Report of the **Implementation Group on Folic Acid Food Fortification to the Department of Health and Children**

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FOREWORD

It is a pleasure to present this final report from the Implementation Group on Folic Acid Food Fortification to the Minister for Health and Children, Mary Harney, T.D.

The group was charged with the implementation of the recommendations of the National Committee on Folic Acid Food Fortification (NCFAFF). This task was successfully carried out, but one of the key issues for the group was to put on hold the proposal for mandatory fortification of bread with folic acid. The NCFAFF recommended that in order to reduce the number of pregnancies affected by neural tube defects (NTDs) in Ireland, all bread on the market should be fortified with folic acid on a mandatory basis. The reasons for putting this recommendation on hold are threefold.

Firstly, we have seen a reduction in the incidence of NTDs in Ireland in recent years. As a result of the work of this group we now have, for the first time in Ireland, accurate data on the national incidence of NTDs which is currently 0.93 per 1,000 births. This reduction in the incidence is likely to be due to an increased amount of folic acid in the food supply, resulting from increased voluntary fortification by manufacturers.

Secondly, work of the group included monitoring and surveillance of dietary folic acid in the Irish population. Results show that there has been an increase of about 30% in the amount of folic acid in the Irish diet over the past 2-3 years as a result of an increase in voluntary fortification of foods. The net effect of an increase in dietary folic acid is that the folate status of the Irish population has improved. Monitoring plasma and red cell folate levels demonstrated a reduction in folate deficiency across all population groups when compared to levels in previous years. A key issue for the group is that controls on voluntary fortification should be strengthened.

Thirdly, new scientific studies that are based on laboratory experiments suggest a relationship between high folic acid intakes and cancer risk. However, epidemiological data and human studies relating to cancer risk are inconsistent and not conclusive. New data are likely to be available in 2009 that will allow for an assessment of folic acid intake levels in relation to cancer risk to be carried out. Therefore, the recommendation from this group is to await the publication of such data so that all decisions are based on the best and most up-to-date scientific evidence available.

The importance of health promotion and public awareness campaigns to promote the consumption of 400 µg of folic acid every day by women of childbearing age who are sexually active was recognised by the group. There is a need for such campaigns to continue in order to protect against the risk of NTDs.

I would like to thank the members of the group for their invaluable contributions and for giving freely of their time to bring this work to a successful conclusion.

Alan Reilly
Chair of Implementation Group on Folic Acid Food Fortification
December 2008

EXECUTIVE SUMMARY

An implementation group, set up by the Minister for Health and Children, was charged with putting the recommendations of the National Committee on Folic Acid Food Fortification (NCFAFF) into operation (NCFAFF, 2006). This report covers work carried out between November 2006 and June 2008, and updates the Interim Report of September 2007.

In 2006, the NCFAFF presented a strong case for mandatory fortification of bread on the Irish market at a level of 120 µg/100 g. Since this recommendation was made, new scientific laboratory studies have emerged that suggest a link between high intake levels of folic acid with risks of cancer. However, epidemiological data and human studies relating to folic acid and cancer risk are inconsistent and not conclusive.

It is the view of the implementation group that all new data must be evaluated and that the decision to proceed with mandatory fortification of bread with folic acid should be based on the best and most up-to-date scientific evidence available. Therefore, the decision to introduce mandatory fortification of bread in Ireland should be put on hold until definitive data are available on the safety of this initiative. New data are likely to be available in 2009 that will allow for an assessment of folic acid intake levels in relation to cancer risk to be carried out. Therefore, the recommendation from this group is to await the publication of such data so that all decisions are based on the best and most up-to-date scientific evidence available.

The implementation group recommends legislation be introduced to establish a national registry of congenital anomalies, and that a system for national surveillance of all congenital anomalies be set up by the Health Service Executive (HSE). Provisions for establishing this national register should be included in the proposed Health Information Bill. This would allow national monitoring of neural tube defects (NTDs) on an on-going basis, which is currently not happening in Ireland. The suggested legislation would be similar to that currently in existence for the provision of information to the National Cancer Registry, or that for the provision of information under the Infectious Diseases Regulations.

Surveillance and monitoring work, carried out as part of the work of the implementation group, has provided definitive data on the national incidence of NTDs. It was recognised that the collection of reliable data on the actual incidence of NTDs in Ireland was essential for assessing the effectiveness of the planned mandatory folic acid food fortification programme. This work, organised by the Coombe Women's Hospital and the Food Safety Authority of Ireland (FSAI), collected data on NTDs diagnosed in all 21 obstetric units in Ireland from January 2005 to December 2006. The total number of births in Ireland for the two-year period was 125,279 (61,042 in 2005 and 64,237 in 2006). During this period, a total of 116 NTDs were recorded which gives an incidence rate of 0.93 per 1,000 births. Of these, there were 58 anencephalics (50%), 47 cases of spina bifida (40.5%), nine encephaloceles (7.8%) and two cases (1.7%) of exencephaly/acrania. Forty four (38%) of the cases did not come to term. Overall, these data have shown a significant reduction has occurred in the prevalence of NTDs in Ireland over recent years.

Prior to fortification, monitoring the actual blood levels of folate within various population sub-groups was seen as a priority by the implementation group, in order to establish a baseline estimate of folate status pre-fortification and to ensure its safety. This work, organised by the School of Biochemistry and Immunology, Trinity College, Dublin and the FSAI, was conducted in 2006. Approximately 1,500 blood samples from different age and sex groups were analysed for plasma and red cell folate levels, vitamin B_{12} and homocysteine levels and the determination of the 677C→T genotype. Monitoring results show that blood folate levels in all sub-groups of the Irish population examined have increased substantially in recent years, indicating that folate deficiency is uncommon, affecting less than 2% of people examined in this study. A key finding is that 93% of women aged 16 to 40 years of age have adequate folate status and over half of these women have blood folate levels that are optimal for preventing NTDs.

The improved folate status of the Irish population is probably due to the widespread consumption of foods fortified with folic acid and, to a lesser extent, to use of multivitamin supplements containing folic acid. This increased amount of folic acid intake in the diet is also the most likely reason for the observed decrease in the numbers of NTDs observed over 2005 and 2006.

During 2006 and 2007, folic acid levels in breads on the Irish market were monitored by the FSAI and the Public Analyst's Laboratory, Galway. Results show that about 25% of breads sold nationally are fortified on a voluntary basis with folic acid to varying levels. Experimental work carried out in collaboration with the food industry demonstrates that in order to achieve optimal levels in bread, folic acid should be added to flour and not during the bread-making process. Additionally, up to 30% of folic acid is lost during bread baking and this loss would need to be factored in to a mandatory fortification programme.

An assessment of fortified foods on the Irish market was also undertaken by the FSAI. More than 200 products were identified, which shows that the range of folic acid fortified foods is increasing. While previously only breakfast cereals were voluntarily fortified, now the range of fortified food includes soups, cereal bars, fruit juices, milk, fat spreads and yogurts. In addition, the levels of folic acid added to foods are now significantly higher than those of a few years ago; some as high as 1,000 µg folic acid per 100 g of food. This increase in voluntary folic acid fortification of food on the Irish market explains the higher folate status of the Irish population. In light of the wider range of products currently fortified with folic acid, the FSAI has re-evaluated food consumption data to determine the amount of folic acid consumed. Among women of childbearing age (the target group) it is now estimated that mean intakes of folic acid has increased to 90 µg per day which represents a 26% increase in intake over the past few years. This increased consumption of folic acid is in addition to intakes of naturally occurring food folate, which are unlikely to have changed significantly.

A comprehensive review of all health information, resources and folic acid health promotion and public awareness campaigns has been completed as part of the work of the implementation group. While recognising the improved folate status of the Irish population, there is still an ongoing need for effective programmes that promote the consumption of 400 µg of folic acid every day by women of childbearing age who are sexually active. It is generally agreed that the target figure of 400 µg of folic acid will prevent all folate/folic acid related NTDs. A request has been made to the Department of Health and Children to include key policy on folic acid supplementation and fortification in the forthcoming national nutrition policy. The implementation group also recommends that resources be provided in the 2009 estimates to support a comprehensive folic acid public education programme. Such health promotion activities should include school children.

CHAPTER 1: INTRODUCTION

In July 2006, the Minister for Health and Children accepted the recommendations of the National Committee for Folic Acid Food Fortification (NCFAFF, 2006). In order to move forward with these recommendations, the Minister set up an implementation group which was charged with putting the recommendations of the NCFAFF into operation. The FSAI was requested to chair the group. This report covers work carried out between November 2006 and June 2008, and updates the Interim Report of September 2007.

1.1 Background on Rationale for a Mandatory Folic Acid Food Fortification Programme in Ireland

In July 2006, all available evidence indicated that a mandatory folic acid food fortification programme would contribute significantly to a reduction in the numbers of babies born with NTDs in Ireland (NCFAFF, 2006). For over 30 years, Ireland has been generally recognised as having one of the highest rate of pregnancies affected by NTDs in the world. The cause of this particular vulnerability to the development of NTDs in Ireland has a genetic basis. Recent studies have shown that almost 50% of Irish people have variations in their genetic make-up which involves genes that influence how folate is metabolised in the body. Such genetic variations may account for as many as 26% of NTD-affected births in Ireland (Kirke *et al*, 2004). The situation in other countries is different in that the overall incidence of NTDs is lower and many of the affected pregnancies are terminated. Termination of pregnancy is illegal in Ireland and as a consequence, Ireland has a much higher burden of disease when compared to other countries. Research carried out by the Health Research Board shows that the proportion of reported pregnancies affected by NTDs that are delivered as live births is much higher in Dublin (81%) compared with other centres, for example Paris (11%) and North London (13%) (Kirke, 2006). This places a greater onus on Ireland to maximise the primary prevention of these serious birth defects.

Since 1991, it has been known that up to 70% of NTDs can be prevented by the consumption of folic acid prior to and at the time of conception. From the mid-1990s, Ireland, in common with many other countries, had a policy of advising women of childbearing age to take folic acid supplements. However, experience has shown that this policy has only had a marginal impact on NTD rates. This is mainly due to the fact that up to 50% of pregnancies are unplanned and that folic acid must be taken at the very early stages of pregnancy. Taking folic acid when pregnancy is suspected is generally too late to prevent NTDs because the neural tube is completely developed 21–28 days post conception.

Mandatory fortification of foods has been introduced in about 40 countries worldwide. The practice of mandatory folic acid fortification of cereal grains and flour was introduced in North America in 1998. Research has shown that such food fortification has resulted in a 20–70% reduction in the rate of pregnancies affected by NTDs. The level of folic acid fortification of bread proposed for Ireland (120 µg per 100 g of bread) would deliver a similar amount of folic acid to women of childbearing age to that provided to North American women through their mandatory fortification of flour.

1. 2 Work Plan for Implementation of a Mandatory Folic Acid Food Fortification Programme in Ireland

There were seven main recommendations made by the NCFAFF, all of which relate to the main recommendation that most bread in Ireland should be fortified with 120 µg folic acid per 100 g bread. The work plan for implementation of the mandatory folic acid food fortification programme involved carrying out all of the actions included in the recommendations. The recommendations and actions required are outlined in the Executive Summary of the NCFAFF Report, 2006 (Appendix 1).

In accordance with these recommendations, the Minister for Health and Children set up an implementation group in October 2006 to oversee the operational issues associated with mandatory folic acid fortification of bread and to report on progress. The implementation group developed a work programme and established working groups to address actions required under specific recommendations. Each working group was chaired by a member of the implementation group and experts were invited onto working groups by the chairs as appropriate.

Work was carried out in the following broad areas:

1) assessment of legislation for the mandatory addition of folic acid to most breads in Ireland and to establish a National Congenital Birth Defects Register

2) monitoring and surveillance work to determine the exact numbers of pregnancies affected by NTDs nationally; to determine the folate status of the Irish population; and to determine folic acid levels in foods on the Irish market

3) to determine the appropriate point during the bread-making process where the addition of folic acid should occur, and to estimate the amount of folic acid that needs to be added to achieve the desired level of 120 µg folic acid per 100 g bread

4) address the new health promotion requirements of health professionals and the general public regarding the need for women to take folic acid supplements

5) review of scientific developments relevant to folic acid food fortification—especially regarding emerging information on the links between elevated levels of folic acid intake and cancer.

1.3 Priorities in Preparatory Work Pre-fortification

A priority for the implementation group was to establish baselines on several important indicators of folate status before initiating a mandatory folic acid food fortification programme in Ireland. These indicators included:

1) accurate information on the numbers of pregnancies affected by NTDs

2) information on the blood levels of folate (folate status) of the Irish population

3) assessment of current folic acid intakes from fortified foods and supplements

4) assessment of current level of folic acid fortification of bread marketed in Ireland; and

5) determination of the optimum point of addition of folic acid during the bread-making process and assessment of losses of folic acid during baking.

Through the experience of others, the need for a science-based plan for monitoring (as part of mandatory folic acid food fortification) has been highlighted as being critically important. One of the key lessons learned during the implementation of national folic acid food fortification programmes in the US and Canada concerned the need for a comprehensive evaluation plan to monitor the effectiveness and safety of the fortification intervention (Rosenberg, 2005). In 2006, the NCFAFF recognised that some of the data available to them needed to be up-dated to ensure Ireland had an accurate pre-fortification assessment of all factors that would be affected by mandatory folic acid fortification. All of the actions prioritised by the implementation group concerned collecting any outstanding information and establishing a reliable picture of pre-fortification Ireland against which the effects of mandatory folic acid fortification of most bread can be measured.

1. 4 Evaluation of Scientific Developments Relevant to Folic Acid Fortification

Safety is the most important consideration involved in proceeding with mandatory folic acid fortification of bread in Ireland. At the time of making the recommendation to proceed with mandatory folic acid food fortification, the only established risk of excessive folic acid intake was the potential to mask symptoms of anaemia[1] associated with vitamin B_{12} deficiency. However, there is an internationally recognised safe upper level (tolerable upper level or UL) of folic acid intake established in relation to the risk of masking vitamin B_{12} deficiency. The level of folic acid fortification recommended (120 µg folic acid/100 g of most bread in Ireland) took account of this safe upper level and ensured there was a margin between the levels of folic acid that could potentially be consumed by the highest bread eaters and the UL. The main role of the implementation group was to ensure a safe level of folic acid distribution across all population sub-groups. The detailed pre-fortification monitoring assessments (level of voluntary folic acid food fortification, blood folate levels within population sub-groups and precise information on the prevalence of pregnancies affected by NTDs) were crucial for estimating the level of folic acid needed.

All other potential effects of folic acid food fortification had been examined in detail by the NCFAFF. There was good evidence that folic acid food fortification does not increase the rate of twinning or interact with drug treatments. However, there was insufficient evidence for the Committee to clarify some of the other potential effects reported in the literature. Therefore, the implementation group continued to evaluate scientific developments, especially in relation to the following:

1) **cardiovascular disease**—folic acid food fortification is associated with a reduced risk of stroke (Wang *et al*, 2007; HOPE, 2006; Spence *et al*, 2005) and may, through its homocysteine-lowering effects, exert some protection against coronary heart disease (Wald *et al*, 2006)

2) **cancer risk**—for some time, available evidence on dietary intakes of folate (natural and synthetic) pointed to a protective effect against cancer (Kim, 2004a and b). However, the emergence of new data indicate that the amount of folic acid consumed (adequate but not excessive) may be particularly important in ensuring beneficial, and not negative, effects in relation to cancer risk (Mason *et al*, 2007). These recent data emphasises the importance of ensuring a safe level of folic acid intake across the population.

New data on these areas are reviewed in relation to the plan for mandatory folic acid fortification in Chapter 8 of this report.

[1] Megaloblastic anaemia affects size of red blood cells and is one of the first signs of deficiency of vitamin B_{12}, but very large doses of folic acid can correct this anaemia. With the masking of this important sign of B_{12} deficiency, irreversible neurological damage due to B_{12} deficiency proceeds unnoticed.

CHAPTER 2: ASSESSMENT OF LEGISLATION

Two of the key recommendations from the NCFAFF (2006), related to the development of legislation for the mandatory addition of folic acid to most bread in Ireland, and for the establishment of a National Registry of Congenital Anomalies.

2.1 Legislation for the Mandatory Addition of Folic Acid to Bread

In the report of the NCFAFF in 2006, a strong case was made for mandatory fortification of most bread on the Irish market at a level of 120 µg/100 g of bread. Since this recommendation was made, new scientific evidence has emerged that possibly links high intake levels of folic acid with risks of various types of cancer, particularly colon cancer (Cole *et al*, 2007; Mason *et a*l, 2007 and 2008). These new data question the safety of folic acid food fortification.

It is the view of the implementation group that all new evidence must be evaluated and that the decision to proceed with mandatory fortification of bread with folic acid should be based on the best and most up-to-date scientific evidence available. New studies are under way and results will be available in 2009 that should clarify if there is an increased risk that links dietary folic acid with cancer. The decision to introduce mandatory fortification of bread in Ireland should be put on hold until definitive data are available on the safety of this initiative.

2.2 Legislation for the Establishment of a National Registry of Congenital Anomalies

In addition to recommending that legislation be introduced for the mandatory fortification of bread with folic acid, the report recommended that a national registry of all pregnancies affected by NTDs should be established and that there should be national surveillance of all congenital anomalies. This would allow monitoring of NTDs on an ongoing basis.

2.2.1 Reasons for congenital birth defects surveillance

Of the 63,000 annual births in Ireland, there are approximately 1,400 congenital anomalies. The reasons for establishing a congenital anomaly register include the facilitation of planning and the evaluation of health services. Examples include the monitoring of NTD rates in the evaluation of primary prevention strategies such as periconceptional supplementation to prevent NTDs; and monitoring rates of congenital rubella syndrome rates in the evaluation of vaccination against rubella to prevent congenital rubella syndrome. Congenital anomaly registers could also have a role in planning of paediatric, rehabilitative and other services.

Population-based registries are a particularly powerful tool for the evaluation of health services. They represent the experience of a whole community, not just the outcomes of specialist units which may serve only a selected group of women or children, or which may have atypical human or financial resources. Many birth defect registries in Europe have been set up to provide a mechanism for the audit of pre-natal screening practice. In addition to providing post-natal data on congenital anomalies, the registry can provide pre-natal diagnosis data, as well as related information about pre-natal screening methods. Such a register would also be a powerful tool for research and surveillance of the environmental causes of congenital anomalies, and could give an early warning of new teratogenic (harmful to the developing foetus) exposures. In this way, birth defects are registered not only for their intrinsic importance, but as indicators of other adverse pregnancy outcomes linked to teratogenic exposures such as spontaneous abortions and neurobehavioural outcomes that are not as easily detectable using routinely gathered statistical data.

In its progress report to the Department of Health and Children in 2008, the FSAI recommended that legislation be drafted to allow for active surveillance of congenital anomalies in Ireland. The suggested legislation is analogous to that currently in existence for the mandatory provision of information under the Infectious Diseases Regulations.

2.2.2 Specific requirements for Congenital Births Defects Register in Ireland: why legislation is necessary for congenital anomaly surveillance?

Data protection rules place restrictions on the obtaining and processing of personal and sensitive data. In an ideal world, consent from the individual to obtain and process their personal health/morbidity data should be sought and obtained. There are specific exceptions to these data protection rules, only applicable in situations where it is impossible for the subject to give consent, e.g. due to incapacity, or in an emergency situation. For the purposes of gathering data for a disease registry, e.g. a congenital birth defects surveillance registry, these specific data protection exceptions are not relevant.

There are a range of practical issues that arise with the three main possibilities for data collection:

1) consent—experience has shown, in the field of congenital anomalies, as well as others, that participation rates can be reduced by up to 20% when consent is sought. This has been the experience in congenital anomaly registries in Europe, e.g. the Netherlands, and in studies of specific congenital anomalies in Ireland. As some congenital anomalies are rare, e.g. five per year, missing one or two through absence of consent can greatly diminish ascertainment. Difficulties inherent in seeking consent, as outlined, can thus result in data that are inaccurate and open to serious misinterpretation, or virtually unusable

2) anonymisation—there have been suggestions that the use of a number of anonymised items of data may be sufficient to allow surveillance of congenital anomalies. In practice however, this has been found to be an ineffective means of accurate data collection and it is insufficient for validation of cases due to the large number of annual births. This method is not used by any of the 30 congenital anomaly registries throughout Europe. Using the name and address of the patient is essential for validation of cases, thus ensuring that each case is not double-counted or that no case is missed. These personalised data are also necessary in the investigation of congenital birth defects that cluster in a geographical area or occur at a particular time (temporal or geographical clusters of anomaly). For instance, an increase in the number of Down Syndrome cases in a small geographical area would be missed without personalised data. Anonymisation is an inadequate and ineffective method in data collection for congenital anomalies

3) legislation—because of data protection rules and participation problems when consent is sought, a more effective means of gathering congenital anomaly data is necessary. Legislation provides the most effective backdrop for data collection in the health arena. In general, legislation can be in two forms allowing for the gathering of specific personalised health/morbidity data by the authorised body and that requiring mandatory reporting to the authorised body of personalised health/morbidity.

In Ireland, surveillance of infectious diseases is covered by mandatory reporting legislation. On the other hand, legislation covering cancer surveillance and gathering of personalised data for cervical and breast cancer screening is not mandatory. In some European countries, such as Finland, there is legislation requiring mandatory reporting of congenital anomalies. From work completed by the implementation group it is clear that the reporting system required for a National Congenital Anomaly Register in Ireland needs to be mandatory. It is widely recognised that the voluntary reporting system in place in Ireland for many decades for recording congenital birth defects (EUROCAT) underestimated the true prevalence. This is mostly due to the non-availability of termination of pregnancy in Ireland.

The Department of Health and Children's report "Health Information - A National Strategy", published in 2004, proposed a legislative framework for surveillance of congenital anomalies (as well as other morbidities). As with other disease surveillance, legislation is therefore necessary to ensure effective collection of data on congenital anomalies in Ireland. Legislation along the lines of that covering the surveillance of infectious diseases, which requires mandatory reporting of cases, would likely be the most appropriate. In addition to legislation, adequate resources must be allocated to ensure comprehensive recording of all cases. From the work of the implementation group it is clear that a robust system will require some resources at each obstetric centre in Ireland to facilitate identification of cases from ultrasound, birth, paediatric medical records and pathology data. In addition, central cross checking data sources of cases recorded will be required.

Having considered the issues, the implementation group recommends that legislation should be introduced as part of the proposed Health Information Bill that will require the HSE to establish a National Congenital Anomaly Registry. This should include requirements for the HSE to:

(a) on a mandatory basis identify, collect, classify, record, store and analyse information relating to the incidence and prevalence of congenital anomalies in Ireland

(b) collect, classify, record and store information in relation to each newly diagnosed individual congenital anomaly and in relation to the pregnancy wherein that congenital anomaly occurred

(c) promote and facilitate the use of the data thus collected in approved research projects and in the planning and management of services

(d) publish an annual report based on the activities of the Registry; and

(e) furnish advice, information and assistance in relation to any aspect of such service to the Minister.

CHAPTER 3: MONITORING THE INCIDENCE OF NEURAL TUBE DEFECTS IN IRELAND

3.1 Background

Bringing about a reduction in the number of pregnancies affected by NTDs is the primary objective of mandatory folic acid food fortification of bread in Ireland. However, accurate data on rates of pregnancies affected by NTDs in Ireland are crucial for assessing the effectiveness of the fortification programme. The report from the NCFAFF (2006) showed that such data are lacking mainly because Ireland has never had a national register for collecting national data on congenital birth defects. From the limited data available, the incidence of NTDs in Ireland has been estimated to have declined over the past two decades from a high of 4.8 per 1,000 births in 1980 to a rate of between 0.8–1.5 in every 1,000 births in 2001 (McDonnell *et al*, 2004). The actual true incidence of affected pregnancies in Ireland is certain to be higher due to the lack of information on all affected pregnancies. Procurement of data that provides a reliable assessment of all pregnancies in Ireland affected by NTDs was a priority recommendation of the NCFAFF in 2006.

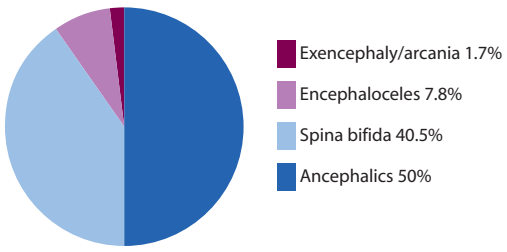
3.2 Monitoring the Incidence of NTDs

The implementation group recognised that the collection of reliable data on the actual incidence of NTDs in Ireland was essential for assessing the effectiveness of the planned mandatory folic acid food fortification programme. These data were required urgently because accurate information on numbers of pregnancies affected by NTDs *before* the implementation of mandatory folic acid fortification is critical for establishing an accurate baseline against which changes could be measured.

In order to obtain accurate data on the incidence of NTDs in Ireland, a monitoring project was initiated for a two-year period (2005 to 2006) and data were collected on NTDs diagnosed in all 21 obstetric units in Ireland. The project was led by Dr Sean Daly, Master of the Coombe Women's Hospital, and funded by the FSAI. All NTDs that were diagnosed ante-natally were confirmed by ultrasound reports. Cases were assigned a unique identity code and those referred from referral centres were followed-up to ensure they were not counted twice. Information was collected on the type of NTD and pregnancy outcome. National birth statistics were retrieved for the two-year period and used to calculate rate of pregnancies in Ireland that were affected by NTDs.

The total number of births in Ireland for the two-year period, January 2005 to December 2006, was 125,279 (61,042 in 2005 and 64,237 in 2006). During this period a total of 116 NTDs were recorded which gives an incidence rate of 0.93 per 1,000 births. Of these there were 58 anencephalics (50%), 47 cases of spina bifida (40.5%), nine encephaloceles (7.8%) and two cases (1.7%) of exencephaly/acrania (Figure 1). Fifteen (12.9%) of the cases identified died *in utero*. Over these two years, 29 (25%) of the NTD-affected pregnancies were terminated—most of these cases were anencephalic.

Figure 1: NTD-affected Births, January 2005 to December 2006 (n=116)



It is surprising to find that spina bifida accounted for only 40% of all NTDs, although these are the cases which are easiest to identify. The proportion of the more severe anencephaly cases is higher than previously observed in Ireland. Babies born with anencephaly will not survive. The data collected in 2005 and 2006 show that the majority of terminated pregnancies were affected by anencephaly. It is therefore concluded that the higher rates of anencephaly recorded in 2005 and 2006 are likely to be due to the inclusion, for the first time, of data on terminations.

Overall, the data have shown that a significant reduction has occurred in the prevalence of NTDs in Ireland over recent years. Data on affected births from 2001 suggested that the rate of NTDs was between 0.8 and 1.5 per 1,000 births. However, this was recognised as being an under-estimate of the actual numbers of pregnancies affected because there were no data available on the numbers that were terminated (termination is not a legal option in Ireland). Monitoring rates of pregnancies affected (rather than births only) during 2005 and 2006 indicate that 25% (29 cases) were terminated. Assuming a similar termination rate for affected pregnancies in 2001 (i.e. the addition of an extra 25% of cases to the numbers of affected births actually recorded for 2001) indicate that over the five year period from 2001 to 2006, rates of NTD-affected pregnancies have fallen from between 1.25–1.88 to 0.93.

A number of factors may be involved in the observed decrease in rates of affected pregnancies. In recent years, there has been a substantial increase in the amount of folic acid in the Irish food supply due mainly to the increased range and numbers of foods voluntarily fortified and the amounts of folic acid added to foods has also increased dramatically (discussed elsewhere in this report). In addition, the increased proportion of births to non-indigenous Irish can be expected to dilute the rate of pregnancies affected by NTDs. Because of particular genetic traits, the Irish as a race are particularly vulnerable to the development of NTDs. Finally, although rates of NTDs fluctuate as is apparent in all of the EUROCAT registries (Botto *et al*, 2005), this is an unlikely explanation for the decreased rate observed.

These data provide an accurate baseline on the incidence of NTDs in Ireland which was necessary to obtain prior to the introduction of a mandatory folic acid food fortification programme. These data also provide strong evidence on the need to include information on all affected pregnancies in order to have accurate ascertainment of NTD rates.

3.3 Conclusions

1) **Over the last five years, the rate of pregnancies affected by NTDs in Ireland has significantly declined. This is likely to be due to an increased amount of folic acid in the food supply from the increasing range of foods voluntarily fortified and the greater amounts of folic acid added.**

2) **The accurate monitoring of the numbers of pregnancies affected by NTDs needs to continue—especially considering the variability and fluctuating amounts of voluntary folic acid food fortification. It is essential that this monitoring records total number of pregnancies affected rather than births only and emphasises the need to establish a Congenital Birth Defect Register where reporting of cases is mandatory.**

CHAPTER 4: FOLATE STATUS OF THE IRISH POPULATION

4.1 Background

Monitoring actual blood levels of folate within various population sub-groups was recognised as a priority by the implementation group prior to fortification, as this establishes a baseline estimate of folate status pre-fortification. After the introduction of a mandatory food fortification programme, a sharp rise would be expected in blood parameters such as serum and red cell folate within a relatively short timeframe. Therefore, blood status of these indicators can provide an early estimation of the impact of food fortification in the various population subgroups. In addition, long-term blood monitoring of actual blood levels of folate is important as it provides excellent surveillance of the exposure of various population sub-groups (women of childbearing age, the elderly and children) to folic acid in the food supply. More comprehensive blood monitoring of parameters closely related to folate (vitamin B_{12}, homocysteine and genetic markers of folate metabolism) provides quantifiable information on the effects of folic acid food fortification on various age and sex groups within the population.

The assessment of blood folate status was also recognised by the implementation group as being a critical component of monitoring to determine the impact of the fortification programme and to ensure its safety. Therefore, assessment of blood levels of folate and other relevant parameters in various population sub-groups prior to initiation of the fortification programme was considered an important priority.

4.2 Folate Status of Sub-groups of the Irish Population

The objective of the project was to provide background data on the folate status of specific sectors of the Irish population in preparation for the introduction of folic acid fortification in Ireland. A valid approximation of the population's folate status was obtained by sampling particular age and sex groups in the greater Dublin area. The sub-groups of the population who were considered to be most relevant and where they were recruited is outlined below:

1) **non-pregnant, healthy adult women, aged 16 to 65 years old**—this group (*n*496) were mainly recruited from women presenting for routine gynecological testing in the out-patient clinics in the Coombe Women's Hospital. Women of childbearing age were defined as being aged between 16 and 40 years. Some women recruited from the Irish Blood Transfusion Board (see 3 below) and several girls over the age of 16 years recruited from the children's hospital out-patient clinics (see 2 below) were included in this childbearing age group

2) **children up to 16 years old**—this group (*n*215: 110 males and 105 females) were recruited from those having blood drawn for medical reasons that do not impact on folate status. They were all attending the out-patient clinics in the children's wing of Adelaide and Meath National Children's Hospital, Tallaght. The group included five boys who were 16 years of age.

3) **healthy adult men, aged 16–65**—this group (*n*292) were recruited from platelet donors through the Irish Blood Transfusion Board

4) **healthy older adults**—this group (*n*462) were recruited from healthy, free-living elderly men and women participants of the Dublin Healthy Ageing Study. The average age of this group was 75 years and the age range was 65 to 95 years. Just over half (55%) of this group were female. Blood samples from this sub-group were limited to serum samples only[2].

Samples were collected during 2006 and 2007.

[2] Recruitment of older healthy adults for this project from General Practitioner clinics was attempted but this proved too difficult. The cohort of participants in the Dublin Healthy Ageing Study was an opportunistic sample.

4.3 Blood Parameters Assessed

The following blood parameters were assessed on all samples with the exception of the elderly adult cohort[3]:

1) total serum (or plasma) folate which is indicative of levels of circulating folate/folic acid. While it is indicative of folate status, levels of plasma folate can be impacted by the level of folate/folic acid in the last meal. There is concern that high circulating levels of plasma folate may be in the form of folic acid ('free folic acid') and that this may be associated with increased risk of cancer[4]

2) red cell folate which is indicative of folate status

3) total serum (or plasma) vitamin B_{12} which is a marker of B_{12} status

4) total serum (or plasma) homocysteine (tHcy) which is recognised as a risk factor for heart disease and can be indicative of folate status as levels tHcy rise when folate status is reduced

5) determine 5,10-Methylenetetrahydrofolate Reductase 677C→T genotype, a common polymorphism of the gene encoding for an important enzyme involved in folate metabolism—this polymorphism is associated with increased risk of developing NTDs.

4.4 Analytical Methods

Collection and analysis of blood samples was undertaken by the Vitamin Research Laboratory, Trinity College, Dublin. Appropriate intra- and inter-assay quality control estimations were carried out throughout the entire period of analysis.

Estimates of total serum (or plasma) folate concentrations were carried out using the laboratory's validated microtitre plate microbiological assay based on the growth of chloramphenicol resistant *Lactobacillus rhamnosus*. Estimates of red cell folate concentrations were provided using the same validated microtitre plate microbiological assay. Estimates of total serum (or plasma) vitamin B_{12} concentrations were provided using the laboratory's in-house microbiological assay based on the growth of colistin resistant *Lactobacillus delbruckii*. Estimates of total serum (or plasma) homocysteine (tHcy) concentrations were provided using the laboratory's automated IMX analyser.

For analysis of the 5,10-Methylenetetrahydrofolate Reductase 677C→T genotype, DNA was extracted from the cell pellets using the well established commercially available Qiagen™ Mini-AMP extraction kits. The 677C→T genotype was then determined using a well documented method based on PCR amplification of DNA followed by RFLP with HinF1 restriction enzyme. Suitable ongoing quality control procedures were carried out, including repeats of a random 10% of individuals.

4.5 Age, Gender and Use of Folic Acid Supplements and Fortified Food

With the exception of the older adult cohort from the Healthy Ageing Study, data on age, gender and use of folic acid supplements and fortified food (factors which could strongly impact on folate status) was collected by questionnaire from individuals donating a blood sample.

1) Age was recorded in one of eight categories: (1) <3 years, (2) 3–7 years, (3) 8–15 years, (4) 16–25 years, (5) 26–40 years, (6) 41–65 years, (7) 66–75 years and (8) 76+ years.

2) Folic acid supplement use (either folic acid alone or as part of a multivitamin preparation) was recorded and, in users, frequency of intake categorised as everyday, several times/week, several times/month, or occasionally. Regular users were defined as taking folic acid containing supplements everyday or several times a week.

3) Fortified foods consumption (fortified breakfast cereals, fortified milk, fortified fat spreads) was recorded and, among users, frequency of intake was categorised as everyday, several times/week, several times/month, or occasionally. Although information was collected on bread intakes, consumption patterns of folic acid fortified brands were too complex to include in the assessment of fortified food intake. Therefore, regular users of folic acid fortified foods were defined as those taking brands of breakfast cereals, milk and fat spreads that were fortified with folic acid every day or several times a week.

[3] Due to the limited blood samples available for the older adult cohort, assessment was restricted to 1, 3, and 4 above.
[4] See Section 8.6. Factors influencing a protective role or a harmful role of folic acid in the development of cancer.

4.6 Folate Status of Healthy Adult Women, Including Those of Childbearing Age

A summary of the blood concentrations of all parameters assessed in women is given in Table 1, with data presented according to three age ranges—younger (16–25 years old) and older (26–40 years old) women of childbearing age and older women (41–60 years old). The data on folate status, as indicated by red cell folate and plasma folate, show that on average (median) the blood folate levels of these healthy adult women were well within the normal range. There was no difference between the three age groups of women in the status of any of the variables (plasma folate, red cell folate, B_{12} or homocysteine [tHcy]), although the well known increase in tHcy with increasing age was evident. Finally, the average (median) plasma folate level (8.3 ng/ml) found in these Irish women is substantially lower than the levels reported recently in American women where average levels reported are 12.5 ng/ml in women (NHANES data reported by Pfeiffer *et al,* 2007).

Table 1: Average (Median [range]*) Blood Concentrations of Parameters Assessed in Non-pregnant Healthy Adult Women

Age Range	Plasma Folate ng/ml Median [Range]	Red Cell Folate ng/ml Median [Range]	Plasma tHcy µmol/L Median [Range]	Plasma B_{12} ng/L Median [Range]
16–25 years (*n*39)	9.32 [2.6–82.8]	473 [56–843]	8.64 [6.0–14.2]	316 [123–541]
26–40 years (*n*220)	7.71 [1.4–35.0]	409 [149–1162]	9.60 [4.0–37.3]	307 [89–916]
41–65 years (*n*237)	8.55 [1.1–38.2]	418 [119–1703]	9.71 [5.0–46.0]	327 [109–808]

*Data were not-normally distributed and are presented as medians and interquartile ranges.

The distributions of plasma folate and red cell folate in women are shown in Tables 2 and 3. Less than 2% of women had deficient folate status (<2 ng/ml plasma folate or <150 ng/ml red cell folate) and just 5% to 7% were either deficient or borderline deficient (i.e. below 3 ng/ml of plasma folate or below 200 ng/ml red cell folate). Over 45% of the cohort had red cell folate levels that were lower than 400 ng/ml—a level previously shown to provide protection against NTDs (Daly *et al*, 1995). Nonetheless, an adequate status, if not optimal for preventing NTDs, was evident in 93% of women. As shown in Tables 2 and 3, just under 9% of women had high circulating levels of plasma folate (>20 ng/ml) and just under 3% having red cell folate levels in excess of 1,000 ng/ml.

The data on red cell folate indicate that the folate status of Irish women has increased significantly in recent years. In 1998, red cell folate levels assessed in adolescent schoolgirls (aged 14 to 17 years) in the same laboratory found 5% were folate deficient (i.e. red cell folate <150 ng/ml) (Ryan *et al*, 1998). The data reported here indicate that only 1% of women are now folate deficient (Table 3). Similarly, the proportion of women of childbearing age (16 to 40 years) that now have optimal levels of folate in terms of protection against the occurrence of NTDs (i.e. red cell folate >400 ng/ml) has more than doubled since 1998 (52% in 2007, compared with 25% in 1998).

Despite the rise in folate status, circulating levels of folate are lower in Irish women compared to women in the US, where mandatory folic acid fortification of flour has been in place for ten years. Comparing the recent blood data on distribution of plasma folate status of Irish women (Table 2) to similar data on American women, the proportion of Irish women with folate deficiency/borderline-deficiency is higher. According to recent US data, only 0.6% of women between the ages of 15 and 45 had serum folate <3 ng/ml (Pfeiffer *et al*, 2007). The red cell folate data are not comparable with US data due to difference in methodology used in the two countries.

Table 2: Distribution of Plasma Folate in Women

Plasma Folate	All Women (aged 16–60) *n*496	Women of Childbearing Age (aged 16–40) *n*259	Excluding Current Vitamin Users (aged 16–40) *n*214
Deficient ≤ 2 ng/ml (4.5 nM)	1.4%	1.5%	1.6%
Possible Deficiency 2–3 ng/ml	5.6%	5.4%	7.2%
Low-Adequate 3–10 ng/ml	51.6%	54.0%	56.0%
Adequate-High 10–20 ng/ml	32.7%	32.0%	29.5%
High >20 ng/ml (45 nM)	8.7%	6.9%	5.7%

Table 3: Distribution of Red Cell Folate in Women

Red Cell Folate	All Women *n*495	Women of Childbearing Age (aged 16–40) *n*259	Excluding Current Vitamin Users (aged 16–40) *n*214
Deficient ≤ 150 ng/ml (340 nM)	1.2%	0.8%	0.5%
Possible Deficiency 151–200 ng/ml	3.8%	5.4%	6.5%
Low-Adequate 201–400 ng/ml	41.2%	41.3%	41.6%
Adequate-High 401–1,000 ng/ml	50.9%	51.0%	50.0%
High >1,000 ng/ml (2,265 nM)	2.8%	1.2%	1.4%

Overall, 55% of women reported multivitamin use and of these, 28% were current users; but this differed across age groups, with some 38% of reported users in the 40–65 age bracket compared with 17% of 16–25 years old (Table 4). Surprisingly, exclusion of current vitamin users (defined as those who took multivitamins or folic acid everyday or several times a week) had only a small effect on folate status in this cohort (Tables 2 and 3), perhaps indicating that good folate status was achieved by many women through increased dietary folate and folic acid fortified food intake.

The reported use of fortified foods in this cohort is presented in Table 5. This indicates a widespread consumption of foods that contain folic acid, with about 80% of women taking cereals and 40–50% taking supermilk or fortified spreads. There was little difference by age group in the reported use of these fortified foods (data not shown). A recent study in Northern Ireland has shown that voluntary food fortification with folic acid can result in a substantial improvement in folate status in an Irish population (Hoey *et al*, 2007). The findings for healthy non-pregnant women in this study suggest that folic acid fortified food contributes significantly to folate status in Ireland.

Table 4: Multivitamin Use in Women

Age Range	% of Cohort	Multivitamin Users*	Current Users*
16–25 years	7.4% *n*34	41.2% *n*14	16.7%
26–40 years	45.8% *n*210	57.1% *n*120	24.1%
41–65 years	46.8% *n*215	55.8% *n*120	35.3%
Total	**100.0% *n*459**	**55.3% *n*254**	**28.3%**

* Values are calculated as the percentage of women in the same age range

Table 5: Fortified Food Intake Among Women

	Yes	No
Folic acid fortified cereal	81.4%	18.6%
Folic acid fortified fat spread	48.1%	51.9%
Folic acid fortified milk	40.5%	59.5%

4.7 Folate Status of Children

A summary of the blood concentrations of all parameters assessed in children is given in Table 6, with data presented according to three age ranges: infants and toddlers (aged up to two years), young children (aged three to seven years) and adolescents (aged eight to 15/16 years). Again, the average (median) folate levels were found to be well within the normal range for all age groups. There were no differences found between males and females for any of the parameters measured so all analyses were carried out on girls and boys combined. There were significant differences found according to age where with increasing age plasma folate, red cell folate and B_{12} levels declined and tHcy increased. Similar trends have been shown for children within these age groups in America and elsewhere (Chen *et al*, 2007; Pfeiffer *et al*, 2007; Monsen *et al*, 2003).

Table 6: Average (Median [range]*) Blood Concentrations of Parameters Assessed in Children

Age Range	Plasma Folate ng/ml Median [Range]	Red Cell Folate ng/ml Median [Range]	Plasma tHcy µmol/L Median [Range]	Plasma B_{12} ng/L Median [Range]
Up to 2 years *n*46	17.13 [1.9–39.5]	713 [333–1618]	6.69 [4.0–15.7]	675 [159–1818]
3–7 years *n*69	14.49 [3.2–43.7]	584 [242–1016]	6.64 [4.3–14.8]	624 [203–1442]
8–15 years *n*105	9.28 [1.4–32.6]	405 [86–873]	7.86 [3.9–20.3]	455 [141–1529]
ANOVA	P<0.0001	P<0.0001	P<0.0001	P<0.0001

Note: Data were not-normally distributed and are presented as medians and interquartile ranges

Overall, 43% of children reported multivitamin use and 18% were current users, as defined by use everyday or several times a week (Table 7). There was no reported use of folic acid alone among children. The relatively high current multivitamin use was possibly affected by the mid-winter timing of the collection and the fact that the children were recruited from those attending a hospital out-patient department. Such children may be more likely to be given supplements compared with the general child population. As expected, supplement use was lower among children ≤2 years old (9%) than in the 3–7 and 8–15 year groups (28% and 15% respectively).

Table 7: Multivitamin Use in Children

Age Range	% of Cohort	Multivitamin users*	Current users*
≤ 2 years	21% *n*46	20% *n*9	9%
3–7 years	31% *n*69	57% *n*39	28%
8–15 years	48% *n*105	45% *n*47	15%
Total	**100%** (*n*220)	**43%** (*n*95)	**18%**

* Values are calculated as the percentage of children in the same age range

The distributions of plasma folate and red cell folate in children are shown in Tables 8 and 9. Less than 1% of children were deficient and only 2.2% of children were in the borderline deficient range for folate status (i.e. plasma folate concentrations lower than 3 ng/ml or red cell folate below 200 ng/ml). However, a surprising finding in this study was the substantial number of children with high levels of circulating folate (plasma folate). As many as 25% of the children had plasma folate concentrations in the highest measurement category (>20 ng/ml), and this was not changed substantially by removing current vitamin users. These values indicate that the proportion of children with high levels of plasma folate is higher than that found in the USA, where folic acid fortification of flour has been in place since 1998. In the two most recent National Health and Nutrition Examination Survey (NHANES) cohorts (2001–2002 and 2003–2004) in the USA, the prevalence of serum folate over 20 ng/ml was close to 20%. Further research investigating how much circulating plasma folate is in unmetabolised form (free-folic acid), the extent of exposure (length of time) and how this relates to folic acid intake from fortified foods and supplements, needs to be carried out.

Overall, however, exclusion of current vitamin use in this study had almost no effect on plasma or red cell folate distributions. Again, this suggests that high folate status is the result of increased folic acid fortified food intake and possibly dietary food sources of natural folate. The reported use of fortified foods in this cohort is presented in Table 10. Over 90% of children above three years old consumed folic acid fortified cereals and in addition there was an increasing use of folic acid fortified milk and folic acid fortified fat spreads with increasing age-group.

Table 8: Distribution of Plasma Folate in Children

Plasma Folate	All Children *n*220	Age Range 3–15 years *n*174	Age Range 3–15 Years Excluding Current Vitamin Users *n*139
Deficient ≤ 2 ng/ml	0.9%	0.6%	0.7%
Possible Deficiency 2–3 ng/ml	1.4%	1.7%	2.2%
Low-Adequate 3–10 ng/ml	34.1%	38.5%	42.4%
Adequate-High 10–20 ng/ml	39.1%	39.1%	38.2%
High >20 ng/ml	24.5%	20.1%	16.5%

Table 9: Distribution of Red Cell Folate (RCF) in Children

Red Cell Folate	All Children *n*218	Age Range 3–15 Years *n*174	Age Range 3–15 Years Excluding Current Vitamin Users *n*138
Deficient ≤ 150 ng/ml	0.5%	0.6%	0.7%
Possible Deficiency 151–200 ng/ml	1.8%	2.3%	2.2%
Low-Adequate 201–400 ng/ml	27.5%	32.2%	34.5%
Adequate-High 401–1,000 ng/ml	66.5%	64.4%	62.6%
High >1,000 ng/ml	3.7%	0.5%	0%

Table 10: Fortified Food Intake among Children

Age	Folic Acid Fortified Cereal Users	Folic Acid Fortified Milk Users	Folic Acid Fortified Fat Spread Users
0–2 years	83%	11%	13%
3–7 years	94%	15%	12%
8–15 years	91%	22%	36%

4.8 Folate Status of Adult Males up to Age 65

A summary of the blood concentrations of all parameters assessed in healthy adult men under the age of 65 years is given in Table 11, with data presented according to three age ranges—adolescents and young men (16 to 25 years old), young men (26 to 40 years old) and middle aged men (41 to 65 years old). Average (median) plasma folate was considerably lower in men compared with the women or children but the average red cell folate level was similar. There was a trend towards higher tHcy with increasing age. There was also an apparent increase in red cell folate with age but this could well be an artefact of the low number of samples collected from the youngest age category, i.e. the adolescent and young men aged 16 to 25 years.

Table 11: Average (Median [range]*) Blood Concentrations of Parameters Assessed in Men

Age Range (years)	Plasma Folate ng/ml Median [Range]	Red Cell Folate ng/ml Median [Range]	Plasma tHcy µmol/L Median [Range]	Plasma B_{12} ng/L Median [Range]
16–25 *n*7	6.00 [3.3–9.3]	331 [308–588]	10.63 [8.4–14.3]	331 [281–526]
26–40 *n*93	5.72 [1.2–31.7]	459 [205–1100]	11.78 [6.8–18.7]	306 [172–668]
41–65 *n*192	6.67 [1.9–33.5]	499 [160–1271]	12.50 [7.0–21.0]	[126–968]
ANOVA	P=0.10	P=0.057	P=0.055	P=0.14

Note: Data were not-normally distributed and are presented as medians and interquartile ranges

The distributions of circulating folate (plasma folate) and red cell folate in the men under 65 years old are shown in Table 12. Despite lower plasma folate status, no men had red cell folate concentrations in the deficient range, 1% were in the borderline deficient range while some 70% were well within the adequate range. A much lower proportion of men under 65 years (just over 2% had high circulating folate levels (i.e. plasma folate >20 ng/ml) compared with children (25%) and women (6–9%).

Table 12: Distribution of Plasma Folate and Red Cell Folate in Men

Plasma Folate	Adult Males Age ≤ 65 *n*292	Red Cell Folate	Adult Males Age ≤ 65 *n*292
Deficient ≤ 2 ng/ml	1.7%	**Deficient** ≤ 150 ng/ml	0%
Possible Deficiency 2–3 ng/ml	11.6%	**Possible Deficiency** 151–200 ng/ml	1.3%
Low-Adequate 3–10 ng/ml	63.4%	**Low-Adequate** 201–400 ng/ml	33.6%
Adequate-High 10–20 ng/ml	20.9%	**Adequate-High** 401–1,000 ng/ml	64.4%
High >20 ng/ml	2.4%	**High** >1,000 ng/ml	0.7%

4.9 Folate Status Older Adults

A summary of the blood concentrations of all parameters assessed in healthy older adults (>65 years old) is given in Table 13. As seen in the other sub-groups above, average (median) levels were well within the normal range. Circulating (plasma) folate levels were lower in men than in women, as has been observed in other studies. However, no red cell folate data, which provides more reliable information on actual folate status, were available for these older adults. Men had higher tHcy compared with women while their vitamin B_{12} levels were similar.

Table 13: Blood Metabolite Concentrations in People over 65 years

Gender	Plasma Folate ng/ml Median [Range]	Red Cell Folate ng/ml Median [Range]	Plasma tHcy µmol/L Median [Range]	Plasma B_{12} ng/L Median [Range]
Male *n*187	12.50 [1.8–71.1]	**	12.92 [6.5–48.5]	306 [53–855]
Female *n*236	15.51 [1.7–288.6]	492** [227–1023]	11.56 [5.8–38.2]	342 [98–1190]
All Participants* *n*462	13.98 [1.7–346.3]	492 [227–1023]	12.32 [5.8–48.5]	324 [53–1190]

Note: Data were not-normally distributed and are presented as medians and interquartile ranges.
* Includes an additional 39 elderly participants with unreported gender
** Samples were not taken for red cell folate in the Dublin Healthy Ageing Study, where the majority of results were obtained. The results for females in this cohort relate to 15 elderly women recruited in the Coombe Women's Hospital

The distribution of circulating folate (plasma folate) levels is shown in Table 14. Unexpectedly, a high proportion of these older adults (just over a third) were found to have circulating (plasma) folate concentrations above 20 ng/ml. This is particularly

surprising given the low rates of reported use of multivitamins and fortified foods by this group (Table 15). This study did not determine how much of the circulating (plasma) folate was in the form of unmetabolised folic acid. However, given the high levels found among this cohort of older adults, it is likely a significant proportion will be in unmetabolised form, which may accelerate growth of existing cancerous tumours (see Chapter 8). Considering that older adults are at high risk of having cancerous tumours, the finding that over a third had high circulating (plasma) folate levels is a matter of concern. Further work should determine how much circulating (plasma) folate is in unmetabolised form, the extent of exposure (in terms of length of time levels are high) and how this relates to folic acid from fortified foods and supplements.

Table 14: Distribution of Plasma Folate in People over 65 Years

Plasma Folate*	Mixed Adults Age 65–95 years *n*462
Deficient ≤ 2 ng/ml	0.7%
Possible Deficiency 2–3 ng/ml	1.8%
Low-Adequate 3–10 ng/ml	31.5%
Adequate-High 10–20 ng/ml	31.7%
High >20 ng/ml	34.3%

*Samples were not taken for red cell folate (RCF) in the Dublin Healthy Ageing Study, where the majority of results for adults over 65 years were obtained. RCF results are available for 15 elderly women recruited in the Coombe Women's Hospital but the distributions are not included because of the small number

Table 15: Multivitamin and Fortified Food Use in Older Adults

	Yes	No
Multivitamins	11.4%	88.6%
Fortified Foods	23.8%	76.2%

In older adults, the distribution of vitamin B_{12} is of particular interest and this is outlined in Table 16. Some 16% of older people had deficient or possibly deficient B_{12} status. A combination of low vitamin B_{12} and very high folate status has recently been highlighted as a risk factor for more rapid decline of cognitive function (Morris *et al*, 2007), so the current results for older people may be of concern and warrant further monitoring (Chapter 8, Section 8.3).

Table 16: Distribution of Vitamin B_{12} in People over 65 Years

Vitamin B_{12}	Mixed Adults Age >65 years
Deficient ≤ 150 pg/ml	7.3%
Possible Deficiency 151–200 pg/ml	8.7%
Low-Adequate 201–300 pg/ml	27.1%
Adequate-High 301–1,000 pg/ml	56.5%
High >1,000 pg/ml	0.4%

4.10 General Comments

The folate status across the different population sub-groups examined are outlined for comparison in Tables 17 and 18. Plasma folate provides information on blood circulating folate levels, but can be distorted by recent food intake (levels will respond to increased folate intake—especially folic acid in food or supplements). Therefore, it must be noted that since none of these blood samples were fasting samples, the plasma folate levels outlined in Table 17 could have been increased by recent intake of a folic acid fortified meal—for example, the children may have had a folic acid fortified cereal for breakfast a few hours before their blood samples were drawn. Further work needs to determine how much of this is in unmetabolised form and how this relates to folic acid intake from fortified foods and supplements.

Table 17: Summary of Plasma Folate Distributions in all Cohorts

	All Women Age≤ 65 *n*496	All Children Age<16 *n*220	All Men Age≤ 65 *n*292	Older adults 65 to 95 years *n*462
Deficient ≤ 2 ng/ml	1.4%	0.9%	1.7%	0.7%
Possible deficiency 2–3 ng/ml	5.6%	1.4%	11.6%	1.8%
Low-Adequate 3–10 ng/ml	51.6%	34.1%	63.4%	31.5%
Adequate-High 10–20 ng/ml	32.7%	39.1%	20.9%	31.7%
High >20 ng/ml	8.7%	24.5%	2.4%	34.3%

The red cell folate levels outlined in Table 18 provide a more reliable indication of actual folate status. However, the red cell folate distributions found in this study suggest that widespread use of voluntary fortified foods has made an important impact on the general folate status of the population. It is noteworthy that the main target group for folic acid food fortification, women of childbearing age, tended to have lower folate status than other groups as determined by red cell folate levels.

Table 18: Summary of Red Cell Folate Distributions in all Cohorts*

	All Women Age≤ 65 *n*495	All Children Age<16 *n*218	All Men Age≤ 65 *n*292
Deficient ≤ 150 ng/ml	1.2%	0.5%	0%
Possible Deficiency 151–200 ng/ml	3.8%	1.8%	1.3%
Low-Adequate 201–400 ng/ml	41.2%	27.5%	33.6%
Adequate-High 401–1,000 ng/ml	50.9%	66.5%	64.4%
High >1,000 ng/ml	2.8%	3.7%	0.7%

*No red cell folate data are available for older adults (66–95 years)

In general, the vitamin B_{12} status of the study group was adequate, although every cohort, including children, contained some samples suggesting low vitamin B_{12} status. As expected and discussed earlier, older adults had the highest prevalence of low B_{12} and this should be watched carefully in the context of increasing trends of food fortification with folic acid.

4.11 Additional Findings

4.11.1 Homocysteine (tHcy) levels and risk of cardiovascular disease

The plasma tHcy concentration is a sensitive marker of low folate or vitamin B_{12} status, with lower status giving rise to higher plasma tHcy levels. This was evident in this study (data not shown). Of interest was the finding that among children and older adults where folate levels were high, vitamin B_{12} emerged as the more important determinant of tHcy. In general, tHcy values above 15 µmol/L are indicative of hyperhomocysteinemia, which is a risk factor for cardiovascular disease. Using this cut-off, the prevalence of hyper homocysteinemia was highest among older adults (affecting 29%) and lowest in children (affecting <1%), while it was intermediate in men under 65 years of age (affecting 13%) and among women of childbearing age (affecting 5%).

4.11.2 Impact of genetic make-up on folate status and tHcy

The other factor that has an impact on both folate and tHcy is the prevalence of a genetic variation in one of the genes involved in how the body handles folate (i.e. the MTHFR 677 C→T polymorphism). The MTHFR genotype was assessed in this study. Table 19 summarises the average (median) blood folate and tHcy levels according to MTHFR genotype for children, men and women younger than 65 years of age in this study. Overall, 12.6% of subjects were homozygous for the TT variant—a prevalence level that is comparable with that found in previous studies carried out in Ireland. Consistent with many studies to date, homozygosity for the TT variant was found in this study to be associated with lower red cell folate concentrations and higher plasma homocysteine (Table 19). As shown previously, TT individuals represent the group that remain at most risk of low folate status in the Irish population (Molloy *et al*, 1997).

Table 19: Effect of the MTHFR 677C→T polymorphism on Folate Related Blood Metabolites

Genotype	Plasma Folate ng/ml Median [Range]	Red Cell Folate ng/ml Median [Range]	Plasma tHcy µmol/L Median [Range]
CC n447 (41.8%)	8.30 [1.1–35.4]	475 [56–1618]	9.15 [3.9–25.0]
CT n488 (45.6%)	7.97 [1.5–92.8]	449 [149–1703]	10.14 [3.3–40.4]
TT n135 (12.6%)	7.86 [1.4–38.2]	358 [86–1132]	10.17 [4.6–46.0]
ANOVA	P=0.53	P<0.0001	P<0.0001

Note: Data are combined for all participants on whom genotype was available. Genotype analysis was not carried out on older adults in the Dublin Healthy Ageing Study. Data were not-normally distributed and are presented as medians and interquartile ranges.

4.12 Conclusions and Recommendations

1. Blood folate levels in all sub groups of the Irish population examined have increased substantially in recent years.

2. Folate deficiency is uncommon affecting less than 2% of people examined in this study.

3. An unexpected finding in this study was the high proportions of children (a quarter) and older adults (over a third) with high circulating levels of folate (measured as plasma folate in this study). However, given the high levels found, it is likely a significant proportion will be in unmetabolised form, which may accelerate growth of existing cancerous tumours (see Chapter 8). Older adults are a high risk group for the existence of cancerous tumours.

4. Further work is urgently needed to determine how much circulating (plasma) folate is in unmetabolised form. This is almost certainly associated with the levels of folic acid consumed from fortified foods and supplements. Good information on folic acid intake and how this relates to length of time for which blood circulating levels of unmetabolised folic acid extend, is urgently required to reduce such exposure to a minimum. This would retain most if not all of the benefits (i.e. prevention of NTDs and possibly cardiovascular disease) but reducing the cancer risk to a minimum.

   The high proportion of people with high folate status found in this study is most likely due to a widespread voluntary folic acid fortification of food.

   However, the target population sub-group for folic acid food fortification—women of childbearing age, tended to have lower folate status (as determined by red cell folate levels) compared with other groups in this study.

CHAPTER 5: EVALUATING THE CURRENT STATUS OF VOLUNTARY FOOD FORTIFICATION AND ITS IMPACT ON FOLIC ACID INTAKE

5.1 Background

The food supply is continuously evolving and over the past few years there has been an upsurge in foods that are voluntarily fortified with folic acid on the Irish market. Regular information on food consumption and dietary folate intakes of all population sub-groups is essential for managing the effectiveness and safety of folic acid food fortification.

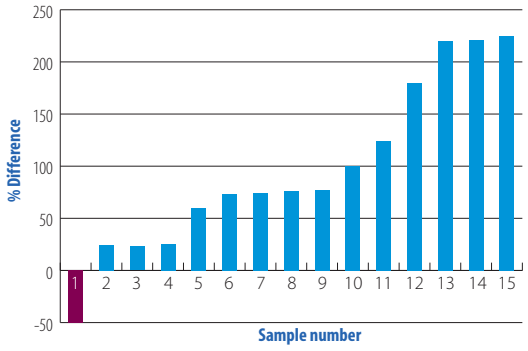
5.2 Bread Survey

Monitoring by the FSAI has shown that approximately 25% of bread on the Irish market is already fortified with folic acid on a voluntary basis. This voluntary addition of folic acid in bread is mainly carried out through fortification of the flour improver mix, which is a specific mix of additives that are added into flour during bread-making. The monitoring programme to establish a baseline analysis of the actual folic acid content of all bread currently on the Irish market was considered necessary prior to the initiation of mandatory fortification. The actual folic acid content of bread as analysed was then compared with the amount of folic acid declared on the label where declaration was made.

Three surveys of bread on retail sale on the Irish market were conducted during 2006 and 2007. Samples of the all types of bread on sale in Ireland were collected—including bread from large and small bakeries, both branded and supermarket private label. Samples were sent to the Public Analyst's Laboratory in Galway and analysed for folic acid content. Results on the actual content of folic acid were compared with the amounts of folic acid declared on labels, where possible. Discussions of findings were held with representatives from the bread industry to inform conclusions reached.

In all, 249 loaves of bread were analysed for folic acid content. Claimed folic acid concentrations, when declared (*n*15, 6.0%), were between 50 and 300 µg per 100 g bread. However, 19.7% of loaves tested (*n*49) actually contained folic acid at concentrations ranging from 23–670 µg per 100 g bread[5]. Results showed that, in general, where folic acid was added and declared (*n*15), actual levels were in excess of the declared levels: between 23 and 224% more folic acid than declared (Figure 2). The survey also showed illegalities in the labelling of fortified bread, where a small number of samples (*n*9, 3.6%) contained folic acid but were not labelled as such (i.e. folic acid was not listed in the ingredient list or on the label) and three samples (1.2%) were labelled as containing folic acid but did not contain folic acid on analysis.

Figure 2: Difference between Labelled Folic Acid Content and Measured Folic Acid Content of Bread



From these results, it was concluded that adding folic acid at the baking stage via the flour improver was not a method of addition that could be sufficiently controlled to deliver a consistent, safe programme of mandatory fortification of bread. Not only would there be significant risk of the addition of excessive amounts of folic acid (over-fortification) but it would not be practical to monitor and enforce compliance throughout the diverse baking industry. Meetings with the industry representative confirmed their preference for the fortification of flour used to make bread.

[5] One sample contained 5,096 µg of folic acid per 100 g bread and this was investigated separately. Investigations revealed a systematic error in the quantity of folic acid added to the flour improver by the improver supplier following confusion over the agreed specification. This issue was resolved by the manufacturer and validated in subsequent FSAI testing.

5.3 Voluntary Food Fortification

During July and August 2007, a survey of all the major supermarket chains was carried out to estimate the extent of voluntary folic acid food fortification. All items of folic acid fortified food were identified and the amount of folic acid declared per 100 g (or 100 ml) of food product noted from the label. These data were used to update the national adult survey database used to estimate the effect of mandatory fortification of most bread with folic acid on total folic acid intakes.

In addition, sample meal and snack plans were constructed using average portion sizes of a selection of these voluntarily fortified foods to estimate the facility of achieving the recommended 400 µg folic acid intake. Folic acid content of average portion sizes was transcribed from product labels, where this information was provided (available on 19 [68%] of the 28 products). Where food labelling only provided folic acid content per 100 g/ml (as in nine [32%] food products) the amount of folic acid provided was calculated for average portions derived from food portion sizes (Food Standards Agency, 2002). The variation in fortification of different brands of the same food products, e.g. folic acid fortified fruit juice and non-fortified fruit juice, was accounted for by using market share data of the fortified brands to enable a probabilistic modelling[6] approach.

In all, 211 food products were found to be voluntarily fortified with folic acid (Table 20).

Table 20: Voluntary Folic Acid Fortification of Food in August 2007: Food Categories, Fortification Levels and Market Share of Fortified Foods on Irish Market

Food Category	Number of Brands	Declared Folic Acid Fortification Levels (µg/100 g)	Percentage of the Category Market Held by Fortified Products **
Breakfast Cereal	104	110–571	60%
Cereal Bars	27	100–250	29%
Fat Spreads*	15	100–1,000	16%
Milk	4	30–70	5%
Juice	11	0.07–100	5%
Dried Soup	4	18–30	13%
Yoghurt	2	40–200	1%
White Bread	38	30–300	6%
Brown Bread			2%
Wholemeal Bread			17%

*Follow-up checks in May 2008 identified 50% less folic acid in several brands of fortified fat spreads (August 2007 fortified at 1,000 µg/100 g and in May 2008 fortified at 500 µg/100 g)
** Based on volume sales 2007 (data supplied by TNS)

Currently, women of childbearing age are advised to take 400 µg of folic acid every day to prevent NTDs. The question of whether this intake could be achieved through choosing foods voluntarily fortified with folic acid was examined by the implementation group.

A daily intake of 400 µg of folic acid could be easily achieved by incorporating foods voluntarily fortified with folic acid into meal plans. As shown in Table 21, using just 28 of the 211 branded food products that were voluntarily fortified with folic acid, various food intake combinations commonly used at breakfast, lunch, dinner and snacks yield intakes of approximately 400 µg of folic acid.

Folic acid content per average portion size was calculated using the folic acid content declared per 100 g of food product or, if provided, folic acid content per average portion. Some of the difficulties consumers face in estimating their actual intake of folic acid from information on food labels identified included:

1) most breakfast cereal brands provided folic acid content per 3 of cereal *with* 125 ml of semi-skimmed milk, which does not facilitate variation around type or amount of milk actually used.

2) almost a third of the folic acid fortified products selected did not state folic acid content per average portion on the food label

3) consumers need to check food labels regularly to keep up with changing levels of addition of folic acid.

[6] Probabilistic modelling, in this context, involved replacing single values for folic acid in a certain food with distributions of values that reflect the variation of folic acid concentration in all such foods on the Irish market. For example, in the database, the folic acid intake from Kellogg's cornflakes would have been replaced by random samples of folic acid concentrations of all cornflakes, fortified or unfortified, on the Irish market, resulting in a distribution of possible intakes instead of a single intake value.

Table 21: Daily Meal and Snack Plans Incorporating Foods Voluntarily Fortified with Folic Acid so that Intakes of 400 µg Folic Acid is Achieved

Scenario	Folic Acid Fortified Foods (portion size = weight in g or ml)		Folic Acid (µg)
1	**Breakfast**	**Brand A breakfast cereal** (medium bowl [40 g])	80
		with **Brand A milk** (3 tablespoons [100 ml])	70
		Brand A multivitamin juice (medium glass [200 ml])	200
	Snack	**Brand A cereal bar** (23.5 g)	44
	TOTAL		**394**
2	**Breakfast**	**Brand A breakfast cereal** (medium bowl [40 g])	33.6
		Brand B multivitamin juice (medium glass [200 ml)]	200
	Snack	**Brand A yoghurt drink** (100 g)	200
	TOTAL		**433.6**
3	**Breakfast**	**Brand A breakfast cereal** (medium bowl [40 g]) with non-fortified milk)	133.6
	Lunch	**Brand A soup** (medium mug as prepared with water [215 ml])	38.7
		Brand A white sliced bread (2 slices [60 g])	30
		Brand B fat spread (2 small single portions [20 g])	200
	TOTAL		**402.3**
4	**Breakfast**	**Brand B breakfast cereal** (medium bowl [40 g]) with non-fortified milk)	132
		Brand B orange juice (medium glass [200 ml])	52
	Lunch	**Brand A fat spread** on a medium bread roll (12 g)	120
		Brand B milk (1 tablespoon [30 ml] in a mug of tea)	9
	Snack	**Brand A flavoured milk drink** (medium glass [200 ml])	126
	TOTAL		**439**
5	**Breakfast**	**Brand C breakfast cereal** (medium bowl [40 g])	160
		with Brand C milk (3 tablespoons [100 g])	70
	Lunch	**Brand A baked beans** (small can [150 g])	30
		Brand C fat spread (2 small single portions [20 g]) on 2 slices of toast (non-fortified bread)	100
	Snack	**Brand A cereal bar** (23 g)	33.12
	TOTAL		**393.12**
6	**Breakfast**	**Brand D breakfast cereal** (medium bowl [40 g])	68
		with Brand C milk (3 tablespoons [100 ml])	70
		Brand C multivitamin juice (medium glass [200 ml])	80
	Snack	**Brand B cereal bar** (37 g)	50.32
		Brand B yoghurt drink (90 g)	36
	Dinner	**Brand E fat spread** (10 g) with mashed potato	100
	TOTAL		**404.32**
7	**Breakfast**	**Brand A wholegrain bread** (2 slices [60 g])	180
		with **Brand D fat spread** (2 small single portions [20 g])	40
	Lunch	**Brand A powdered soup** (medium bowl as prepared with water [220 ml])	66
	Dinner	**Brand A milk** (medium glass [200 ml])	140
	TOTAL		**426**

All these factors contribute to the difficulty in determining folic acid content per average portion of fortified foods. In addition, the bread analysis survey (where folic acid was found to exceed declared levels by between 23 and 224%) indicates that actual intakes of folic acid from these menus could exceed 400 µg.

In summary, the feasibility whereby women could use foods voluntarily fortified with folic acid by manufacturers to achieve the recommended 400 µg of folic acid every day is questionable for several reasons:

1) folic acid intake must be calculated in terms of actual portions consumed from food labelling information, yet according to labelling rules, folic acid content must be listed per 100 g (or 100 ml) of food product. It is almost impossible for the average consumer to estimate the amount of folic acid provided in the actual portion they consume because this requires (a) knowledge of portion sizes and (b) calculations to translate information per 100 g to the weight of the portion. This is recognised by food manufacturers leading many to voluntarily provide nutrition

information per average portion size, e.g. two thirds of fortified foods selected provided nutrition information per average portion size. However, there are no specific rules governing average portion size information and this leads to inconsistencies that the consumer must negotiate. For example, the amount of folic acid provided by the food product itself (i.e. the cereal without milk added); or how large or small is the portion (lack of information on how many portions are provided in an entire package of food)

2) folic acid content of foods within the same food category of products varies considerably both between brands and even within brands, e.g. breakfast cereals ranged from 126 µg/100 g to 334 µg/100 g within the same brand while fortified fat spreads ranged from 100 µg/100 g to 1,000 µg/100 g. Thus, natural variation in food choice makes it challenging for women to achieve the same folic acid intake on a regular basis

3) the *ad hoc* nature of voluntary folic acid food fortification, where manufacturers can initiate, discontinue or change fortification of foods at any time, makes label checking essential every time a food is purchased

4) there is likely to be differences between the labelled amount of folic acid and the actual amount of folic acid in foods which lead to variable intakes.

5.4 Re-evaluation of Appropriateness of the Level of Folic Acid to be Used in Mandatory Fortification of Most Bread in Ireland

The update of the 1999 adult food intake database with current levels of voluntary folic acid fortification of food, demonstrated a significant increase in folic acid intakes. The probabilistic modelling analysis and testing of the appropriateness of the proposed level of mandatory folic acid fortification, showed that estimated folic acid intakes, based on 2007 food fortification patterns, had increased to 90 µg/person/day for women aged 18–50 years from 67 µg/person/day for the same age group based on 1999 food fortification patterns (Table 22). In 1999, over a third (35%, *n*130) of women aged 18 to 50 years (*n*369) did not consume any folic acid fortified foods. Updating the 1999 data in terms of 2007 folic acid food fortification reduced this proportion of women to less than 2% (7/369 women). In relation to the UL of 1,000 µg for folic acid, there was a large gap between the folic acid intakes of the highest folic acid consumers (99th percentile folic acid intakes) among all groups, i.e. 519 µg/day for women of childbearing age, 383 µg/day for older women and 299 µg/day for men (Table 22 b). However, this analysis did not account for an increased use of folic acid containing supplements that may have occurred between 1999 and 2007.

Table 22: Folic Acid Intakes of Adults in Ireland after Food Intake Data Collected in 1999 is Updated to Account for Increased Voluntary Folic Acid Food Fortification of Food Brands in 2007

(a) Estimated mean intakes of folic acid, natural folates and total folate among adults in 1999 (µg/day)

	Females 18-50 Years *n*368			Females 50-64 years *n*114			Males 50-64 years *n*127		
	Folic Acid	Natural Folate	Total Folate	Folic Acid	Natural Folate	Total Folate	Folic Acid	Natural Folate	Total Folate
Mean	67	201	268	48	201	249	31	295	326

(b) Estimated mean intakes of folic acid, natural folate and total folate after using 1999 data updated for voluntary folic acid food fortification in 2007 (µg/day)

	Females 18-50 Years *n*369			Females 50-64 years *n*114			Males 50-64 years *n*127		
	Folic Acid*	Natural Folate	Total Folate	Folic Acid*	Natural Folate	Total Folate	Folic Acid*	Natural Folate	Total Folate
Mean	90	203	292	68	202	271	75	296	371
Median	52	199	260	46	198	247	56	274	342
99th percentile	519	347	754	383	382	654	299	608	723

* Supplement usage was not updated from 1999 usage patterns: it is likely that there is higher usage and greater availability of supplements containing folic acid in 2007 compared with 1999.

Analysis of the types of fortified food products that contributed most to folic acid intake among the adult groups is shown in Table 23. The principal food categories responsible for the increased estimates of intake in 2007 were fat spreads and bread products, which were not fortified in 1999. In fact, the contribution of fat spreads was particularly large in older men (Table 23). It was not possible to update the food consumption database for changes in food supplement use, or content, in this work and hence, it is likely that folic acid exposure is underestimated in this analysis.

Table 23: Contribution (µg/day) of Folic Acid Fortified Foods that have Contributed Most to the Increased Folic Acid Intake Among Adults in Ireland in 2007

Folic Acid (µg/day)	Females 18–50 years		Females 51–64 years		Males 51–64 years	
	1999	2007	1999	2007	1999	2007
Food Supplements*	36	36	18	18	9	9
Breakfast Cereals	28	20	23	16	18	16
Fortified Fat Spreads	0	16	0	18	0	27
Fortified Milk	2	6	6	6	1	7
Fortified Bread	0	8	0	5	0	11
Total	67	90	48	68	31	75

* Supplement usage was not updated from 1999 usage patterns: it is likely that there is higher usage and greater availability of supplements containing folic acid in 2007 compared with 1999.

This work demonstrated that the likely exposure of the population to folic acid from voluntary fortification of food was larger than previously estimated by the NCFAFF (2006). It also showed that continued proliferation of voluntary fortification of foods could, at some point, lead certain 'at risk' population groups towards higher exposures closer to the UL for folic acid.

Nutrition labelling of foodstuffs is voluntary, but is compulsory where a nutrition or health claim is made on the label or in cases where foods have been fortified. Consequently, it is not possible for consumers to make informed choices regarding the foods they consume. However, the requirement for nutrition labelling of all foodstuffs is likely to become mandatory in the near future under the ongoing revision of EU food labelling laws.

5.5 Conclusions

1) **Flour is the preferred route for folic acid addition on a mandatory basis both for ease of enforcement, control and compliance.**

2) **Maximum limits for voluntary folic acid addition to food need to be agreed at European level as well as possible restrictions on the categories of food to which folic acid can be added.**

3) **Legislative controls need to be established for the tolerances within which actual nutrient concentrations in foods may differ from the declared concentration of nutrients on the label.**

4) **Nutrition labelling that provides information in terms of average portion size, in a consistent format, is required to enable shrewd use of voluntarily fortified foods to achieve public health nutrition.**

CHAPTER 6: TECHNICAL AND INDUSTRY RELATED ISSUES

6.1 Background

Initial thinking around the process of fortification of bread was to add folic acid at the baking stage. This would allow control of the types of bread that were fortified and would exclude other bakery products. As a result, consumers would be offered choice. Choice was a desirable aspect of the mandatory folic acid fortification programme that was clearly identified in the report of the NCFAFF (2006). However, discussions in Chapter 5 describe why the addition of folic acid at the baking stage, via fortification of flour improver, is not a method that will deliver consistent, safe, mandatory fortification of bread. Fortification of bread-making flour is the preferred choice of the baking and milling industry and the enforcement agencies. Fortification operated at the flour-milling stage is controllable and compliance and enforcement are simpler. However, fortification of flour introduces a number of challenges including reduced consumer choice, potential barriers to trade, and variation of folic acid in bread made from fortified flour.

When folic acid is added to flour, it must be introduced at a concentration that will ensure that the final amount of folic acid delivered to the consumer in bread is optimal. Folic acid is inactivated by heat, oxidation and other processing and storage effects (Morgan, 1996). Because bread has a short shelf-life, the overriding processing effect is heat inactivation of folic acid. Since recipes and baking processes are variable by bread type and recipe, fortification of flour at a given concentration of folic acid will result in bread with a variable folic acid concentration. Therefore, the desired 120 µg/100 g bread with a tight tolerance is not achievable by this fortification method.

Odlums Ltd are the main millers in Ireland. Discussions have determined that commercial bread-flour can be fortified without fortifying other flours, either commercial or retail. It is also possible to fortify white bread flour and exclude wholemeal flour if so desired. However, there is some limited cross-over in the use of bread flour in other bakery products by smaller bakeries which would introduce a small 'halo' effect of folic acid fortified foods containing flour. It is not possible to quantify the extent of this effect but it is considered to be small and will require monitoring.

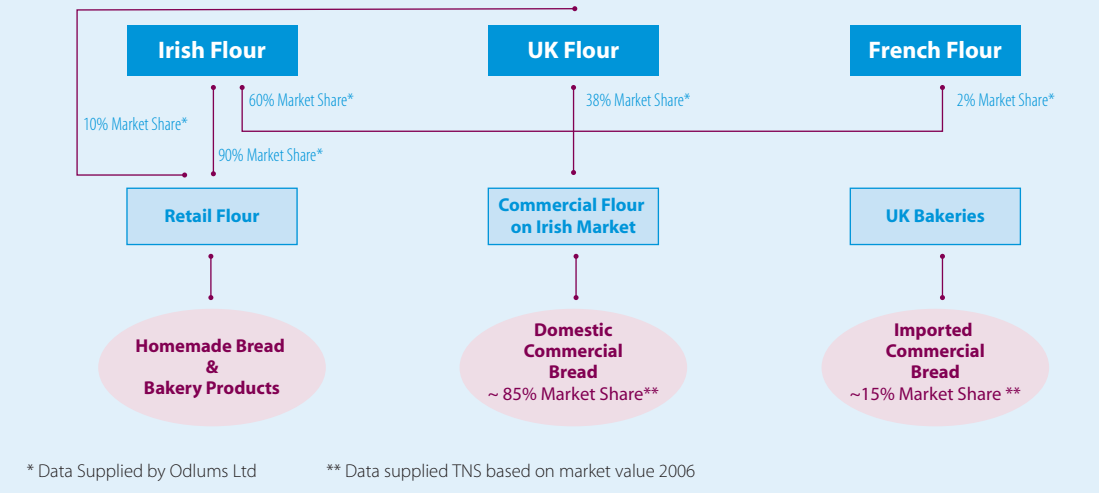
Irish milled flour and Irish produced bread do not control 100% of the Irish market. Consequently, a decision has to be made whether to legislate to ensure that imported flour and bread contain folic acid. Clearly, such measures have to be sanctioned by the European Union and could be opposed by Member States exporting these products to Ireland. Such action is only likely to be defended successfully if there is a demonstrable public health benefit to the imposition of necessary trade restrictions.

6.2 The Irish Flour and Bread Market

Discussions with bakers, millers and retailers in Ireland and the analysis of market share data allowed the implementation group to build a picture of the Irish flour and bread market (Figure 3).

Odlums produce around 98% of flour milled nationally, comprising 90% of the retail flour and 60% of the commercial flour sold in Ireland. UK based mills supply around 38% of the commercial flour, mainly from Northern Ireland, and the remaining 10% of the retail flour. About 2% of commercial flour is supplied by French mills, predominantly to Cuisine de France. The main bakers in Ireland are represented by the Irish Bread Bakers Association. Two bakers, Brennans and Irish Pride, together hold around 38% share of the Irish wrapped bread market. Around 25% of this market belongs to private label, half of which belongs to Dunnes Stores and Tesco Ireland. The remaining 47% of the market share is divided up amongst a number of Irish and UK bakeries and smaller private label brands. It is estimated that approximately 15% of the Irish wrapped bread market is held by bread originating in the UK, with the remaining 85% held by bread produced in Ireland.

Figure 3: The Irish Flour and Bread Market



The contribution of imported bread and flour is significant. Modelling exercises conducted by the FSAI estimate that if imported bread and flour are allowed into Ireland without folic acid added, then approximately 40% of the Irish bread market would not be fortified. If a mandatory fortification programme is to be successful it would be necessary to legislate and require imported bread and flour to be fortified at a given concentration of folic acid if the desired public health benefits were to be realised.

6.3 Establishing the Appropriate Fortification Concentration of Folic Acid in Flour

The FSAI, Odlums and the Public Analyst's Laboratory, Galway collaborated in bread-baking trails with flour containing folic acid. These trials were conducted in the pilot bakery in Odlums using scaled down equipment used in industrial baking. There is limited literature on the effects of baking on folic acid, but studies have demonstrated losses between 12% and 25% caused by heat (Gujska and Majewska, 2005; Kariluoto *et al*, 2004). Baking trials were conducted on four bread types: white pan, wholemeal pan, brown soda bread, and white baguette. Recipes and process conditions were selected based on known industrial baking norms. Inactivation of folic acid during baking was variable within bread type and between bread types (Table 24).

Table 24: Inactivation of Folic Acid by Bread Baking

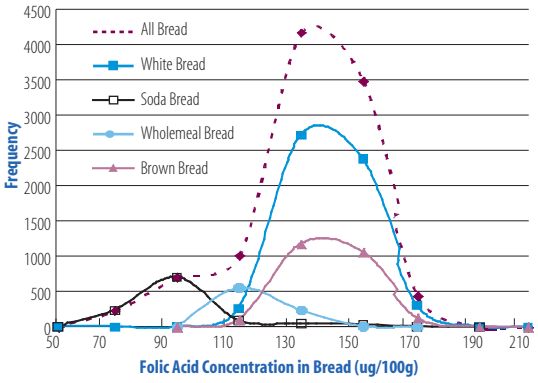
Bread Type	Mean Percentage Inactivation	95% confidence level
White Pan Bead (n15)	20.4%	+/- 3.4%
Wholemeal Pan Bread (n15)	31.6%	+/- 1.4%
White Baguette (n15)	20.0%	+/- 1.8%
Brown Soda Bread (n15)	28.2%	+/- 4.5%

Clearly, folic acid is inactivated more in wholemeal bread and soda bread than in white pan bread and white baguettes. A higher inactivation rate was expected in soda bread due to the highly alkaline conditions in the dough caused by the raising agent. However, the high inactivation rate in wholemeal bread is unexpected but consistent across two independent trials.

These preliminary trail results suggest that a flour fortification level of 240 µg/100 g flour would be sufficient to deliver an average bread folic acid level of 120 µg/100 g bread. Unfortunately, the tolerance to which flour could be fortified is difficult to determine on a pilot scale. Pilot trials using small scale equipment have demonstrated that variation in the target value for folic acid fortification of flour may be larger than +/- 5% as estimated by Odlums. This aspect will need further research before enacting appropriate values in legislation.

Market share data on the Irish bread market (TNS, 2007) show that white bread has a 55% share of the market, with wholemeal bread and soda bread having a 7% and 27% share respectively. The remainder is brown bread with an 11% share. Given this consumption pattern, the different folic acid inactivation rates on baking and the different bread recipes, the variation in folic acid concentration around the mean value will be large for all Irish made bread. Figure 4 shows an estimate of this variation from modelling work conducted by the FSAI.

Figure 4: Estimated Distribution of Folic Acid in Bread Manufactured in Ireland from Flour Fortified with Folic Acid at 240 µg/100 g Flour



The modelling estimated that the mean concentration of folic acid in bread made in Ireland from fortified flour (240 µg/100 g flour) was 122.6 µg/100 g (95%CI +/- 0.39 µg/100 g) and 90% of bread made in Ireland would contain folic acid at a concentration between 76 and 149 µg/100 g. This assumes that imported flour would be fortified at the same level as Irish flour and does not account for import of unfortified bread.

However, Figure 4 shows a bimodal distribution of folic acid in bread. The lower peak was contributed by the soda bread because inactivation of folic acid is high and the flour content of bread is low. The upper peak resulted from the other bread types; however, the lower shoulder of that peak was composed predominantly by the wholemeal bread, where inactivation of folic acid is highest. Therefore, a consumer's intake of folic acid is dependent on the predominant form of bread consumed. Those consuming mostly soda bread (mean 81.2 µg/100 g, 95%CI +/- 1.01 µg/100 g) or wholemeal bread (mean 107.9 µg/100 g, 95%CI +/- 0.46 µg/100 g) will receive less folic acid than those consuming white bread (mean 129.7 µg/100 g, 95% CI +/- 0.32 µg/100 g) and brown bread (mean 129.8 µg/100 g, 95%CI +/- 0.47 µg/100 g).

6.4 Conclusions

Fortification of flour is the easiest method for fortification of bread with folic acid both in terms of compliance and enforcement. However, the consequence of this is a reduction in the amount of bread available unfortified in Ireland, therefore restricting consumer choice. This could be facilitated by mandatory labelling if a product is made with fortified flour. However, in UK, it has been suggested that wholemeal flour could be left unfortified, which would create choice. This option is available in Ireland; however, the advantages and disadvantages of this would need to be considered carefully in the context of a balanced diet.

Trials have shown that bread made from fortified flour will contain variable amounts of folic acid and therefore it will not be possible to deliver the target concentration of 120 µg/100 g in bread with a tight tolerance. It is also clear that the potential impact of imported flour and bread on folic acid intake is significant; therefore to achieve the required health benefits of a mandatory food fortification programme with folic acid, imported flour and bread will need to be fortified. This will need to be carefully considered as it may create a potential barrier to trade.

Folic acid intake modelling work will be necessary to investigate the impact on health of the estimated folic acid concentrations in bread made in Ireland, as well as the consequences of allowing imported flour and bread to remain unfortified. Such modelling is also necessary to determine the necessity for mandatory controls of voluntary fortification of foods that could result in unsafe intakes in some population groups when all bread is fortified. In summary:

1) **flour will need to be fortified with folic acid to a target concentration of 240 µg/100 g with a tolerance to be determined by industrial scale trials**

2) **national legislation will be required for the mandatory fortification of imported bread and bread making flour tat levels of 240 µg/100 g for flour and 120 µg/100 g for bread**

3) **modelling of folic acid intake is conducted to determine the health effects of mandatory fortification of bread with folic acid and to investigate the need for parallel controls on voluntary food fortification with folic acid.**

CHAPTER 7: HEALTH PROMOTION

7.1 Background

Mandatory folic acid food fortification will result in an even distribution of folic acid intake throughout the population and will ensure that almost all women of childbearing age who are sexually active will consume some folic acid every day. However, it is estimated that fortifying bread at a level of 120 µg/100 g will provide just 25% of the 400 µg of folic acid women need every day to ensure babies in Ireland are protected against most NTDs. Fortifying breads at higher levels may provide women with greater amounts of folic acid, but would most likely result in over-exposing others, particularly those who eat a lot of bread—the proposed carrier food for folic acid. Therefore, even with a mandatory folic acid food fortification programme in place, there is an ongoing need for effective programmes that promote the consumption of 400 µg of folic acid every day by women of childbearing age who are sexually active. Similar to other developed countries, over half of all pregnancies in Ireland are unplanned. This, together with the fact that NTDs develop at a very early stage in pregnancy, necessitated adoption of the national policy that all women of childbearing age, who are sexually active, are advised to take 400 µg of folic acid every day; a supplement providing 400 µg of folic acid is recommended.

Three of the seven recommendations made by the NCFAFF (2006) relate to the need for effective health promotion programmes to ensure optimal uptake of the advice for folic acid by women in the target group (Appendix 1). The issues raised are:

1) that all health promotion information on folic acid is accurate and clear in promoting the on-going importance of ensuring women in the target group take 400 µg of folic acid every day

2) that all health professionals are well informed about the continuing need to promote folic acid intake by women who are sexually active after implementation of mandatory folic acid fortification

3) there is access by all women to 400 µg folic acid supplements and to identify barriers to folic acid supplementation such as cost and availability

4) that there is awareness among health professionals about the optimal 400 µg dose of folic acid and the risks associated with high dose supplements in terms of masking B_{12} deficiency

5) best practice in Ireland regarding the promotion of folic acid intake by women in the target group.

A Health Promotion Working Group was established as part of the implementation group to develop a plan and coordinate activities around the implementation of these recommendations. The main areas of activity of this working group included:

1) reviews of folic acid literature/resources, both hard copy and web-based, and an analysis of their compatibility with the current policy on folic acid

2) evaluations of previous folic acid campaigns reviewed and difficulties encountered by organisations in promoting the 'folic acid policy message' collated. Identification of models of good practice from an education and social marketing perspective

3) primary qualitative research, supported with low income groups, to identify barriers to folic acid supplementation. Research funded by *safe*food and supported by the FSAI and the HSE

4) engagement with pharmaceutical sector and key stakeholders on proposed folic acid education and communication proposals

5) request to the Department of Health and Children to include key policy on folic acid supplementation and fortification in the forthcoming national nutrition policy and to include the resourcing of a comprehensive folic acid public education programme in the 2009 estimates process.

7.2 Approach Used

A thorough review of all available literature and web-based advice on folic acid supplementation in Ireland was undertaken. Information was collected from the public sector, non-governmental organisations (NGOs) and the private sector, such as; health services, health professionals, health information services (Voluntary Health Insurance, Irish National and Dietetic Institute, Irish Association for Spina Bifida and Hydrocephalus etc.) and manufacturers. All of these resources were analysed in terms of their compatibility with the current policy of advising all women of childbearing age, who are sexually active to take 400 µg of folic acid. Two series of 'frequently asked questions' were formulated for health professionals and the general public to provide the basis for new resources development.

A review of the availability of folic acid supplements was undertaken, which included a review of websites selling folic acid supplements as well as field work researching the availability of folic acid supplements in pharmacies, health food stores and supermarkets.

A sub-sample of multivitamin supplements on sale in Ireland was examined for folic acid content. This sample included all multivitamin supplements notified to the FSAI since 2003, as well as a random sample of those available in pharmacies.

7.2.1 Identification of good practice in effective promotion of folic acid use by women

Previous folic acid campaigns were reviewed for information on difficulties encountered by organisations in promoting the 'folic acid policy message'. This included corresponding with key organisations such as: the Irish College of General Practitioners; pharmacists; the National Youth Health Programme; the Social, Personal and Health Education (SPHE) Support Service; the National Council for Curriculum Assessment; the Crisis Pregnancy Agency; the Health Promotion Agency Northern Ireland; the Irish, Nutrition & Dietetic Institute; and the Department of Health and Children. The evaluation of the "Folic Acid—Today and Everyday" campaign, undertaken in 2005, was reviewed. This information was used to develop models of good practice from an education and social marketing perspective.

7.2.2 Engagement of key stakeholders

A list of key stakeholders (including health care professionals, youth organisations, statutory bodies, NGOs and consumer groups relevant to this area) was compiled, which will be used as the basis for communication of the revised promotional materials relating to folic acid. Direct engagement has taken place with the pharmaceutical sector and key stakeholders on proposed folic acid education and communication proposals.

7.2.3 Identification of barriers to use of folic acid supplements by women from socio-economically disadvantage groups in Ireland

Primary qualitative research was undertaken to identify barriers to use of folic acid supplements by women from disadvantaged backgrounds[7]. Thirty six women, aged 18 to 30 years, participated in six focus groups in the Cork city area. Each focus group comprised of members from an established community group who came together around either youth services, or training and/or work. Women were recruited through their community and group leaders. Focus groups ranged in size from four to eight participants and were recorded digitally.

Three focus groups, comprising of a total of 19 participants (13 of whom were mothers), were conducted in disadvantaged areas and a further three focus groups, comprising of 17 participants (none of whom were mothers) were conducted in advantaged areas. Although this does reflect the fact that women from more advantaged backgrounds are having children later, attempts are currently being made to recruit a group of mothers aged 18–30 years from an established community group in an advantaged area. On completion of all focus groups, transcripts will be analysed for major themes and a final report will be published.

7.2.4 Develop a plan for the Department of Health and Children

A request was made to the Department of Health and Children to include key policy on folic acid supplementation and fortification in the forthcoming national nutrition policy, and to include the resourcing of a comprehensive folic acid public education programme in the 2009 estimates process.

[7] This research was funded by *safe*food and supported by the FSAI and the HSE.

7.3 Findings and Discussion

7.3.1 Review of all written and web-based literature and resources

The literature review highlighted that some information in the public domain is outdated and requires revision to bring it into line with the core health message regarding folic acid. In response to this need, 'frequently asked questions' were formulated for health professionals as well as the general public to provide the basis for new resources development.

The review of the availability of folic acid supplements as well as the levels of folic acid in multivitamin supplements provided the information required to develop scientifically sound advice as part of the 'frequently asked questions'.

7.3.2 Identification of good practice in effective promotion of folic acid use by women

Information gained through the literature review that informed the report of the NCFAFF, and the review of previous folic acid campaigns regarding difficulties encountered by organisations in promoting the 'folic acid policy message', is being taken into consideration in the development of models of good practice from an education and social marketing perspective. In addition, the research undertaken to investigate the barriers to taking folic acid supplements among women from low socio-economic groups will also contribute to best practice. New and novel media channels including digital (in particular, texting services) and the internet have recently gained prominence in the context of Irish health promotion campaigns. Evidence based approaches will be used to formulate the proposed campaign, including the use of formative research, focus groups, key stakeholder involvement, testing of proposed materials and the use of appropriate media.

7.3.3 Engagement of key stakeholders

The list of key stakeholders will be used as the basis for initial communication of the revised promotional materials relating to folic acid, in order that maximum penetration of the target audience can be achieved. The key stakeholders will be important from an advocacy perspective at policy and programme levels.

The SPHE Management Committee has agreed to include key health messages with regard to the importance of including folic acid in young people's diet. This will be emphasised at teacher in-service days, and in contact with schools. The Steering Group on Food and Nutrition Guidelines (Healthy Eating Guidelines) for post primary schools has included folic acid in its current draft and will consult further with the Folic Acid Implementation Committee. The National Youth Health Programme has also included folic acid on its agenda.

7.3.4 Identification of barriers to use of folic acid supplements by women from socio-economically disadvantage groups in Ireland

Primary qualitative research was undertaken to identify barriers to use of folic acid supplements by women from disadvantaged backgrounds[8]. While this research is ongoing, the identification of focus groups has indicated that women from less advantaged backgrounds are having their children earlier than those from more advantaged backgrounds. This will be taken into consideration in targeting key health promotion messages, key stakeholders and in the roll-out of the proposed campaign.

From a health inequalities perspective, it is particularly important to consider the barriers identified and the fact that folic acid is available free of charge to medical card holders through the General Medical Services (GMS) scheme.

7.3.5 Develop a plan for the Department of Health and Children

A request was made to the Department of Health and Children to include key policy on folic acid supplementation and fortification in the forthcoming national nutrition policy, and to include the resourcing of a comprehensive folic acid public education programme in the 2009 estimates process.

[8] This research was funded by *safe*food and supported by the FSAI and the HSE.

7.4 Conclusions and Recommendations

A comprehensive review of all health information, resources and folic acid health promotion and public awareness campaigns has been completed. Primary research into the barriers to taking folic acid supplements among women from low socio-economic and ethnic minority groups has been undertaken. Key health messages and information resources on the promotion of folic acid supplementation have been developed. A database of key health, organisational and consumer stakeholders has been compiled to ensure comprehensive distribution of this key health policy. Key organisations have been engaged with and commitments obtained to include folic acid health.

The working group has made the following recommendations:

1) **a comprehensive health promotion and social marketing campaign be resourced and undertaken**

2) **the campaign will be fully integrated and will consider the use of a full spectrum of media channels available, i.e. TV, radio, outdoor, digital, online, information line, direct marketing, consumer publications, professional journals and PR. The use of texting services and popular online web pages should be considered in engaging with young people. In addition, key settings such as education, the workplace and the community should be targeted**

3) **the campaign will develop tailored messages and media to target the wide range of consumers, utilising the evidence arising from the primary qualitative research undertaken to identify barriers to the use of folic acid supplements by women from disadvantaged backgrounds**

4) **the resources identified are widely distributed and available to health care professionals who have a key role to play in advocating for folic acid supplementation. Engagement with professional bodies to identify the most efficient education programmes, e.g. Continuing Medical Education, should be explored. Pre- and post-graduate programmes should include folic acid supplementation**

5) **evidence based social marketing methods should be used to target consumers, and in particular those at risk of health inequalities, to ensure the uptake of folic acid supplementation. In particular, the use of new methods of marketing, such as digital, texting and online, should be fully exploited to ensure maximum uptake**

6) **the availability of folic acid free of charge on the GMS scheme should be highlighted to health care professionals and consumer groups**

7) **particular consideration should be given to new populations with distinct cultural and lifestyle practices.**

CHAPTER 8: SCIENTIFIC DEVELOPMENTS

1.1 Background

The NCFAFF (2006) carried out a thorough risk: benefit analysis prior to recommending that a mandatory folic acid fortification programme be introduced in Ireland. Since 2006, the implementation group has continued to monitor scientific developments relevant to risks and benefits of folic acid food fortification. New research results in three key areas were addressed by the implementation group:

1) **cardiovascular disease**—folic acid food fortification is associated with a lower risk of stroke and may protect against cardiovascular disease through its homocysteine-lowering effects. Given the high rate of cardiovascular disease in Ireland, any effect of folic acid food fortification would have major health implications for public health

2) **cognitive function of older adults**—in association with its homocysteine-lowering effects, folic acid food fortification may be associated with improved cognitive function in older adults (Malouf *et al*, 2003; Durga *et al*, 2007). Considering that older adults are the vulnerable group to the only established risk from excessive folic acid intake—masking of vitamin B_{12} deficiency—protection of cognitive impairment in this age group may be important in addressing the risks borne by this population sub-group

3) **cancer risk**—folic acid food fortification has long been considered to be protective against cancer, especially colorectal cancer (Kim, 2004). However, the emergence of new data (Cole *et al*, 2007; Mason *et al*, 2007 and 2008) (note Lancet 2008 letters) indicates that the amount of folic acid consumed may be particularly important in ensuring beneficial effects in relation to cancer risk. This recent data emphasises the importance of ensuring a safe level of folic acid intake across the population.

While developments relating to folic acid food fortification in cardiovascular disease and cognitive function of older adults were not of major concern, the emerging issues in cancer risk were significant.

In terms of association of folic acid and cancer, evidence from human and animal research involving all levels of study design (randomised controlled trials, interventions studies, observational studies, and studies of cell culture) was explored. Where possible, mechanisms whereby folate nutrition exerts protective or harmful effects were elucidated, including the identification of factors that may influence the direction of effect. The relevance of these findings for folic acid food fortification in Ireland—both the current voluntary addition of folic acid to a range of foods and the planned mandatory fortification programme—were explored. Finally, conclusions were drawn in relation to folic acid food fortification and the planned mandatory fortification of most bread in Ireland.

8.2 Cardiovascular Disease

Folic acid food fortification is associated with a reduced risk of stroke (Wang *et al*, 2007; HOPE, 2006; Spence *et al*, 2005). While observational data indicate that low folate intake levels are a risk factor for cardiovascular disease, the evidence from trials is not consistent. Trials carried out to date have been underpowered and so definitive evidence on the possible protective effects against coronary heart disease require meta-analysis of the results of recent and on-going trials (Clarke *et al*, 2007; Smith *et al*, 2008; Wald *et al*, 2006). The implementation group notes that information from such meta-analysis is expected to become available in early 2009.

8.3 Cognitive Function of Older Adults

Cognitive function declines with age—especially cognitive domains related to memory and information processing speed. Folic acid consumption, with or without vitamin B_{12}, may help prevent cognitive impairment in the elderly (Malouf *et al*, 2003). A randomised, double-blind, placebo controlled trial involving over 800 older adults (aged 50–70 years) in the Netherlands found that daily consumption of supplements of 800 µg folic acid over three years significantly improved cognitive function (Durga *et al*, 2007). Improvements in this trial involved domains of cognitive function associated with ageing and these changes were associated with significant reductions in homocysteine.

A recent follow-up study of almost 1,000 older adults (>65 years) reported that higher dietary intakes of folate and folic acid were associated with a decreased risk of Alzheimers disease independent of intakes of vitamins B_{12} and B_6 (Luchsinger *et al*, 2007). However, a recent analysis of the relationship between blood levels of folate and vitamin B_{12} and measures of cognitive function in older adult participants in the 1999–2002 US National health and Nutrition Examination Survey (>60 years, *n*1,459) found that the protective effects of folate on cognitive function were dependent on adequate vitamin B_{12} status (Morris *et al*, 2007). In this study, low vitamin B_{12} status was defined as blood B_{12} concentration <148 pmol/L *or* serum methylmalonic acid concentration > the reference range (60–210 nMol/L), while high folate status was defined as a blood (serum) folate concentration >59 nMol/L. Older adults with low blood levels of B_{12} and high blood levels of folate were at higher risk of anaemia and impaired cognitive function. However, when blood levels of B_{12} were normal, high blood levels of folate were protective of cognitive function. The authors of this study consider the previously proposed effects of free (unmetabolised) folic acid in the circulation (as a result of repeated high intakes of fortified food or supplements) which may cause rapid deterioration of the central nervous system (Reynolds, 2002). On the other hand, the beneficial effects of high folic acid intakes, where B_{12} levels were normal, support the idea that many older adults would actually benefit from more folate and folic acid food fortification (Morris *et al*, 2007).

In summary, this recent study indicates a 'good' and 'not so good' effect of folic acid food fortification. On the 'good' side if B_{12} status is good, then folic acid (even at high intake levels) is protective of cognitive function (Morris *et al*, 2007; Smith, 2007). However, if B_{12} status is not good, the associated cognitive impairment can be made worse by high intakes of folic acid (Morris *et al*, 2007; Smith, 2007).

Collectively, these recent reports indicate a beneficial effect of folic acid food fortification on cognitive function of older adults—as long as their B_{12} status is adequate. Thus, the main implication for folic acid food fortification is to emphasise the importance of ensuring older adults with low B_{12} status are identified and treated.

8.4 Folic Acid and the Risk of Cancer Development

For some time folate has been considered to have a role in cancer prevention. Folate deficiency has been linked to the risk of cancer in humans—including cancer of the colon, other parts of the gastrointestinal tract, pancreas and breast (Kim, 2006). Some, but not all, retrospective studies suggest higher dietary intake levels and higher blood markers of folate status are generally associated with a reduced risk of malignant disease, especially involving the colorectum (Ulrich, 2007; Ulrich and Potter, 2007; Kim, 2006). This situation led to a definitive study to test the chemopreventive effect of folate on colorectal adenomas or polyps (well established precursors of colorectal cancer [adenocarcinoma]). This six year randomised controlled trial, involving people with a recent history of these lesions, found that supplementation with a high dose of folic acid (1mg/day) did not prevent the re-occurrence of adenomas. Conversely, this study demonstrated that high dose folic acid supplementation significantly increased the number of adenomas by 44% (RR=1.44, CI=1.03–2.02) and increased the incidence of advanced lesions (Cole *et al*, 2007). The publication of this study provides support for the theory (arising from animal studies) that folate (folic acid) nutrition has a dual role in the development of cancer—a harmful role in addition to the protective role that is well documented in previous studies.

In addition to the randomised controlled trial of high dose folic acid supplements on colorectal cancer risk (Cole *et al*, 2007), an epidemiological study of time trends for colorectal cancer incidence in the US and Canada speculate that the introduction of folic acid fortification may have been responsible for an observed increase in colorectal cancer incidence (Mason *et al*, 2007). This study shows that mandatory fortification of foods with folic acid occurred at about the same time as non-significant increases in colorectal cancer incidence (Mason *et al*, 2007). However, it has been noted that if the increased rate of colorectal cancer was caused by folic acid fortification, the effect of folic acid on progression of cancer would have had to have been immediate (Bayston *et al*, 2007 and 2008). It was also noted by Scientific Advisory Committee on Nutrition (SACN, 2007) of the Food Standards Agency in the UK, that the timing of changes in the blood folate concentration of the American population was also not clearly consistent with changes in colorectal cancer incidence. However, SACN rejects the argument made by others (Bayston *et al,* 2007 and 2008) that the increases in colorectal cancer incidence rates can be explained by changes in screening practices (SACN, 2007). The reality is that time trends in national cancer incidence rates can be affected by many different factors and fluctuations in rates over a few years are observed from time to time.

In summary, the new research linking folic acid intakes with cancer risk requires careful consideration. The implementation group noted that the amount of folic acid given to people involved in the randomised controlled trial carried out by Cole *et al* (2007) was ten times greater than the amount the proposed mandatory folic acid fortification programme of bread in Ireland will deliver (>1,100 µg vs. 110 µg folic acid, respectively). Nonetheless, a thorough review of all aspects of this potential risk was carried out and the findings are discussed in this section.

8.5 Folic Acid and the Development of Cancer: Mechanisms for a Protective Role and a Harmful Role

8.5.1 The role of folate in DNA replication and repair

Folate is a coenzyme that carries one-carbon unit and is of critical importance in the metabolism of amino acids and nucleotides needed for DNA (Scott and Weir, 1998) (see Appendix 2 for technical information). The cancer-preventive role of folate is mainly attributed to its central role in the synthesis, replication and repair of DNA (Ulrich and Potter, 2007; Ulrich 2007). However, the precise mechanism whereby folate status might affect DNA function is currently not well defined (Ulrich and Potter, 2007; Ulrich, 2007). Previously, the protective effects of dietary folate, including folic acid, were considered to be related to ensuring an adequate amount of folate for DNA metabolism (Ulrich and Potter, 2007; Ulrich, 2007). However, emerging evidence indicates that the mechanisms are much more complex and that a dual role exists for folate nutrition in cancer development—one that is protective against new tumour development and another that may promote growth of existing tumours (see Appendix 2 for technical details). Understanding the elements that produce these diverging effects is essential for ensuring optimal and safe intake levels of folic acid for all in the population.

8.5.2 The dual role of folate in cancer development

1) Cancer prevention—studies of cell biology and studies involving animals provide strong evidence that folate adequacy is protective against cancer through the central role it plays in DNA function and repair. Such studies indicate that where there is folate deficiency, DNA repair and function is upset. The resulting abnormal patterns in DNA function are among the most common identified in the development of cancer.

2) Cancer promotion—cancer cells have much higher rates of growth and replication (division into multiple cells) compared with normal, healthy cells. These higher growth rates require accelerated DNA synthesis. Therefore, the key role that folate plays in DNA synthesis and replication, makes folate a potential growth promoter of cancerous cells. Studies of cell biology have shown that cancer cells tend to increase their uptake of folate. This explains why chemical agents that have an anti-folate effect are the basis for chemotherapy for cancer (Farber, 1949; Heinle and Welch, 1948).

8.6 Factors Influencing a Protective Role or a Harmful Role of Folic Acid in the Development of Cancer

8.6.1 The importance of the amount of folate consumed in cancer development

Two recent studies of postmenopausal breast cancer risk and folate nutrition highlight the importance of the amount of folate consumed in determining safety or risk (Stolzenberg-Solomen *et al*, 2006; Ericson *et al*, 2007; Ulrich, 2007). In 2006, the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial (PLCO) involving 25,400 women (aged >55 years) reported that very high folate intakes, attributed to excessive supplement use (folic acid >400 µg/day), may generally be harmful rather than beneficial in breast cancer development (Stolzenberg-Solomen *et al*, 2006). Conversely, in a more recent report from the Swedish Malmo Diet and Cancer study (cohort of 11,699 women, aged >50 years), women with the highest folate intakes were found to have over 40% lower risk of breast cancer compared with women with the lowest folate intakes (Ericson *et al*, 2007). Differences in the amount of folate consumed (and, presumably, in their folate status) among these two cohorts of women may explain the divergent outcomes in breast cancer risk associated with folate intake (Ulrich, 2007).

The protective associations of folate against breast cancer in the Swedish study were stronger than those reported from previous cohort studies, which mainly involved women in the US. Previous American studies have generally shown no relation between folate intake and breast cancer alone (Ulrich, 2007; Kim, 2006). However, a consistent finding in all of these studies is the protective effect of folate against the development of breast cancer among women with high alcohol intakes (Ulrich, 2007; Kim, 2006). The interaction of folate and alcohol is plausible. Alcohol interferes with how the body absorbs and utilises folate (Halsted *et al*, 2002) and a high consumption of alcohol is a well known risk factor for breast cancer.

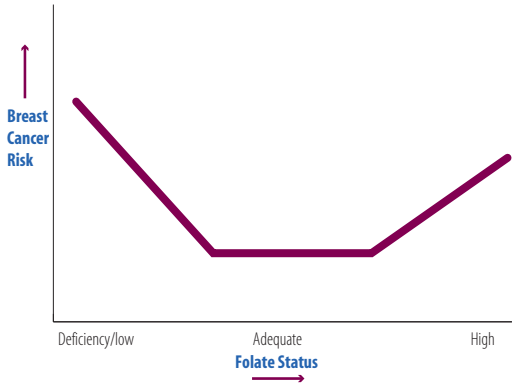
It has been suggested that the much stronger protective effects of folate against breast cancer in the Swedish study compared with previous American studies, is possibly related to the likelihood that the Swedish women had a lower folate status (Ulrich, 2007). If only very low folate status increases the risk of invasive breast cancer, then a protective effect of folate would only be detected in populations where significant numbers of women have low folate status (Ulrich, 2007). The PLCO study findings that excessive folic acid intakes increased risk of breast cancer, however, raise the possibility that intakes that exceed an adequate level are no longer protective but become harmful (Ulrich, 2007). The risk of developing breast cancer was significantly increased by 19% (HR 1.19; 95% CI 1.01–1.41; *p* trend=0.04) in women reporting supplemental folic acid intakes >400 µg/day compared with those reporting no supplement intake (Stolzenberg-Solomen *et al*, 2006; Ulrich, 2007). The differences in level of folate intake among high consumers in the Swedish study, where only 19% of the women were supplement users, compared with high consumers in the US PLCO cohort, are considerable (Ulrich, 2007). Women with the highest intakes of folate (those with intakes in the top fifth [quintile]) in the Swedish cohort was >349 µg/day (Ericson *et al*, 2007), whereas the highest quintile in the American PLCO cohort was more than double this at >853 µg/day (Stolzenberg-Solomen *et al*, 2007; Ulrich, 2007).

Considering these recent studies and evidence from other epidemiological studies, it has been suggested that the relation between folate and postmenopausal breast cancer may be non-linear (Ulrich, 2007). According to this theory:

1) optimal folate status for breast cancer prevention is where levels are adequate (above the level of deficiency) but not excessive, and

2) highest risks for developing breast cancer are at the two extremes of folate status—at low levels and at high levels (Figure 5).

Figure 5: Adequate (but not excessive) Folate Status may be Optimal for Breast Cancer Prevention (adapted with permission from Ulrich, 2007)



8.6.2 Factors affecting achievement of 'adequate folate status'

1) **Genetic factors** (See Appendix 2 for technical notes)—genetic variation involving genes that are involved in the absorption, transport and metabolism of folate would be expected to affect the folate status of individuals, even though they are exposed to the same amount of folate in the diet. There is a growing body of evidence implicating differential cancer risks associated with the polymorphisms in the folate metabolic pathway (MTHFR C677T and A1298C). In the case of colorectal cancer, some research indicates that the two polymorphisms might protect against colorectal adenomas developing into cancer (Huang *et al*, 2007); while other research indicates differential vulnerability to type of colorectal tumour (Hubner *et al*, 2007; Chang *et al*, 2007). Thus, 'gene-nutrient interactions' will influence the level of dietary folate or folic acid that is optimal for individuals in terms of preventing cancer. Awareness of the distribution of genetic polymorphisms affecting folate metabolism are useful for gauging the effects of folic acid food fortification.

2) **Folic acid vs folate** (see Appendix 2 for technical notes)—natural folates and the synthetic form, folic acid (used in fortified foods and supplements), differ in terms of rates of absorption (bioavailability) and metabolism (see Chapter 3 Report of the NCFAFF, 2006). When ingested in amounts that relate to those available naturally in the diet, folic acid is readily absorbed and metabolised in the liver to the polyglutamate forms, which are indistinguishable from those derived from naturally occurring folates in food. However, when ingested in pharmacological amounts (i.e. exceeding the amount that could be provided by the diet), the absorption and metabolism of folic acid differs from that of naturally occurring forms of the vitamin.

In addition to being more bioavailable (more readily absorbed from the intestine into the body), intakes of large doses of folic acid result in free folic acid in blood plasma. This happens because the capacity within the small intestine is limited for converting folic acid to the natural form of folate found in the body (5-methyl –THF). If single doses of folic acid exceed 200 µg, they are not metabolised immediately resulting in unaltered folic acid circulating in the blood (Kelly *et al,* 1997; McPartlin *et al,* 1997)—a phenomenon not encountered from consumption of natural folates.

Therefore, the form of the vitamin folate is an important factor in determining the achievement of an 'adequate folate status' for the prevention of cancer. Folic acid is the form always used for food fortification and supplements. As indicated by the NCFAFF (2006), these differences emphasise the importance of ensuring dietary, rather than pharmacological, amounts are the levels used for food fortification and supplements.

8.7 Folic Acid Food Fortification in Ireland: Finding a Safe Balance between too Little and too Much

8.7.1 The need to avoid too little folic acid

During the 1970s and 1980s, before voluntary addition of folic acid to foods (such as breakfast cereals), a high rate of pregnancies in Ireland were affected by NTDs. For example, the crude birth prevalence per 10,000 in Dublin was as high as 46.9 in 1980 (McDonnell *et al*, 1999) and represented one of the highest rates in Europe. During the 1980s and early 1990s, voluntary addition of folic acid to foods became more common in Ireland, and during this time there was a decline in the rate of births in Ireland affected by NTDs (McDonnell *et al*, 1999). This decline was evident in other countries also (Botto *et al*, 2005) where falling rates were partly, but not fully, explained by more widespread pre-natal diagnosis and selective termination of affected pregnancies (EUROCAT Working Group, 1991). Because such secondary prevention (pre-natal screening and termination of affected pregnancies) is not a legal option in Ireland, the increase in folic acid in the diet is believed to be the most significant factor in inducing the decline in NTDs. Since the mid-1990s, national health policy in Ireland, similar to a lot of other countries, advised women to take a folic acid supplement and to choose folic acid fortified foods to prevent birth defects.

The trends in rates of pregnancies affected by NTDs in Ireland indicate that natural forms of folate may not be sufficient to meet the needs of Irish women of childbearing age. The marked geographic variation in European trends of NTD rates demonstrates that Ireland and the UK have been particularly vulnerable to the development of these birth defects for many decades (EUROCAT Working Group, 1991). Research has shown there is a genetic basis for this vulnerability to NTDs in Ireland (see Appendix 2: Genetic basis for beneficial effects of folic acid in the prevention of NTDs in Ireland). Furthermore, these studies indicate that increasing dietary folic acid intakes can be particularly effective in the prevention of NTDs in Ireland. The genetic variation, which affects almost half (48%) of all pregnancies in Ireland (Kirke *et al*, 2004), is associated with lower blood folate status (Molloy *et al*, 1997; Mills *et al*, 1995).

In summary, removing all folic acid from the diet of people living in Ireland would most likely result in sharp increases in preventable birth defects (NTDs). The new data linking folate nutrition and cancer emphasise the need to ensure folate deficiency is avoided. However, the new data emphasise the need to ensure a safe level of folic acid and that no sub-group is exposed to excessive intake levels. Mandatory folic acid food fortification of a staple food (bread) represents the most effective and safe means of ensuring an even distribution of folic acid within the population. The lowest effective dose can be used and monitoring high consumers of the one food category used is relatively straight-forward. Contrary to this, voluntary folic acid fortification is not easily monitored or controlled (see below and Chapter 5).

8.7.2 The need to avoid too much folic acid

Natural forms of food folate are not readily absorbed by the body and so the safe upper limit only applies to the synthetic form of the vitamin—folic acid. The safe upper limit independently established by two expert panels (EU Scientific Committee on Food, 2000; US Institute of Medicine's Food and Nutrition Board, 1998) relates to the risk of excessive amounts of folic acid to mask vitamin B_{12} deficiency. This is set at 1,000 µg/day for adults; with values extrapolated from this for children which take account of their smaller body size. While these tolerable upper level (UL) values were not established in relation to cancer risk, they serve as a practical guide for maximum dietary intake levels in the absence of more specific criteria.

Over the past five years, in particular, the range of foods voluntarily fortified with folic acid has increased substantially. In tandem with this, the *amount* of folic acid added to various foods has also increased—but this varies considerably between different food categories (see Chapter 5). The nature of voluntary fortification is *ad hoc* in that the manufacturer decides when to initiate or discontinue the fortification and on the level of folic acid added. New rules have been introduced at European level to regulate voluntary fortification[9] but implementation of controls is at an early stage. Maximum levels of nutrient addition are yet to be established and there are no indications on whether the range of foods that can be fortified will be controlled. Monitoring for high consumption of folic acid fortified is very

[9] Regulation (EC) No 1925/2006 (OJ L404, p26, 30/12/2006) of the European Parliament and of the Council of 20 December 2006 on the addition of vitamins and minerals and of certain other substances to foods. Amended by *Regulation (EC) No 108/2008* (OJ L39, p11, 13/02/2008) of the European Parliament and of the Council of 15 January 2008.

difficult in this situation as people with relatively low food intakes but who choose mainly fortified brands inadvertently consume excessive amounts of folic acid.

In addition to fortified foods, use of supplements providing folic acid has to be considered. The US PLCO study found a link between excessive folic acid intakes (attributed to supplement use) and an increased risk of breast cancer. (Stolzenberg-Solomen *et al*, 2006). To prevent birth defects, women of childbearing age are advised to take a supplement providing 400 µg of folic acid, per day. However, consumers may be exposed to excessive folic acid intake if they choose a high dose supplement, or through multiple doses if they consume a range of different supplements. From a regulatory perspective, the European Commission is in the process of setting maximum and minimum limits for the amounts of vitamins (including folic acid) and minerals present in food supplements[10].

In summary, control of both voluntary folic acid fortification and of the amount of folic acid available in supplements, is imperative to ensure upper safe levels of folic acid are not exceeded. Monitoring the extent and causes of excessive folic acid consumption is very difficult given the extent of current voluntary folic acid fortification and range of food supplements available. Assessment of blood level folate status of various population sub-groups is important in this situation as a more precise indication of actual exposure is provided (see Chapter 4).

8.8 Conclusions and Recommendations

1) **Given the genetic make-up of the Irish population, some folic acid food fortification is likely to be necessary to ensure adequate intake and to prevent pregnancies being affected by preventable NTDs.**

2) **To minimise cancer risk, it is best to have an adequate, but not excessive, intake of folate—this probably requires some folic acid food fortification.**

3) **To preserve cognitive function in older adults, identification and treatment of low B_{12} status together with ensuring folate intakes are adequate (probably involving some folic acid food fortification) is desirable.**

4) **Controls need to be established to ensure a safe level of folic acid food fortification in Ireland so that no population sub-group is exposed to deficient or excessive intake levels.**

5) **Monitoring dietary intakes of folate, folic acid, B_{12}, and other related B vitamins, along with blood markers of these nutrients, should continue in order to assess exposure of various population sub-groups. This is particularly important given the predominance of voluntary folic acid food fortification, which has an uneven distribution within the population.**

6) **To ensure an even intake of folic acid across all population sub-groups, controls have to be developed for voluntary fortification, both in terms of limits on range of foods that can be fortified and on maximum amounts of folic acid that can be added to fortified foods.**

7) **Controls also need to be implemented on the amount of folic acid provided in supplements.**

[10] Directive 2002/46/EC of the European Parliament and of the Council of 10 June 2002 on the approximation of the laws of the Member States relating to food supplements.

APPENDIX 1. RECOMMENDATIONS OF THE NATIONAL COMMITTEE ON FOLIC ACID FOOD FORTIFICATION, 2006

The recommendations of the Committee are:

Recommendation 1: Policy Aspects of Folic Acid Food Fortification

- All bread (white, wholemeal and brown) manufactured or marketed in Ireland, with the exception of minor bread products, should be fortified on a mandatory basis with folic acid at a level which provides 120 µg per 100 g of bread as consumed.
- Consumer choice should be accommodated by excluding minor bread products as well as retail flour, from the mandatory fortification programme.
- An implementation group should be established to oversee the operational issues associated with these recommendations and to advise the Minister for Health and Children on progress.

Recommendation 2: Legislation and Folic Acid Food Fortification

- The Department of Health and Children should make new regulations that would introduce mandatory fortification of all bread marketed in Ireland, with the exception of minor bread products.
- The new regulations should provide for upper and lower tolerance limits around 120 µg folic acid per 100 g bread which will be set by the implementation group following consultation with the industry.
- The new regulations should address labelling and health and nutrition claims for breads fortified with folic acid.

Recommendation 3: Technical Aspects and Folic Acid Food Fortification

- The implementation group should consult with industry to determine the most appropriate point(s) in the bread-making process to add folic acid.
- Technical guidance and codes of practice should be developed to support ongoing quality assurance of the folic acid fortification process.
- External assessment procedures should be put in place to monitor the folic acid fortification levels of all fortified breads, compliance with the labelling format and health claims provided for in food regulations.
- An adequate lead in time before enactment of the legislation on folic acid fortification should be given to allow for adequate preparation by industry.

Recommendation 4: Monitoring the Effects of Folic Acid Food Fortification

- An assessment of all pregnancies affected by NTDs, including those that do not reach term, should be undertaken immediately to establish a baseline for monitoring.
- A national congenital birth defects register that records all pregnancies affected by birth defects, including those that do not reach term, should be established without delay for ongoing surveillance purposes.
- To assess the impact of the fortification programme on the population, the measurement of blood parameters relevant to folate status of all age/sex groups should be undertaken immediately to establish a baseline for monitoring and this should be repeated at regular intervals.
- Dietary intakes of the B vitamin, folate, in all population sub-groups should be monitored regularly and these assessments should distinguish between intakes of naturally occurring food folate and folic acid from fortified foodstuffs and supplements.

- Monitoring of folic acid levels in breads should be included as part of the national food monitoring and surveillance programme.
- Monitoring of folic acid levels in foods that are voluntarily fortified with folic acid and in supplements available in Ireland, should be included as part of the national food monitoring and surveillance programme.
- The implementation group should report to the Minister for Health and Children on the overall impact of the fortification programme as identified by the monitoring programmes.

Recommendation 5: Health Professionals and Folic Acid Food Fortification

- All relevant health professionals should be updated on the implications of the mandatory folic acid food fortification programme and on the need to continue to advise women of childbearing age who are sexually active to take folic acid supplements.
- Written and web-based material outlining the implications of the folic acid fortification programme for women's health should be made widely available by health professional representative bodies and agencies.
- The implementation group should address barriers to folic acid supplement use, including cost and availability.
- All relevant health professionals should be aware that high dose folic acid supplements may increase the risk of masking B_{12} deficiency.

Recommendation 6: Folic Acid Supplements

- While the level of addition of folic acid to bread will contribute to a reduction in the incidence of NTDs, it will not, however, provide women who could become pregnant and are sexually active, with the optimal level recommended for protection of their pregnancies. Therefore, the policy of recommending folic acid supplements for women needs to continue.

Recommendation 7: Health Promotion Needs

- Awareness of the need for women of childbearing age who are sexually active to take folic acid supplements should be actively and vigorously promoted through a national integrated health promotion programme involving all stakeholders across all settings.
- Awareness of the need for women of childbearing age who are sexually active to take folic acid supplements should be promoted by the relevant Government departments.

APPENDIX 2. FOLIC ACID AND THE DEVELOPMENT OF CANCER—MECHANISMS FOR A PROTECTIVE ROLE AND A HARMFUL ROLE

A2.1 Technical Outline of the Role of Folate in DNA Replication and Repair

Folate and other B vitamins (B_6 and B_{12}) function as co-enzymes in one carbon metabolism which is critical for synthesis and methylation of DNA (Scott and Weir, 1998). Folate is required for the conversion of homocysteine into methionine and eventually into *S*-adenosylmethionine (SAM), which is the primary donor of methyl groups for most methylation reactions—including DNA methylation (Scott and Weir, 1998). Folate also assists in the synthesis of purines and thymidylate for DNA synthesis (Scott and Weir, 1998). DNA methylation is central to gene silencing and, probably, to the suppression of repetitive DNA of viral origin which comprises considerable parts of the genome (Ulrich, 2007; Laird, 2005). Deficiency of folate, or the other B vitamins involved in these one-carbon reactions, may interfere with DNA methylation and synthesis, leading to aberrant gene expression and DNA instability, and the eventual development of birth defects and cancer (Davis and Uthus, 2004; Kim, 2006; Lin *et al*, 2008). However, the precise influence of folate status on DNA methylation is not well defined—thus the affects of folate status on cell biology are unclear (Ulrich, 2007).

A2.2 Technical Outline of the Dual Role of Folate in Cancer Development: The Importance of Timing and Dose

Despite the central function of folate in maintaining DNA integrity and stability, the folate nutrition may have dual effects on cancer development (Kim, 2006; Ulrich and Potter, 2007; Ulrich, 2007). Several lines of experimental data (studies involving animals and cell cultures) indicate that both the *timing* and *dose* of folate supplementation during carcinogenesis (the development of cancer) matters (Ulrich and Potter, 2007; Ulrich, 2007; Kim, 2006; Song *et al*, 2000a and b). Although increases in folic acid intake before the existence of pre-cancerous lesions (such as polyps [adenomas] or aberrant crypt foci [microscopic adenomas]) can prevent tumour development, once pre-cancerous lesions are present, supplementation with folic acid may increase tumour growth (Ulrich, 2007). Kim (2006) outlined this potential for a dual modulatory role for folate on carcinogeneisis:

'In normal tissues, folate deficiency is associated with DNA strand breaks, impaired DNA repair, increased mutations and aberrant DNA methylation—thereby predisposing them to neoplastic transformation. Folate supplementation can correct some of these defects induced by folate deficiency, thereby preventing or suppressing neoplastic transformation. In contrast, in neoplastic cells, where DNA replication and cell division are occurring at an accelerated rate, folate depletion causes ineffective DNA synthesis, resulting in inhibition of tumour growth and progression, which is the basis for antifolate-based cancer chemotherapy' (Kim, 2006).

A2.3 Technical Outline of Factors Affecting Achievement of 'Adequate Folate Status'

A2.3.1 Genetic factors

Several genetic polymorphisms in the folate metabolic pathway (MTHFR C677T and A1298C) interact with folate, folic acid and other related nutrients, and alcohol. Such genetic variation in genes that are involved in the absorption, transport, metabolism and excretion of nutrients have been shown to modify cancer risk and also to modulate the effect of nutrients and related compounds on cancer risk (Kim, 2006; Rebbeck *et al*, 2004). Evidence is accumulating that these polymorphisms influence risk of cancer (Huang *et al*, 2007; Zintzaras, 2006). In the case of colorectal cancer, some research indicates that the two polymorphisms might protect against colorectal adenomas developing into cancer (Huang *et al*, 2007); while other research indicates differential vulnerability to type of colorectal tumour (Hubner *et al*, 2007; Chang *et al*, 2007). Thus, although individuals within a population might be exposed to a similar level of dietary folate or folic acid, the level of folate or its metabolites in the target tissue—and the functional effects in the target tissue—could be very different due to genetic or epigenetic variation.

This 'gene-nutrient interaction' will obviously influence the level of dietary folate or folic acid that is optimal for individuals in terms of preventing cancer.

A2.3.2 Folic acid vs folate

Differences in the bioavailability and metabolism of folic acid and folate are factors that affect the achievement of 'adequate folate status,' in terms of optimal intake for prevention of cancer. When natural folates are consumed, absorption into the body occurs in the small intestine through the action of an enzyme, folate conjugase. This enzyme breaks down the natural folate polyglutamates to monoglutamates. This step is not required for absorption of folic acid because it is already in monoglutamate form, which makes folic acid more bioavailable (more readily absorbed from the intestine into the body).

For transport to all cells in the body through the blood circulatory system, the folate monoglutamates are usually all converted to one form, 5 methyl-tetrahydrofolate (5-methyl-THF), which passes by diffusion from blood into all body cells. However, if large doses of folic acid are consumed the mechanisms converting monoglutamates to 5-methyl-THF are saturated and free folic acid appears in plasma. This happens because the capacity for converting folic acid to 5-methyl-THF in the small intestine is limited. If single doses of folic acid exceed 200 µg, they are not metabolised immediately resulting in unmetabolised folic acid circulating in the blood (Kelly *et al*, 1997; McPartlin *et al*, 1997)—a phenomenon not encountered from consumption of natural folates.

Cells in the body cannot retain the form of folate usually delivered by blood (5- methyl-THF). Therefore, through the action of enzymes that also need vitamin B_{12} to work, 5-methyl-THF in the cells is converted to THF, which is a form that can be retained. In fact, THF is the active substrate for synthesis of forms of the folate vitamin needed by the body (THF-polyglutamates). Normally, any unaltered (or 'free') folic acid delivered to cells by the blood stream is also converted by these vitamin B_{12}-dependent enzymes to THF inside the cells, or is excreted in urine.

Therefore, the form of the vitamin folate is an important factor in determining the achievement of an 'adequate folate status' for the prevention of cancer. Folic acid is the form always used for food fortification and supplements. As indicated by the NCFAFF (2006), these differences emphasise the importance of ensuring dietary, rather than pharmacological, amounts are the levels used for food fortification and supplements.

A2.4 Genetic Basis for Beneficial Effects of Folic Acid in the Prevention of NTDs in Ireland

A significant association has been shown between mothers of children with NTDs and a common variation in the gene coding for an enzyme involved in folate metabolism (Whitehead *et al*, 1995; van der Put *et al*, 1995). The enzyme involved is 5,10-methlylenetetrahydrofolate reductase (MTHFR). The genetic variation codes for a thermolabile (temperature sensitive) variant of the enzyme, which only has about 50% of the enzymatic activity. A study based in Dublin found low red blood folate (red cell folate) levels in people with this genetic variation (Molloy *et al*, 1997), and they also have elevated homoscysteine blood levels (Kang *et al*, 1988), which may increase their risk of coronary heart disease (Kang *et al*, 1991). Previous work in Dublin has shown that low folate and raised homocysteine levels in early pregnancy are risk factors for the development of NTDs (Mills *et al*, 1995).

A2.5 Irish Population at Particular Genetic Risk for Development of NTDs

The normal gene encoding for MTHFR is referred to as CC, which indicates that both chromosomes in the pair have the normal gene. Variations within the gene for MTHFR can involve just one of the chromosomes and is referred to as CT (heterozygosity for the T allele of the C677T polymorphism); or it may involve both chromosomes and is referred to as TT (homozycosity for the T allele of the C677T polymorphism). While the CT polymorphism (heterozygous) is more common, occurring in more than a third (38%) of the Irish population, the TT polymorphism is rarer, only occurring in about 10% of the Irish population. Until last year, it was thought that risk of developing NTDs was only associated with the rarer TT polymorphism (Botto *et al*, 2000). However a recent study involving over 300 survivors of NTDs in Ireland, found that the CT polymorphism is also associated with risk of developing NTDs (Kirke *et al*, 2004). It was estimated from the findings of this study that up to half of the folate-related NTDs may be explained by this single genetic variant. Most significantly, this study indicates that up to half of all pregnancies in Ireland have the genetic background for risk of developing an NTD.

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Notes

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