid from all sources[6]				Excluding folic acid from fortified breakfast cereals, fat spreads, (including supplements)				Excluding folic acid from fortified breakfast cereals, fat spreads, and supplements			
Age (years)/ status	Level of folic acid fortification (µg/100g of flour)	Average increase in folic acid intake (µg/day)	Mean total folate intakes (µg/day)	Estimated % below RNI 200µg/ day	Estimated number exceeding 1mg/day folic acid[7]*	Estimated number with low vitamin B_{12} status exceeding 1mg/day folic acid**	Mean total folate intakes (µg/day)	% below RNI 200µg/ day	Estimated number exceeding 1mg/day folic acid*	Estimated number with low vitamin B12 status exceeding 1mg/day folic acid**	Mean total folate intakes (µg/day)	% below RNI 200µg/ day	Estimated number exceeding 1mg/day folic acid	Estimated number with low vitamin B_{12} status exceeding 1mg/day folic acid
65-79 adults (FL)	0	-	295	32	3,400	170	217	49	0	0	206	51	0	0
	100	62 (50)	357 (346)	14 (16)	11,200	560	279 (268)	20 (24)	0	0	267 (256)	21 (25)	0	0
	200	123 (101)	419 (396)	5 (7)	23,800 (16,300)	1,190 (820)	341 (318)	7 (11)	0	0	329 (306)	8 (11)	0	0
	300	185 (151)	481 (447)	2 (4)	32,900 (26,800)	1,650 (1,340)	403 (369)	3 (6)	0	0	391 (357)	3 (7)	0	0
	450	278 (227)	573 (523)	1 (2)	98,200 (76,100)	4,910 (3,800)	495 (444)	1 (3)	3,100 (0)	0	483 (432)	1 (3)	0	0
80+ adults (FL)	0	-	262	53	7,700	770	194	71	7,700	770	173	74	0	0
	100	57 (47)	319 (309)	23 (27)	11,600	1,160	250 (240)	36 (41)	7,700	770	230 (219)	37 (42)	0	0
	200	113 (93)	375 (355)	12 (16)	16,100 (11,600)	1,610 (1,160)	307 (287)	19 (24)	9,200	920	286 (266)	19 (25)	0	0
	300	170 (140)	432 (402)	5 (9)	16,100 (11,600)	1,610 (1,160)	363 (333)	8 (13)	9,200	920	343 (313)	9 (13)	0	0
	450	254 (209)	517 (472)	2 (6)	41,100 (25,300)	4,110 (2,520)	448 (403)	3 (8)	13,700 (9,200)	1,360 (920)	427 (382)	4 (9)	0	0
65-79 adults (Inst)	0	-	261	46	0	0	193	60	0	0	192	60	0	0
	100	65 (56)	326 (317)	14 (18)	0	0	258 (249)	24 (30)	0	0	258 (249)	24 (30)	0	0
	200	130 (113)	391 (374)	7	0	0	323 (305)	9 (11)	0	0	323 (305)	9 (11)	0	0
	300	195 (169)	456 (430)	1 (2)	0	0	388 (361)	5 (7)	0	0	388 (361)	5 (7)	0	0
	450	293 (253)	554 (514)	1 (2)	700 (0)	0	486 (446)	1 (2)	0	0	486 (446)	1 (3)	0	0
80+ adults (Inst)	0	-	241	47	0	0	176	71	0	0	172	72	0	0
	100	60 (52)	301 (293)	21 (24)	0	0	236 (228)	30 (37)	0	0	233 (225)	32 (39)	0	0
	200	121 (105)	361 (345)	8 (10)	0	0	296 (280)	14 (19)	0	0	293 (277)	15 (20)	0	0
	300	181 (157)	421 (398)	4 (5)	500 (0)	0	356 (333)	6 (10)	0	0	353 (330)	6 (10)	0	0
	450	271 (236)	512 (476)	1 (2)	1,400 (0)	0	447 (411)	1 (2)	0	0	443 (408)	1 (3)	0	0
65+ adults (FL +Inst)	0	-	285	37	11,100	940	210	55	7,700	770	197	57	0	0
	100	61 (49)	346 (335)	16 (19)	22,800	1,720	271 (260)	24 (28)	7,700	770	257 (246)	25 (29)	0	0
	200	121 (99)	407 (385)	7 (9)	39,900 (27,900)	2,800 (1,980)	332 (310)	10 (14)	9,200	920	318 (296)	11 (14)	0	0
	300	182 (149)	468 (435)	3 (5)	49,500 (38,400)	3,260 (2,500)	392 (360)	4 (8)	9,200	920	379 (346)	4 (8)	0	0
	450	272 (223)	558 (510)	1 (3)	141,400 (101,400)	9,020 (6,320)	483 (434)	1 (4)	16,800 (9,200)	1,360 (920)	469 (420)	2 (4)	0	0

6 Figures including contribution of folic acid from fortified breakfast cereals, fat spreads and supplements are taken as the mean of values for overage included and excluded.

7 Population estimates were taken from 2001 census (National Statistics Census 2001), applying the ratio of free-living individuals to institutional individuals for England and Wales applied to numbers in UK population.

* Figures rounded to the nearest 100 **Figures to the nearest 10

32. Table 16 illustrates intake data across the four different levels of fortification for free-living and institutional adults over 65 years of age. Comparing current folate intakes with intakes post-fortification, shows that each level of fortification progressively reduces the number of adults with folate intakes below the RNI and increases the number exceeding the UL for folic acid (1mg/day).

33. Table 16 suggests that no institutional adults aged 65 years and over currently exceed intakes of 1mg/day folic acid compared to 11,100 free-living adults aged 65 years and over. This difference could be explained by reduced food consumption of individuals living in institutions, because of illness or restrictions on intake. It can be seen from Table 13 that mean folate intake is marginally greater for free-living adults aged 65 years and over than those living in institutions. There is no obvious difference in fortified breakfast cereal or fat spread consumption between free-living groups and institutional groups. However, supplements make a lower contribution to folate intakes for institutional adults aged 65 years and over (weighted mean 1%) compared to free-living adults aged 65 years and over (weighted mean 5%). This could explain why there are fewer individuals with extreme high folic acid intakes in the institutional groups than the free-living groups.

34. The total number of adults aged 65 years and over with low vitamin B12 status currently exceeding intakes of 1mg/day folic acid is estimated to be about 900. This figure rises with increasing doses of flour fortification.

UK population

Table 17: Effect of flour fortification on folate intakes of UK population (figures excluding wholemeal flour shown in brackets)

Age (yrs) and sex group	Increase in folic acid from each fortification dose of 100µg/100g flour (excluding wholemeal) (md/day)[8]	% currently below RNI[9]	% below RNI at fortification level 100µg/100g flour	% below RNI at fortification level 200µg/100g flour	% below RNI at fortification level 300µg/100g flour	% below RNI at fortification level 450µg/100g flour	% with current intakes above UL[10]	% above UL at fortification level 100µg/100g flour	% above UL at fortification level 200µg/100g flour	% above UL at fortification level 300µg/100g flour	% above UL at fortification level 450µg/100g flour
4-6 children	44 (42)	4	2	1	0	0	0.8	3.0 (2.8)	6.9 (6.3)	14.2 (12.1)	31.6 (29.7)
7-10 children	54 (51)	15	4	2	1	1	1.8	2.7	5.0 (4.7)	10.4 (9.1)	23.3 (20.2)
11-13 children	56 (53)	39	18	8 (9)	5	3 (4)	0.0	0.2	0.6	1.5 (1.4)	7.5 (6.5)
14-18 males	70 (67)	20	8 (9)	2	2	1	0	0	0.2	0.9	5.3 (4.9)
14-18 females	51 (48)	50	24 (25)	12 (14)	8 (9)	5 (7)	0.0	0.1	0.1	0.2	1.1
19-34 females	49 (45)	35	20 (21)	12	4 (6)	3 (4)	0.0	0.0	0.2	0.2	1.1
35-49 females	50 (44)	28	12 (15)	6 (9)	4 (5)	3	0.0	0.0	0.0	0.0	0.3
19-34 males	70 (63)	14	5	3	3	0	0.6	0.8 (0.6)	0.8	1.1	3.3 (2.4)
35-49 males	75 (66)	9	5	2 (3)	1	1	0.2	0.2	0.6 (0.2)	1.0 (0.6)	3.7 (3.1)
50-64 adults	63 (55)	17	6 (7)	4	2	1 (2)	0.0	0.0	0.0	0.3 (0.2)	1.7 (1.3)
65-79 adults (Inst)	65 (56)	46	14 (18)	7	1 (2)	1 (2)	0.0	0.0	0.0	0.0	0.6
80+ adults (Inst)	60 (52)	47	21 (24)	8 (10)	4 (5)	1 (2)	0.0	0.0	0.0	0.2 (0)	0.5 (0)
65-79 adults (FL)	62 (50)	32	14 (15)	5 (7)	2 (4)	1 (2)	0.1	0.2	0.4 (0.2)	0.5 (0.4)	1.4 (1.1)
80+ adults (FL)	57 (47)	53	23 (27)	12 (16)	5 (9)	2 (6)	0.4	0.5	0.8 (0.5)	0.8 (0.5)	1.9 (1.2)
Population Mean[11]	57 (51)	23%	10 (11) %	5 (6) %	3%	1 (2) %	0.2%	0.4%	0.8 (0.7)%	1.5 (1.3) %	4.3 (3.7) %

8 Folic acid intake increases by this amount for every 100µg/100g increase in flour fortification.

9 Percentage of individuals with total folate intakes below the RNI set for each age group (Department of Health, 1991).

10 Percentage of individuals with total folic acid intakes above the tolerable upper intake level (UL) for folic acid set for each age group (European Scientific Committee on Foods, 2000).

11 Includes infants aged 0-3 years old in the calculation of population mean, assuming they do not consume flour see paragraph 2.

35. Table 17 shows the effect of fortification on the whole UK population.

- For each additional 100µg of folic acid per 100g flour (including wholemeal) the mean percentage of individuals with intakes below the RNI decreases by about 50%.

- 23% of the population currently have intakes below the RNI. Age groups with the highest percentage of individuals with intakes below the RNI are children aged 11-13 years, women of childbearing age (14-49 years) and adults over 65 years of age.

- With each additional 100µg/100g flour dose of folic acid from flour fortification (including wholemeal) the mean percentage of individuals with intakes above the UL increases by 100%.

- 0.2% of the population currently have intakes above the UL. The population group with the highest percentage of individuals with current folic acid intakes above the UL is children aged 4-10 years (weighted mean 1.4%). (UL set at 300µg/day and 400µg/day folic acid for children aged 4-6 years and 7-10 years respectively, European Scientific Committee on Food, 2000).

- Fortification of flour would contribute an average additional 50µg/day (47mµg/day excluding wholemeal) folic acid to the diets of children aged 4-10 years. This can be compared to the current contribution of fortified breakfast cereals, 56µg/day folic acid; fortified fat spreads, 24µg/day folic acid and supplements 2µg/day folic acid (Table 13).

36. The data presented in tables 13-17 are summarised in tables 10 and 11 of the main report (page 98-99). The effects of mandatory fortification are discussed in detail in the main report (section 8).

References

Clarke R, Grimley Evans J, Schneede J, Nexo E, Bates C, Fletcher A, Prentice A, Johnston C, Ueland PM, Refsum H, Sherliker P, Birks J, Whitlock G, Breeze E & Scott JM. Vitamin B12 and folate deficiency in later life. *Age Ageing* 2004; **33**:34-41.

Daly S, Kirke PN, Molloy AM, Weir DG & Scott JM. Folate levels and neural tube defects implications for prevention. *JAMA* 1995; **274**:1696-1702.

Daly S, Mills JL, Molloy AM, Conley M, Lee YL, Kirke PN, Weir D G, Scott JM. Minimum effective dose of folic acid for food fortification to prevent neural-tube defects. *Lancet* 1997; **350**:1666-1669.

Department of Health. Dietary Reference Values for Food Energy and Nutrients in the United Kingdom. (Report on Health and Social Subjects, No. 41). London: HSMO, 1991.

Department of Health. *Folic Acid and the prevention of disease*. Report on Health and Social Subjects 50. London: TSO, 2000.

European Scientific Committee on Food. Opinion of the Scientific Committee on Food on the Tolerable Upper Intake Level of Folate 2000. Online at http://ec.europa.eu/comm/food/fs/sc/scf/out80e_en.pdf

Expert Group on Vitamins and Minerals. *Safe Upper Levels for Vitamins and Minerals*. FSA: London, 2003.

Finch S, Doyle W, Lowe C, Bates CJ, Prentice A, Smithers G & Clarke PC. National Diet and Nutrition Survey: people aged 65 years or over. *Volume 1: Report of the diet and nutrition survey*. London: TSO, 1998.

Food Standards Agency. *Nutrient Analysis of Breakfast Cereals*. Unpublished 2002.

Gregory J, Lowe S, Bates CJ, Prentice A, Jackson LV, Smithers G, Wenlock R & Farron M. *National Diet and Nutrition Survey: young people aged 4 to 18 years. Volume 1: Report of the diet and nutrition survey*. London: TSO, 2000.

Henderson L, Gregory J, & Swan G. *National Diet and Nutrition Survey: adults aged 19 to 64 years. Volume 1: Types and quantities of foods consumed.* London: TSO, 2002.

Henderson L, Irving K, Gregory J, Bates CJ, Prentice A, Perks J, Swan G & Farron M. *National Diet and Nutrition Survey: adults aged 19 to 64 years. Volume 3: Vitamin and mineral intake and urinary analytes.* London: TSO, 2003.

Hoare J, Henderson L, Bates CJ, Prentice A, Birch M, Swan G, Farron M. *National Diet and Nutrition Survey: adults aged 19-64 years. Volume 5: Summary report.* London: TSO, 2004.

National Statistics Census.
www.statistics.gov.uk/census2001/pop2001/united_kingdom.asp All People part 1: Census 2001, National Report for England and Wales- Part 2, 2001.

Wald NJ, Law MR, Morris JK, Wald DS. Quantifying the effect of folic acid. *Lancet* 2001; **358**:2069-73.

Appendix 1, Annex 2

Data Used

Table 18. Population groups* and sources of data used in the modelling exercise

Age group	NDNS Survey	Year of field work	Sex	Number studied
4-6 years	Gregory *et al* 2000	1997/8	Male & Female	355
7-10 years				482
11-13 years				360
14-18 years			Female	267
			Male	235
19-34 years	Henderson *et al* 2002; Henderson *et al* 2003; Hoare *et al* 2003.	2000/2001	Female	289
			Male	221
35-49 years			Female	379
			Male	303
50-64 years			Male & Female	532
65-79 years Free Living (FL)	Finch *et al* 1998	1994/5	Male & Female	828
65-79 years Institutional (Inst)				121
80+ years Free Living (FL)				447
80+years Institutional (Inst)				291

*Population data for UK aged 4 years and over obtained from 2001 census (National Statistics Census 2001).

References

Finch S, Doyle W, Lowe C, Bates CJ, Prentice A, Smithers G & Clarke PC. *National Diet and Nutrition Survey: people aged 65 years or over. Volume 1: Report of the diet and nutrition survey.* London: TSO, 1998.

Gregory J, Lowe S, Bates CJ, Prentice A, Jackson LV, Smithers G, Wenlock R & Farron M. *National Diet and Nutrition Survey: young people aged 4 to 18 years. Volume 1: Report of the diet and nutrition survey.* London: TSO, 2000.

Henderson L, Gregory J, & Swan G. *National Diet and Nutrition Survey: adults aged 19 to 64 years. Volume 1: Types and quantities of foods consumed.* London: TSO, 2002.

Henderson L, Irving K, Gregory J, Bates CJ, Prentice A, Perks J, Swan G & Farron M. *National Diet and Nutrition Survey: adults aged 19 to 64 years. Volume 3: Vitamin and mineral intake and urinary analytes.* London: TSO, 2003.

Hoare J, Henderson L, Bates CJ, Prentice A, Birch M, Swan G, Farron M. *National Diet and Nutrition Survey: adults aged 19-64 years. Volume 5: Summary report.* London: TSO, 2004.

National Statistics Census.
www.statistics.gov.uk/census2001/pop2001/united_kingdom.asp All People part 1: Census 2001, National Report for England and Wales- Part 2, 2001.

Appendix 2, Annex 2

Assumptions

Percentage Flour in NDNS Food Groups

In order to estimate the impact of flour fortification, the percentage of flour in each of the main NDNS flour containing food groups was estimated. This was the same approach used for previous analysis (Department of Health, 2000). The percent flour content was adjusted to represent more recent recipes. The percentages used are shown in Table 19. The flour content of products not represented in Table 19 was assumed to be zero.

Table 19. Flour content of food products

NDNS Food Group	Estimated % Flour
White Bread	63
Wholemeal bread	60*
Soft grain bread	63
Other breads	55
Pizzas	25
Other cereals, dumplings Yorkshire puddings etc	25
Biscuits	50
Fruit pies	30
Buns Cakes & Pastries	45
Sponge type puddings	30
Other cereal based puddings (crumbles, bread pudding, pancakes, cheesecake trifle etc	10

*Wholemeal flour, exempt from fortification in specific examples.

- Using these food groups excludes some products, which would contain flour in the recipe such as savoury flans, pies, quiches and battered/breaded fish/meat. The contribution of these products to flour consumption was considered to be low and so they were omitted from the analysis.

- The estimated flour content of "other breads" has been adjusted downwards to allow for the inclusion of non-wheat based breads in the category. No attempt has been made to adjust for inclusion of non-wheat flour products in other categories.

Table 20. Assumptions made in modelling exercise

Subject	Assumption	Basis	Source
Breakfast cereals	Label values were used for folate values of fortified cereals.	The FSA 2002 breakfast cereal survey provided label data for the folate content of a number of breakfast cereals. These values were compared against current declared levels of fortification for consistency. The survey also provided analytical values of natural folate for unfortified cereals.	Nutrient Analysis of Breakfast Cereals sampling report (FSA, 2002).
	32% overage applied to the label values for folic acid fortified breakfast cereals.	The 32% overage used was the calculated difference between analytical folate values for various breakfast cereals and their corresponding declared label values. Analytical data was based on analysis of composite samples of up to ten products purchased from different outlets so each composite sample is likely to include products at different stages of shelf life. The difference between the analytical and label values (ie the overage) ranged from 4 to 113%. The average overage estimated for these cereals was 32%. Therefore an overage of 32% was added to fortified cereals to represent hypothetical levels of folic acid on consumption.	Nutrient Analysis of Breakfast Cereals sampling report (FSA, 2002)
	Folic acid content is uniform throughout the product.	Folic acid is in the cook-in premix, so is uniformly dispersed through the product and would not be concentrated in the dust.	Personal communication with Cereal Partners 2006
Fat Spreads	One third of reduced and low fat spread consumers, use spreads fortified with folic acid at a level of 1000µg/100g. It was assumed these consumers are the third with the highest folate intakes before fortification.	• Products in all of Unilever's Flora range are fortified at 1000µg/100g. A few other products on the market are fortified at the same or lower levels. • Unilever's flora range occupies 18.5% market share of yellow fat spreads (including butter). • It was assumed folic acid fortified spreads as a total, occupy 20% of the market of yellow fat spreads. • Fortified spreads are all reduced or low fat spreads. • Reduced fat spreads account for around 60% of yellow fat consumption. • Therefore it was estimated that a third of reduced and low fat spreads are fortified.	Personal communication with Unilever 2005, data from adults NDNS 2002/3 and EFS 2003/4
	20% overage applied to fortified reduced or low fat spreads.	Unilever allow a fortification error of 20%. There is no degradation of folic acid in fat spreads over time.	Personal communication with Unilever 2005
Supplements	Supplement intake has not changed since the surveys were carried out.	Data on supplement use has not been adjusted to reflect current intakes, as this would involve further detailed study. No information is available on current supplement use.	
	30% overage applied to all folic acid containing supplements	Average overage of 30% for supplements manufactured in the UK.	Personal communication with trade associations PAGB, CRN, HFMA

Subject	Assumption	Basis	Source
Bread	No bread is currently fortified with folic acid.	Only one brand identified as fortified with folic acid, which holds a fraction of the market share for breads.	Personal communication with bakers and millers and supermarket searches 2005
Bioavailability	Folate and folic acid are assumed to be 100% bioavailable.	No universally known conversion factor, however folic acid in flora fat spreads is known to be 60% bioavailable.	Sanderson *et al*, 2003. Personal communication with Unilever 2005
Imported Flour	All flour is milled in the UK, and therefore subject to fortification.	Imported flour represents an insignificant proportion of flour consumed in the UK.	Personal communication with bakers 2005.
Fortification applies to all wheat flour	All flour used in manufactured products in the given categories (Table 19) is fortified.	Assuming flour fortification will take place at the milling process, all products made with wheat flour milled in the UK will be subject to fortification.	
Wholemeal flour	Wholemeal bread is the only significant source of wholemeal flour.	The majority of wholemeal flour is found in wholemeal bread and other sources are insignificant.	NDNS series
Processing losses	Processing losses of 25%	Research has shown processing losses of between 15-25% in bread. Bread is the main source (69%) of flour in the average UK diet. Losses will be greater for products with greater surface area to volume ratios, so the upper level has been taken.	Johansson *et al*, 2002, Gujska *et al*, 2000, & Kariluoto *et al*, 2004. NDNS series
Intake Data	Dietary intake data from NDNS series represents usual intake.	The NDNS series was based on 4-7 day weighted intake data. This approach may overestimate extremes in folate intake.	
Under reporting	No underreporting		
Children aged 0-3 years	No children aged 0-3 consume food products containing flour.		

References

Department of Health. *Folic Acid and the prevention of disease.* Report on Health and Social Subjects 50. London: TSO, 2000.

Food Standards Agency. *Nutrient Analysis of Breakfast Cereals.* Unpublished 2002.

Gujska E, Majewska K, Effects of baking process on added folic acid and endogenous folates stability in wheat and rye breads. *Plant Foods Human Nutrition* 2000; **60** (2):37-42.

Johansson M, Witthöft C, Bruce Å, Jägerstad M. Study of wheat breakfast rolls fortified with folic acid. *European Journal of Nutrition* 2002; **41**: 279-286.

Kariluoto S, Vahteristo L, Salovaara H, Katina K, Liukkonen K-H, Piironen V. Effect of Baking Method and Fermentation on Folate Content of Rye and Wheat Breads. *American Association of Cereal Chemists* 2004; **81**(1): 134-139.

Sanderson P, McNulty H, Mastroiacovo P, Melse-Boonstra A, Finglas PM & Gregory JF. Folate Bioavailability: UK Food Standards Agency Workshop Report. *British Journal of Nutrition.* 2003; **90**: 473-379.

Finch S, Doyle W, Lowe C, Bates CJ, Prentice A, Smithers G & Clarke PC. *National Diet and Nutrition Survey: people aged 65 years or over. Volume 1: Report of the diet and nutrition survey.* London: TSO, 1998.

Gregory J, Lowe S, Bates CJ, Prentice A, Jackson LV, Smithers G, Wenlock R & Farron M. *National Diet and Nutrition Survey: young people aged 4 to 18 years. Volume 1: Report of the diet and nutrition survey.* London: TSO, 2000.

Henderson L, Gregory J, & Swan G. *National Diet and Nutrition Survey: adults aged 19 to 64 years. Volume 1: Types and quantities of foods consumed.* London: TSO, 2002.

Henderson L, Irving K, Gregory J, Bates CJ, Prentice A, Perks J, Swan G & Farron M. *National Diet and Nutrition Survey: adults aged 19 to 64 years. Volume 3: Vitamin and mineral intake and urinary analytes.* London: TSO, 2003.

Hoare J, Henderson L, Bates CJ, Prentice A, Birch M, Swan G, Farron M. *National Diet and Nutrition Survey: adults aged 19-64 years. Volume 5: Summary report*. London: The Stationery Office 2004.

Annex 3

Dietary reference values for folate and B vitamins

1. The dietary reference values (DRVs) form the basis for assessment of nutritional status; therefore, differences between countries can affect the interpretation of population status. Table 21 shows that the RNIs for riboflavin, vitamin B6 and vitamin B12 are similar in the US and UK, however, there are important differences for folate.

2. In the UK, the RNI for folate is 200µg/d for adults. In the USA, however, the recommended daily allowance (RDA; equivalent to the RNI) for folate is 400µg/d dietary folate equivalents (DFE) for adults (1µg of DFE = 1µg food folate = 0.5µg of folic acid taken on an empty stomach = 0.6µg of folic acid with meals). The primary indicator of status used to estimate the RDA in the USA was red blood cell folate in conjunction with plasma folate and total homocysteine (tHcy) concentrations. In the UK, red blood cell folate and autopsied liver folate concentrations were the primary indicator. In the USA the thresholds used to define adequate folate status were 305nmol/L (140ng/ml) red blood cell folate and 7nmol/L serum folate. In the UK they were 150ng/ml red blood cell folate and liver concentrations above 3µg/g.

3. In the US, a metabolic maintenance study (O'Keefe et al., 1995) was given greatest weight in the recommendation for the estimated average requirement of 320µg/d of DFE. O'Keefe et al., (1995) conducted a controlled metabolic study in which five women were fed for 70 days a diet that provided 319µg/d of DFE (30µg food folates and 170µg folic acid). Three of the five had red blood cell folate concentrations less than 305nmol/L (140ng/ml) red blood cell folate; 7nmol/L serum folate and two of the five had tHcy greater then 16µmol/L. This was interpreted as meaning that approximately half would have adequate folate status if 320µg/d of DFE had been consumed. The RDA was set using a coefficient of variation of 10 percent giving a value of 400µg/d of DFE.

4. In the UK, the RNI for adults was based on Canadian assessments of status (Department of Health, 1991). These showed that the folate content of the liver was consistently greater than 3µg/g with a mean

dietary intake of food folate of 150-200µg/d. In this population no more than 8-10% of adults had red blood cell concentrations below 150ng/ml and a RNI of 200µg/d was therefore set.

5. The NDNS of adults (Henderson et al., 2003; Ruston et al., 2004) reported that red blood cell folate concentrations of less than 350nmol/L were found in 5% of the samples from both men and women, while 1% of men and less than 0.5% of women had a serum folate concentration below 7nmol/L.

6. There is evidence for an increase in folate requirements in late pregnancy and a need to replace amounts secreted into milk during lactation (Department of Health, 1991). In the US, the RDA for pregnancy is 600µg/d of dietary folate equivalents and 500µg/d of dietary folate equivalents for lactation. In the UK, the RNI for pregnancy is 300µg/d folate and for lactation is 260µg/d folate.

Table 21. Comparison of the UK RNI with the US RDA for other B vitamins involved in aspects of folate metabolism

Country	Riboflavin	Vitamin B6	Vitamin B12
UK (RNI)	1.1mg women; 1.3mg men	1.2mg women; 1.4mg men	1.5µg women; 1.5µg men
USA (RDA)	1.1mg women; 1.3mg men	1.1mg women; 1.3mg men	2µg women; 2µg men

Annex 4

Tolerable Upper Intake Levels of Folic Acid in the USA and Europe

Table 22: Food and Nutrition Board, Institute of Medicine, USA (1998)

Age	Tolerable Upper Intake Level (UL) for folic acid (µg/day)
0-12 months	Not possible to establish
1-3 years	300
4-8 years	400
9-13 years	600
14-18 years	800
Pregnancy/Lactation: 14-18 years	800
Pregnancy/Lactation: 19 years and older	1000
Adults	1000

Table 23: European Scientific Committee on Foods (2000)

Age (years)	Tolerable Upper Intake Level (UL) for folic acid (µg/day)
1 – 3	200
4 – 6	300
7 – 10	400
11 – 14	600
15 – 17	800
Adults	1000

Annex 5

NTD-affected pregnancies in the UK

Table 24. Reported numbers and rates of NTD-affected pregnancies in England and Wales

	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004
NTD-affected births													
Number	126	103	82	114	90	81	91	88	123	99	119	121	113
Rate per 10,000 Births	1.8	1.5	1.2	1.8	1.4	1.3	1.4	1.4	2.0	1.7	2.0	1.9	1.8
NTD reported terminations													
Number	NA	NA	NA	336	336	281	305	295	331	288	255	297	254
Rate per 10,000 Births				5.2	5.1	4.3	4.8	4.7	5.5	4.8	4.3	4.8	4.0
Total NTD-affected pregnancies													
Number	NA	NA	NA	450	426	362	396	383	454	387	374	418	367
Rate per 10,000 Births				6.9	6.5	5.6	6.2	6.1	7.5	6.5	6.2	6.7	5.7
Total live and still births	692452	676887	668114	651315	652595	646148	638950	624862	607304	597506	599279	624816	643026

Source of birth data: Office for National Statistics anomaly statistics notifications. A statistical review of congenital anomolies received as part of the England and Wales national Congenital Anomoly System, 2005. Accessible at: http://www.statistics.gov.uk/downloads/theme_health/MB3_No20/MB3_No20.pdf.

Source of termination data: Series AB ONS Abortion statistics 1995-2001; DH Statistical Bulletin - Abortion statistics 2002-2004. Data prior to 1995 are not available at this level of condition, only numbers for the wider grouping – central nervous system.

Table 25. Reported numbers and rates of NTD-affected pregnancies in Wales

	1998	1999	2000	2001	2002	2003	2004
NTD-affected births							
Number	4	14	7	8	11	9	6
Rate per 10,000 Births	1.2	4.3	2.2	2.6	3.6	2.9	1.8
NTD reported terminations							
Number	59	46	42	49	44	35	46
Rate per 10,000 Births	17.5	14.3	13.4	15.9	14.5	11.2	14.2
***Total NTD-affected pregnancies**							
Number	66	62	50	60	58	45	55
Rate per 10,000 Births	19.6	19.2	15.9	19.5	19.1	14.4	16.9
Total live and still births	33620	32266	31449	30771	30369	31330	32504

*Figures include NTD miscarriages.

Source of data: Congenital Anomaly Register and Information Service for Wales (CARIS) (personal communication, 2006)

Table 26. Reported numbers and rates of NTD-affected singleton pregnancies in Scotland

	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	[b]2002	2003
NTD-affected births												
Number	51	42	38	39	44	30	34	33	19	22	22	24
Rate per 10,000 Births[a]	8.1	7.0	6.4	6.7	7.8	5.2	6.1	6.2	3.7	4.4	4.5	4.9
NTD reported terminations												
Number	35	26	37	39	48	35	43	30	25	20	37	25
Rate per 10,000 Births	5.5	4.2	6.2	6.7	8.5	6.1	7.8	5.7	4.9	4.0	7.5	5.1
Total NTD-affected pregnancies												
Number	86	69	75	78	92	65	77	63	44	42	59	49
Rate per 10,000 Births	13.6	11.2	12.5	13.4	16.4	11.4	13.9	11.9	8.6	8.4	12.0	9.9
Total live and still births	63316	61582	59780	58193	56176	57265	55345	52893	51309	50013	49217	49430

[a] All Infants followed up from birth for period of one year to allow detection of anomalies from hospital inpatient records or General Register Office for Scotland death registrations

[b] Notification of births system changed.

Source of birth data: Information and Statistics Division: Scotland. Scottish Perinatal and Infant Mortality & Morbidity Report (2003). Accessible at: http://www.isdscotland.org/spimmr
Termination data source: General Register Office for Scotland and Notifications (to the Chief Medical Officer for Scotland) of abortions performed under the Abortion Act 1967.

Table 27: Reported numbers and rates of NTD-affected births in Northern Ireland

	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003
NTD-affected births												
Number	24	13	23	16	13	14	17	18	15	17	14	11
Rate per 10,000 Births	9.4	5.2	9.5	6.7	5.3	5.8	7.1	7.8	6.9	7.7	6.5	5.1
NTD reported terminations	N/A											
Total NTD-affected pregnancies	N/A											
Total live and still births	25478	24850	24251	23838	24535	24218	23790	22089	21605	22074	21507	21756

Source of birth data: Northern Ireland Child Health Systems. Northern Ireland Department of Health, Social Services and Public Safety (personal communication, 2005)

Annex 6

Folic acid fortification strategies in other countries

Table 28. Examples of folic acid fortification strategies

Country	Fortification position	Information on fortification level
Australia	Voluntary (1996)	Voluntary – includes breakfast cereal, some bread & marmite (levels unavailable). Bread will no longer be fortified voluntarily in line with new legislation.
	Mandatory fortification under consideration	80-180 µg/100g bread as consumed
Belgium	Voluntary	For product to be labelled as fortified with folic acid the daily portion has to contain 15 to 200% of 200µg
Bolivia, Colombia, Paraquay Ecuador	Mandatory fortification	Wheat flour - 60-300µg/100g
Brazil	Legislation pending	150µg/100g wheat & maize flour
Canada	Mandatory (1998)	Mandatory - 150µg/100g white flour, 200µg/100g enriched pasta Voluntary - 150 – 220 µg/100g cornflour
Chile	Mandatory (2000)	220µg/100g flour
Czech Republic	Voluntary	Information on foods unavailable – level 200µg/100g
France	Voluntary	Breakfast cereals and products aimed at children or women – levels unavailable Goats milk at 4.5µg/100g
Germany	Voluntary	Breakfast cereals and products – levels unavailable
Greece	Voluntary	Unavailable
Hungary	Voluntary	Previously fortified @ 60µg/100g bread
Iceland	Voluntary	Breakfast cereals and products 30-700µg/100g, flour and rice 30-100µg/100g
Ireland	Mandatory fortification recommended (pending legislation)	120µg/100g of white, brown and wholemeal bread as consumed
Israel	Voluntary (legislation pending)	Wheat flour (level unknown) Co-fortification with vitamin B_{12} being considered
New Zealand	Voluntary (1996)	Voluntary – includes breakfast cereal, some bread & marmite (levels unavailable). Bread will no longer be fortified voluntarily in line with new legislation
	Mandatory fortification under consideration	80-180µg/100g bread as consumed
UK	Voluntary	Breakfast cereals and products 8-643µg/100g; flora spread products 1000µg /100g.
USA	Mandatory (1998)	140µg/100g grain in food as consumed

Annex 7

Studies considered in relation to folate and risk of cardiovascular disease and cancer

Table 29. Cohort studies investigating an association of circulating folate concentrations with risk of cardiovascular disease

Study (Reference)	Study population	Age range (yrs)	Mean follow-up (yrs)	No of cases	Adjusted relative risk (95% CI)	Trends	Other factors adjusted for in analysis
Morrison *et al.*, 1996	5,056 general population	35-79	15	165 fatal CHD	1.69 (1.10 to 2.61) for lowest v highest quartile of serum folate	NS	Sex, age, smoking, diabetes, serum cholesterol and hypertension
Chasan-Taber *et al.*, 1996	NCC 14,916 male physicians	40-84	7.5	333 MI 333 MC	1.4 (0.9 to 2.3) for lowest 20th percentile v above the 20th percentile of serum folate	NS	Diabetes, angina, hypertension, Quetelet's index, total cholesterol, HDL cholesterol, aspirin use.
Folsom *et al.*, 1998	NCC 15,792 general population	45-64	3.3	232 fatal and non-fatal CHD	1.01 (0.5 to 2.2) for highest v lowest quintile of plasma folate for men 0.39 (0.1 to1.06) for highest v lowest quintile of plasma folate for women	Men p=0.6 Women p=0.003	Sex, age, race, field centre, smoking, total cholesterol, HDL cholesterol, hypertension and diabetes, dietary vitamin intake for energy intake.
Ford *et al.*, 1998	2,657 general population	25-74	19	873 CHD	1.04 (0.86 to 1.26) for lowest v highest quintile of serum folate	NS	Sex, age, race, education, smoking, hypertension, serum cholesterol, BMI, physical activity, diabetes and alcohol consumption and use of supplements.
Hung *et al.*, 2003	2,314 general population	20-90	29	413 fatal CVD of which 229 fatal CHD	Men 0.96 (0.74 to 1.25) and women 1.08 (0.81 to 1.45) for lowest v highest quartile of red blood cell folate	Men p=0.35 Women p=0.21	Age, blood pressure, BMI, serum cholesterol, white cell count, smoking menopause, diabetes, hypertension, alcohol.
Voutilainen *et al.*, 2004	810 men	46-64	7.7	114 cardiac events	0.39 (0.18 to 0.83) for highest v lowest tertile of serum folate. No association between tHcy and acute coronary events was observed.	p=0.016	Age, smoking, BMI, systolic blood pressure, serum LDL and HDL cholesterol, serum lycopene, alpha tocopherol and beta carotene.

MI, myocardial infarctions; BMI, body mass index; NS, not stated; NCC, nested case-control; MC, matched control; CHD, coronary heart disease; CVD, cardiovascular disease

Table 30. Cohort studies investigating an association of dietary folate intakes with risk of cardiovascular disease

Study (Reference)	Study population	Age range (yrs)	Mean follow-up (yrs)	No of cases	Adjusted relative risk (95% CI)	Trends	Other factors adjusted for in analysis
Rimm *et al.*, 1998	80,082 female nurses	30-55	14	939 MI and fatal CHD	0.69 (0.55 to 0.87) for highest v lowest quintile of dietary folate intake	p=0.003	Age, time period, smoking, BMI, postmenopausal hormones, aspirin, vitamin E supplements, exercise, hypertension, parental history of CHD, and intake of polyunsaturated, saturated and trans fat, fibre and alcohol
Voutilainen *et al.*, 2001	1,980 men	42-60	10	199 acute coronary events	0.45 (0.25 to 0.81) for highest v lowest quintile of dietary folate intake in subjects with no previous CHD	No previous CHD p=0.008	Age, total, LDL and HDL cholesterol and triglyceride, smoking, physical activity, hypertension, diabetes, CHD in family, examination years, excretion of nicotine metabolites and nutritional factors.
He *et al.*, 2004	43,732 men	40-75	14	725 incident strokes	0.71 (0.52 to 0.96) for highest v lowest quintile of dietary folate intake. (Association for ischemic, but not hemorrhagic, stroke).	p=0.05	Age, smoking, BMI, physical activity, history of hypertension and hypercholesterolemia, aspirin, alcohol, fibre, potassium, vitamin E and total energy

MI, myocardial infarctions; BMI, body mass index; NS, not stated; NCC, nested case-control; MC, matched control; CHD, coronary heart disease.

Table 31. Trials of homocysteine-lowering vitamin supplements in people with prior CHD, prior stroke or renal disease

Trial (Country)	Fortified population (-/+)	Prior disease	Actual / (scheduled) number randomized	Actual / scheduled duration of treatment (years)	Observed / (estimated) reduction in tHcy (%)	Homocysteine-lowering regimen (mg/d)		
						Folic acid	B12	B6
CHAOS-2† (UK)	-	CHD	1880	2	13	5.0	-	-
SU.FOL.OM3 (France)	-	CHD	(2000)	5	(25)	0.5*	0.02	3
WENBIT (Norway)	-	CHD	3000	3	25%	0.8	0.4	40
NORVIT (Norway)	-	CHD	3749	3	27	0.8	0.4	40
SEARCH (UK)	-	CHD	12064	7	25	2.0	1.0	-
HOPE-2 (Canada)	+	CHD	5522	5	(20)	2.5	1.0	50
WACS (USA)	+	CHD	5442	7.4	(20)	2.5	1.0	50
Su.Fol.O3 (France)	-	Stroke	(1000)	5	(25)	0.5	0.02	3
VITATOPS (Australia)	-	Stroke	(8000)	3	25	2.0	0.5	25
VISP† (USA)	+	Stroke	3680	2	15	2.5	0.4	25
FAVORIT (USA)	+	Renal	(4000)	5	33	2.5	0.4	20
VA Trial (USA)	+	Renal	2056	5	(33)	40.0	0.5	100

Table courtesy of Dr Robert Clarke; † terminated early, see text for details; * 5-MTHF.

Table 32. Cohort studies investigating the association of folate intake with colorectal cancer risk

Study (Reference)	Study population	Age range (yrs)	Mean follow-up (yrs)	No of cases	Adjusted relative risk (95% CI)	Trends	Other factors adjusted for in analysis
Giovannucci *et al.*, 1995	14,931 men Health Professionals' Follow-up Study	40-75	6	205 colon	0.86 (0.54 to 1.36) for highest vs. lowest quintile of folate intake 0.74 (0.47 to 1.17) for multivitamin use for 10 or more years compared to nonusers 3.30 (1.58 to 6.88) for high alcohol (>20g/d) low methyl (methionine and folate) diet vs a low alcohol (<5g/d) high methyl diet in non aspirin users.	p=0.30 NS p<0.01	Age, smoking, physical activity, BMI, aspirin use, multivitamin use, total energy, fat, red meat, vitamin D and calcium intake, family history of CRC, history of polyps/endoscopy
Glynn *et al.*, 1996	NCC 29,133 men smokers Alpha-Tocopherol Beta-Carotene cohort	50-69	5-8	91 colon 53 rectal 276 non-cases	0.51 (0.20 to 1.31) for highest vs. lowest quartile of folate intake for colon cancer. 2.12 (0.43 to 10.54) for highest vs. lowest quartile of folate intake for rectal cancer.	p=0.15 p=0.26	Total energy intake, physical activity, energy-adjusted folate intakes, vitamins A, C and E, fibre, protein, starch, calcium. iron, alcohol intake, smoking, BMI
Giovannucci *et al.*, 1998	88,756 women Nurses' Health Study	30-55	14	442 colon	0.69 (0.52 to 0.93) for highest vs. lowest quartile of folate intake. 0.48 (0.33 to 0.71) for highest vs. lowest quartile of folate intake in women whose methionine intake <1.8g/d. 0.29 (0.15 to 0.56) for multivitamin use for 15 or more years compared to nonusers	p=0.01 p<0.001 p<0.001	Age, aspirin use, physical activity, BMI, smoking, family history of CRC, and red meat, fibre, methionine and fibre intake

Table 32. Cont.

Study (Reference)	Study population	Age range (yrs)	Mean follow-up (yrs)	No of cases	Adjusted relative risk (95% CI)	Trends	Other factors adjusted for in analysis
Su & Arab et al., 2001	10,183 general population NHANES I Epidemiology Follow-up Study	25-74	20	219 colon	0.40 (0.18 to 0.88) for highest v lowest quartile of dietary folate intake in men 0.74 (0.36 to 1.51) for highest v lowest quartile of dietary folate intake in women 2.22 (1.03 to 4.77) for alcohol drinkers (>1.16 drinks/wk) low methyl (methionine and folate) diet vs non-drinkers, high methyl diet in men, but not women.	p=0.03 p=0.70 p=0.05	Age, race, gender, smoking, BMI, family history of colon cancer, intake of fat, fibre, calcium, vitamin B6, vitamin B12, total energy and alcohol
Fuchs et al., 2002	88,758 women Nurses' Health Study	30-55	16	535 colon	0.48 (0.28 to 0.83) for highest vs. lowest quartile of folate intake in women with a family history of colon cancer 0.81 (0.62 to 1.07) for highest vs. lowest quartile of folate intake in women with no family history of colon cancer	p=0.02 for interaction NS	Age, aspirin use, physical activity, BMI, smoking, family history of CRC, postmenopausal oestrogen use, red meat, alcohol, animal fat, vitamins A, C, D, E, methionine and fibre intake
Konings et al., 2002	NCC 120,852 general population Netherlands Cohort Study	55-69	7.3	760 colon 411 rectal 3500 non cases	0.73 (0.46 to 1.17) for highest vs. lowest quintile of folate intake for colon cancer in men 0.68 (0.39 to 1.20) for highest vs. lowest quintile of folate intake for colon cancer in women 0.66 (0.35 to 1.21) for highest vs. lowest quintile of folate intake for rectal cancer in men 1.26 (0.58 to 2.76) for highest vs. lowest quintile of folate intake for rectal cancer in women	p=0.03 p=0.18 p=0.03 p=0.55	Age, energy intake, family history, alcohol, vitamin C, iron and dietary fibre intake

Table 32. Cont.

Study (Reference)	Study population	Age range (yrs)	Mean follow-up (yrs)	No of cases	Adjusted relative risk (95% CI)	Trends	Other factors adjusted for in analysis
Flood et al., 2002	45,264 women Breast Cancer Detection Project Follow-up Study	40-93	8.5	490 colon and rectal	0.86 (0.65 to 1.13) for highest vs. lowest quintile of dietary folate intake for CRC 1.01 (0.75 to 1.35) for highest vs. lowest quintile of total folate intake (includes supplements) for CRC	p=0.14 p=0.67	NSAID use, smoking, education, BMI, physical activity, red meat, alcohol, total fat, vitamins D, grains, methionine, energy, alcohol and fibre intake
Harnack et al., 2002	41,836 women Iowa Women's Health Study	55-69	13	598 colon 123 rectal	1.12 (0.77 to 1.63) for highest vs. lowest quintile of folate intake for colon cancer 0.89 (0.52 to 1.51) for highest third vs lowest third of folate intake for rectal cancer	p=0.67 p=0.44	Age, BMI, oestrogen use, smoking, dietary energy, calcium, and vitamin E
Terry et al., 2002	NCC 56,837 women Canadian National Breast Screening Study	40-59	10.4	295 cases 5334 non-cases	0.6 (0.3 to 1.1) for highest vs. lowest quintile of folate intake for colon cancer 0.7 (0.3 to 1.8) for highest vs. lowest quintile of folate intake for rectal cancer	p=0.41 p=0.36	Age, smoking, BMI, physical activity, education, fat and energy intakes

Table 32. Cont.

Study (Reference)	Study population	Age range (yrs)	Mean follow-up (yrs)	No of cases	Adjusted relative risk (95% CI)	Trends	Other factors adjusted for in analysis
Wei et al., 2004	87,733 women 46,632 men Nurses' Health Study and Health Professionals' Follow-up Study	30-55 40-75	20 14	1,139 colon 339 rectal	0.82 (0.66 to 1.03) for highest vs. lowest quartile of folate intake for colon cancer in women 0.72 (0.45 to 1.16) for highest vs. lowest quartile of folate intake for colon cancer in men 0.82 (0.68 to 0.99) for highest vs. lowest quartile of folate intake for colon cancer in men and women	p=0.04 p=0.57 p=0.06	Age, family history, BMI, physical activity, red meat, processed meat, alcohol, calcium, height, smoking, history of endoscopy and gender in combined cohort
Larsson et al., 2005	61,433 women Swedish Mammography Cohort	40-75	15	547 colon 252 rectal	0.61 (0.41 to 0.91) for highest vs. lowest quintile of folate intake for colon cancer 0.93 (0.55 to 1.56) for highest vs. lowest quintile of folate intake for rectal cancer	p=0.02 p=0.97	Age, BMI, educational level, red meat consumption, total energy intake, energy adjusted intakes of saturated fat, methionine, vitamin B6, ß-carotene, calcium and cereal fibre.

BMI, body mass index; NSAID, non-steroidal anti-inflammatory drugs; NS, not stated; NCC, nested case-control; CRC, colorectal cancer.

Table 33. Cohort studies investigating the association of serum folate concentration with colorectal cancer risk

Study (Reference)	Study population	Age range (yrs)	Mean follow-up (yrs)	No of cases	Adjusted relative risk (95% CI)	Trends	Other factors adjusted for in analysis
Glynn et al., 1996	NCC 29,133 men smokers Alpha-Tocopherol Beta-Carotene cohort	50-69	5-8	91 colon 53 rectal 276 non-cases	0.96 (0.40 to 2.30) for highest vs. lowest quartile of serum folate concentrations for colon cancer. 2.94 (0.84 to 10.33) for highest vs. lowest quartile of serum folate concentrations for rectal cancer.	p=0.83 p=0.10	Total energy intake, physical activity, energy-adjusted folate intakes, vitamins A, C and E, fibre, protein, starch, calcium. iron, alcohol intake, smoking, BMI
Kato et al., 1999	NCC 15,785 women Women's Health Study	NS	3-9	105 colon and rectal 523 non cases	0.52 (0.27 to 0.97) for highest vs. lowest quartile of serum folate concentrations for CRC.	p=0.04	Education, race, religion, physical activity, aspirin use, family history of CRC, history of occult blood testing, alcohol, smoking, energy, macronutrient, fibre, vitamin A, C and E intakes, Quetelet index

NCC, nested case-control.

Table 34. Cohort studies associating breast cancer risk with folate intake

Study (Reference)	Study population	Age range (yrs)	Mean follow-up (yrs)	No of cases	Adjusted relative risk (95% CI)	Trends	Other factors adjusted for in analysis
Zhang et al., 1999	88,818 Nurses' Health Study	30-55	16	3,483	0.93 (0.83 to 1.03) for highest vs. second lowest quintile of folate intake 0.56 (0.41 to 0.79) for highest vs. second lowest quintile of folate intake for alcohol intake more than or equal to 15g/d	P=0.26 P=0.006	Age, total energy intake, parity, age at first birth, family history of breast cancer/disease, alcohol intake, BMI, weight gain/loss, height, age at menopause, age at menarche, HRT use, beta carotene and supplement intake
Sellers et al., 2001	34,387 Iowa Women's Health Study	55-69	12	1,586	1.19 (0.90 to 1.58) for lowest 10th percentile vs. upper 50th percentile of total folate intake 1.59 (1.05 to 2.41) for lowest 10th percentile vs. upper 50th percentile of folate intake for alcohol intake more than 4g/d	NS NS	Age, education, family history of breast cancer, age at menarche, age at menopause, oral contraceptive use, HRT, parity, age at first birth, BMI, waist-to-hip ratio, height, alcohol, smoking, physical activity and other B vitamins
Feigelson et al., 2003	66,561 Cancer Prevention Study II Nutrition Cohort	40-87	5	1,303	1.10 (0.94 to 1.29) for highest vs. lowest quartile of total folate intake 1.33 (0.94 to 1.88) within the lowest quartile of folate intake for highest quartile of alcohol intake (>15g/d) vs. non drinkers.	NS NS	Age, ethanol, methionine, multivitamin use, race, education, family history, breast lump history, mammographic history, HRT use, parity at first birth. Age at menopause, age at menarche, physical activity, BMI, weight gain, energy intake
Sellers et al., 2004	33,552 Iowa Women's Health Study	55-69	14	1823	1.19 (0.98 to 1.45) for lowest 10th percentile vs. upper 50th percentile of dietary folate intake 2.26 (1.59 to 3.21) for lowest 10th percentile vs. upper 50th percentile of dietary folate intake in those with a family history of breast cancer	p=0.20 P=0.005	Age, energy intake, education, age at menarche, age at menopause, oral contraceptive use, HRT, parity, age at first birth, BMI, waist-to-hip ratio, height, smoking, use of folic acid supplementsand physical activity

HRT, hormone replacement therapy; BMI, body mass index; NS, not stated.

Table 35. Cohort studies associating breast cancer risk with serum folate concentration

Study (Reference)	Study population	Age range (yrs)	Mean follow-up (yrs)	No of cases	Adjusted relative risk (95% CI)	Trends	Other factors adjusted for in analysis
Wu et al., 1999	NCC 12,450 women	18-90	NS	133 cases 133 MC	1.08 (0.50 to 2.37) lowest vs. highest quintile of serum folate concentration	p=0.73	Family history, bilateral ovariectomy, age at menarche/menopause/first birth, number of pregnancies, months of breast feeding, oral contraceptive use, HRT, education, BMI and physical exercise
	NCC 14,625 women	18-90	NS	110 cases 110 MC	0.79 (0.33 to 1.90) lowest vs. highest quintile of serum folate concentration	p=0.41	
Zhang et al., 2003	NCC 32,826 women Nurses Health Study	43-69	6-7	712 cases 712 MC	0.73 (0.50 to 1.07) highest vs. lowest quintile of serum folate concentration 0.11 (0.02 to 0.59) for highest vs. lowest quintile of serum folate concentration for alcohol intake more than or equal to 15g/d	p=0.06 p=0.01	Parity, age at first birth, family history of breast cancer/disease, alcohol intake, BMI, age at menopause and menarche

NCC, Nested case-control; NS, not stated; MC, matched-control.

Table 36. Results of secondary outcome of cancer from trials of homocysteine-lowering vitamin supplements in people with prior CHD, prior stroke or renal disease.

Trial (Country)	Fortified population (-/+)	Prior disease	Actual number randomized	Actual duration of treatment (years)	Relative risk (95% CI) for incidence/death of cancer	Trend	Homocysteine-lowering regimen (mg/d)		
							Folic acid	B12	B6
NORVIT* (Norway)	-	CHD	3749	3	1.22 (0.88 to 1.70) cancer incidence (folic acid and B12 vs no folic acid and B12) 1.02 (0.65 to 1.58) cancer incidence for folic acid and ,B12, B6 v placebo	p=0.23 p=0.94	0.8	0.4	40
HOPE-2 (Canada)†	+	CHD	5522	5	1.06 (0.91 to 1.23) cancer incidence 0.99 (0.74 to 1.33) cancer deaths for folic acid and ,B12, B6 v placebo	p=0.47 p= 0.94	2.5	1.0	50

* Incident cases of cancer were recorded as a measure of safety, there was a numerical increase in the risk of cancer among patients assigned to folic acid, but this difference was not significant

† Incidence of cancer and death from cancer were measured as secondary outcomes, there was no significant difference in incident cancers and death from cancer.

Annex 8

Colorectal cancer incidence rates, USA: by age and sex (all races)

*Source: Surveillance, Epidemiology, and End Results (SEER) Program (www.seer.cancer.gov) SEER*Stat Database: Incidence – SEER 9 Regs Public-Use, Nov (1973-2003), National Cancer Institute, DCCCPS, Surveillance Research Program, Cancer Statistics Branch, released April 2006, based on November 2005 submission.*

Based on 9 SEER registries. Age-adjusted to the 2000 US standard population.

Figure 5: 20-54 years

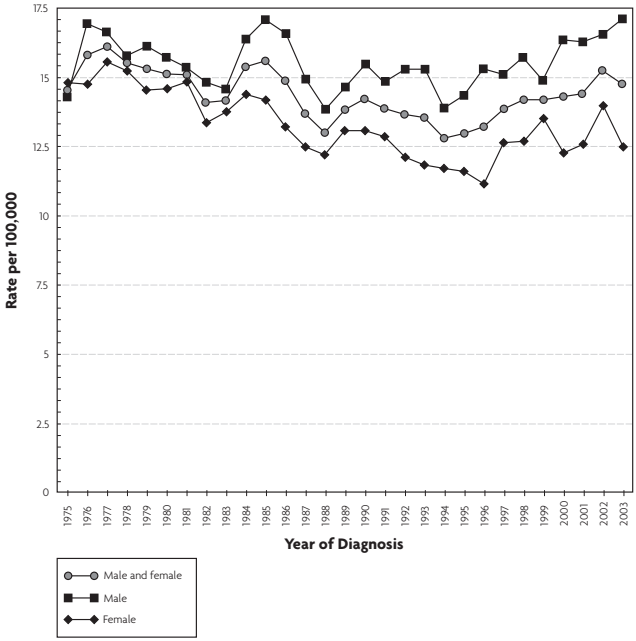


Figure 6: 55-64 years

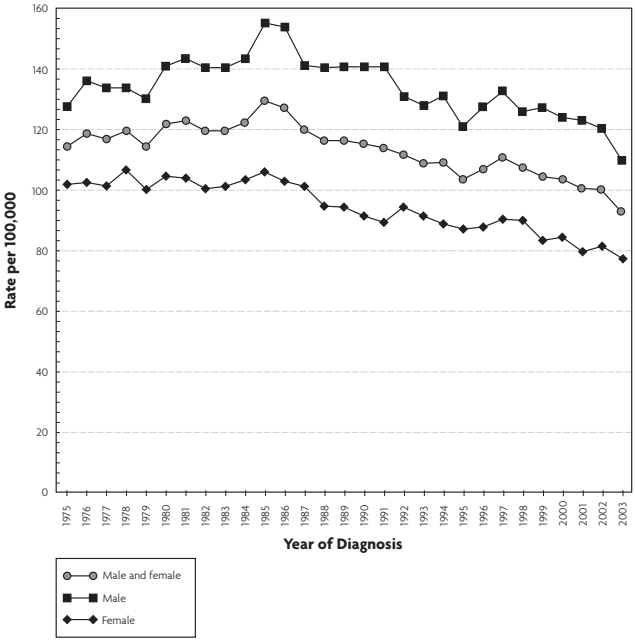


Figure 7: 65-74 years

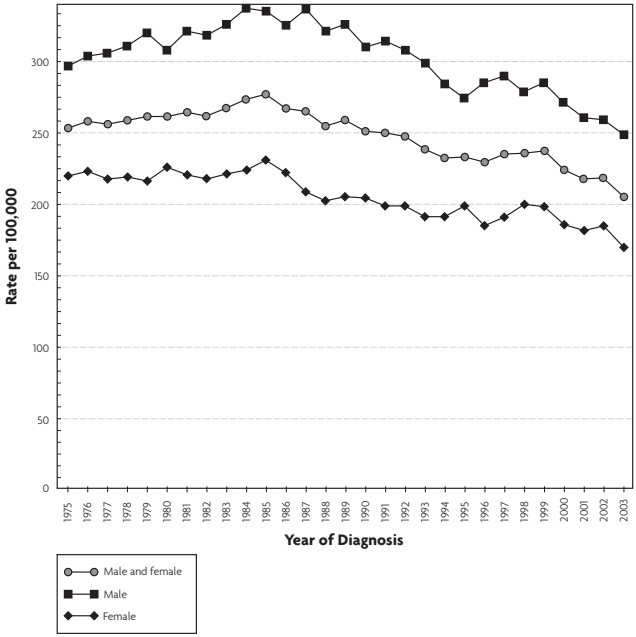


Figure 8: 75 years and over