The Benefits and Risks of Folic Acid Fortification

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In 1998, the Food and Drug Administration (FDA) implemented a policy that required the fortification of enriched grain products with folic acid, the aim being to help prevent neural tube defects in newborns. Increased folic acid intake has been associated with reduced risks of cardiovascular disease and colon cancer, but also carries the danger of masking symptoms of vitamin B-12 deficiency. The fortification policy has been predicted to provide an overall health and economic benefit to society. Since policy implementation, folic acid intake among the general population has increased and the rate of neural tube defects has decreased. More research needs to be done to evaluate the full health and economic benefits and costs of folic acid fortification, as well as to more specifically assess the policy's long-term impact.

Background

Neural tube defects

Between the seventeenth and thirtieth day after conception, the neural tube – which later becomes the spinal cord, brain and skull – forms in the embryo and closes. A neural tube defect (NTD) occurs when the neural tube fails to close properly, leaving the developing brain or spinal cord exposed to amniotic fluid. These defects occur within the first four weeks of pregnancy, and result in malformations of the spine (spina bifida), skull and brain (anencephaly).[1,2]

NTDs are some of the most common birth defects, having affected approximately 2,500 infants,[3] 3 to 5 percent of all infants born annually in the U.S. by

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the early 1990s,[4] and approximately 1,500 NTD-affected fetuses are aborted each year.[5] It is estimated that half of NTD-affected pregnancies are related to inadequate folate intake by the mother during the early weeks of pregnancy.[3]

There are severe health, lifestyle, and economic implications of NTDs. Birth defects overall are the leading cause of infant mortality in the United States and the fifth leading cause of years of potential life lost.[6] These consequences are especially profound in cases of anencephaly and spina bifida, two common types of neural tube defects. All infants with anencephaly die shortly after birth, and most babies born with spina bifida grow to adulthood but suffer from varying degrees of bowel and bladder incontinence, as well as sometimes paralysis.[7] The total economic burden to society of spina bifida is approximately $489 million per year, a total of $294,000 per new case.[6]

Folic acid fortification policy

The extent of the problem for NTDs is of particular interest because they are somewhat preventable. Research in the early 1990s[8,9] established that high intakes of folate or folic acid, two forms of a water-soluble B-vitamin, can significantly reduce the risk of NTDs in newborns. Such results are seen only when consumption is high during the first 4-6 weeks of pregnancy.

Although this situation appears to create a clear opportunity for a successful health policy intervention, the difficulties with this strategy are manifold. For one, reaching the target audience is difficult because it is not possible to identify women who are at an increased risk for an NTD-affected pregnancy by a simple screening test, and because most women are not aware of their pregnancy during the first 4 – 6 weeks. In addition, if women tried to attain these high levels of folic acid through over-the-counter vitamins, they would risk consuming teratogenic and toxic levels of vitamins A and D, which are believed to cause birth defects when taken in excess during pregnancy[10,11] and can result in severe health consequences in non-pregnant persons.[10,12] For example, vitamin A toxicity in non-pregnant persons can cause problems such as severe liver injury and bone pathologies,[10] while that of vitamin D can result in health problems such as impaired renal function and calcium loss to the bone.[12]

The ideal preventive measure using folate therefore appeared to be to increase the folate intake of all women during their entire fertile period. In 1992, the United States Public Health Service recommended that all women of childbearing age who are capable of becoming pregnant consume 400 micrograms (μg) of folate per day, the current recommended dietary allowance. However, it is virtually impossible from a policy perspective to regulate individual diet and multi-vitamin use. The situation – as well as evidence of folate's potential preventive effects on heart disease and colon cancer – led to strong advocacy for the folic acid fortification of foods. Debate ensued, not only due to the uncertainty about the lowest effective dose and the potential health risks of excessive folate intake involved for the elderly,[13-16] but also due to the weight of such a large federal program.

Fortification efforts are not undertaken lightly, and folic acid fortification would be the first large endeavor since the 1940s.

The addition of iodine to salt in 1924 was one of the earliest fortification programs and was successful in helping to prevent goiter, cretinism, and other symptoms of severe iodine deficiency.[3] Vitamin D was added to cow's milk in the 1930's, and mandatory enrichments – including thiamin, niacin, riboflavin, and iron – were made to flours and breads in 1943.[3] All such fortification requirements have been initiated to enhance the quality of food and to help prevent deficiency diseases in the general population.[3]

Folic acid fortification is particularly important because it has the potential for far-reaching health implications. On the one hand, increased intake of folic acid is proven to help reduce NTD risk,[8,9] and may also decrease heart disease and colon cancer risks.[17-28] On the other hand, these benefits are not without potentially negative side effects, as excessive folate intakes may cause neurological damage in elderly persons that have anemia caused by vitamin B-12 deficiency.[13-26] Controversy remained regarding the appropriate level of fortification to meet these various competing health risks and benefits.[29,30]

In 1993, and only in light of the compelling and significant scientific findings relating folate to NTD prevention,[31] the Food and Drug Administration (FDA) decided to fortify the food supply with folic acid. The policy requires that manufacturers add from 0.43 to 1.4 μg of folic acid per pound of product to enriched cereal-grain products, applicable to the following: breads, cereals, flours, corn grits, corn meal, pastas, rice, rolls and buns, farina, macaroni, and noodle products.[31] Research and data from the National Health and Nutrition Examination Surveys and the Framingham Offspring Study, as well as others, indicate that the fortification program has increased the level of folate equivalents consumed in most American adult diets.[32-36]

Health Effects of Fortification

Fortification effects on folate consumption levels

The primary intent of the policy was to increase the percentage of women of child-bearing age that consume the recommended 400 μg/day of folate from 29% to 50%.[7,37-39] The FDA predicted that fortification would cause total folate intake to increase by an average of 100 μg per day per person. Released data from the National Health and Nutrition Examination Surveys (NHANES) indicates that the average age-specific folate intakes have increased by approximately 100 μg/day for both male and female adults (Figure 1) from the early 1990s to the end of the decade.[40,41]

Various studies have tried to specifically estimate the changes in folate intake as a result of the fortification.[32-36, 42-47] The range of research done includes updating pre-policy survey data,[35] analyzing blood folate levels,[33,34,44,46] quantifying food folate levels in comparison to amounts required and labeled,[36,43,47] and assessing the effects on homocysteine levels of short-term consumption of fortified foods.[42,45] The overall conclusion is that folate intake levels have increased for the average person by approximately 100 μg/day, and possibly more.

For example, Lewis and colleagues[35]

NHANESIII: 1988-1994		
	Male	Female
All Ages	317	239
20-29 years	330	241
30-39 years	340	235
40-49 years	327	234
50-59 years	349	238
60-69 years	345	277
70-79 years	306	268
80 years+	323	265

NHANESIV: 1999-2000		
	Male	Female
All Ages	405	319
20-39 years	435	327
40-59 years	431	335
60 years+	387	312

Figure 1: Mean food folate intakes by age and gender; Sources: 22,23

updated two national food consumption surveys – NHANESIII and NHANES '99-'00 – to reflect folate intake as a result of the recent policy as well as to correct consumption estimates for the higher bioavailability of synthetic folic acid.[48-50] Using information about food ingredients and characteristics, they estimated total folate intakes based on food data and dietary supplement use, concluding that average total folate intake has increased for both men and women since the implementation of the policy.[35] They estimated that over 50% of women of childbearing age are in fact now consuming at least 400 µg/day of folic acid. However, the predictability of these results for NTD rates may be questionable, as this data incorporates a new method of measuring folate intake (dietary folate equivalents), which was not considered in studies of NTD risk attributed to folate intake.

Jacques et al.[34] measured plasma folate using blood samples from the Framingham Offspring Study Cohort and found that fortification of enriched grain products with folic acid was associated with substantial improvement in blood folate status among middle-aged and older adults. Evidence also suggests that folic acid has been added in higher quantities than as required by the regulation, possibly doubling the average projected increase from 100 µg/day to 200 µg/day.[32] This intake difference was the same for both supplement and non-supplement users. However, more recent research suggests that this may not be the case.[43,47]

Folate and NTD prevention

Given the magnitude of the fortification policy, there is much interest in assessing how the fortification of the U.S. food supply with folic acid has impacted the rate of NTD-affected pregnancies in the U.S.

population. Research done since the policy implementation indicates that although the specific goal of reducing the rate by 50%[7] has not been met, the rate of NTDs has decreased by approximately 20%-30%.[37,51-54] This lower rate is in fact what would be expected, given the fortification's predicted increase of 100 μg/day of folic acid.[55] However, given that a large percentage of women of childbearing age are still not consuming the recommended 400 μg/day of the vitamin,[35] this reduction is not as high as that originally aimed for by the US Public Health Service in 1992.[7] The high levels of serum folate now found in the target population[46,56] and of folate found in enriched products[36] may, on the other hand, imply a greater decrease in NTDs than that which has been seen.[55] More research needs to be done on the link between folate intake levels, serum folate and NTDs in order to clarify the conflicting evidence.

Folate and heart disease risk

As increased folic acid intake is associated with decreased risk of coronary heart disease (CHD),[17-19] various studies have considered the potential benefits of fortification on CHD prevention.[13,42,45] In a randomized controlled trial of 75 men and women with coronary artery disease, Malinow and colleagues concluded that cereals fortified with folic acid have the potential to increase plasma folate levels and to reduce plasma homocysteine levels, which are believed to reduce the risk of coronary artery disease.[42] In addition, Tice[45] and Tucker[13] and their colleagues projected that fortification would benefit CHD prevention, as long as the folate-CHD link can be proven.

Folate and colon cancer prevention

In the past decade several studies have shown a decreased risk of colon cancer associated with folate intake.[20-28] The originators of the folate-colorectal cancer hypothesis did a case-control study in which they found a lower risk of rectal cancer and a slightly lower risk of colon cancer in association with high folate intakes.[20] Much of the subsequent evidence for the impact of folate on colon cancer comes from the Nurses' Health Study (NHS)[22] and the Health Professionals' Follow-Up Study (HPFS).[21]

In the former analysis, higher folate intake in 1980 was associated with lower risk of colon cancer in 1994, and the use of multi-vitamins containing folic acid showed a significant benefit after 15 years of use, with a risk ratio of 0.25. In HPFS, higher folate intake among men was associated with a moderately decreased risk for colon cancer as well as a protective effect for the increased colon cancer risk associated with high alcohol intake.[21] This evidence would suggest that the fortification program would benefit colon cancer prevention efforts, although more research needs to be done to clarify the folate-colon cancer relationship.

Potential health and economic costs of fortification

Despite the benefits of increased total folate intake, there is some risk that too much of the vitamin may harm certain populations. Extremely high levels of folate intake can mask changes in the nervous system that are caused by vitamin B-12 deficiency, which is particularly a problem for

elderly persons.[13-15] The prevalence of this disease is estimated to be approximately 9 to 23 cases per 100,000 persons,[16,57] or about 10 to 20 percent of the U.S. elderly population.[3] When left untreated, the anemia caused by B-12 deficiency can result in irreversible neurological problems, such as numbness, balance problems, weakness, and paralysis.[16]

The FDA has addressed this issue by recommending that adults do not exceed 1,000 μg of total folate consumed per day, an amount defined as a tolerable upper intake level.[31] As there is uncertainty regarding the exact number of people who may be affected by this type of anemia, it is difficult to predict the fortification's potential consequences on those at risk.[16] Due to the large number of fortified foods on the market, there is the concern that some people who are at risk for B12 deficiency may now be getting too much folate in their diets.[2,31] Recent research does indicate, however, that fortification may have resulted in no major increase in masking of vitamin B-12 deficiency.[58]

Various public health experts have evaluated the societal economic consequences of the fortification. The calculations predict that an overall economic benefit – produced primarily by decreases in NTD births – should result from the fortification.[13,16,39,45,57,59] For example, Tucker and colleagues[13] estimated that, at the level of 140μg/100 g of enriched grain product, the benefits – including predicted reductions in heart disease risk – of folate fortification outweigh the risks associated with the masking of B12 deficiency. The potential costs, however, of negative health effects on certain populations – specifically those with B-12 deficiency – are significant, and could have the potential to outweigh the benefits if substantial benefits (at a break-even level) are not achieved.[59]

Implications for Future Research

Given the safety concerns for children and the elderly, the uncertainty and debate around the minimum effective dose for preventing NTDs and other diseases, the importance of preventing such ailments in a cost-effective manner, and the magnitude of such a fortification program, there is great interest in evaluating the impact of the FDA's policy decision.[1,29-31,35,36] What specifically remains to be known are the long-term health and economic effects of chronic fortified food consumption – on NTDs, and other health outcomes. Now that fortification has been in place for a number of years, these economic implications can be studied further, utilizing the health and economic costs and benefits that have been realized to date.

In particular, due to the uncertainty regarding the risks of excessive folate intake for populations at risk of B-12 deficiency, this issue should be studied further to determine whether – at NTD-reduction levels attained from the policy – the fortification still offers a net health benefit. Such analyses may also consider further policies that could be put into place to remedy the problem, such as fortifying foods with B12.[30,60] This work should incorporate a full health and economic assessment of the policy's total impact on rates of NTDs, CHD and colon cancer, as well as the outcomes for the elderly and children.

Two further policy questions arise as a result of the fortification. One is the

economic evaluation of a potential policy intervention that targets the use of folic-acid-containing multivitamin supplements among the general adult population. This would be of particular interest if it is found that there is a threshold effect of folic acid intake on colon cancer prevention, for example if the risk ratios level off with intakes above a certain daily folate intake level.

In addition, there is ongoing debate in England regarding implementing a similar policy to help prevent NTDs. The primary emphasis of the dispute revolves around the health risks to the elderly. A full cost-benefit analysis of the policy in that country should be conducted that takes into account not only the NTD-prevention benefits and the risks to persons with B-12 deficiency, but also the benefits of folic acid intake on heart disease and colon cancer. Such an analysis could provide tangible information for British policy-makers in making this decision.

Future research goals should be to elaborate on the mechanisms by which folate acts to reduce the risk of colon cancer, as well as to clarify and empirically estimate the dose-response relationship between folate intake and the progression of colon cancer. Increasing the understanding of this relationship would help to more accurately capture the effects of folate on colon cancer and polyp outcomes, as well as the impact of the fortification policy. The results of such work could help guide future decisions regarding such fortification, dietary, clinical, and preventive guidelines. It may also provide motivation for further prospective studies to prove a link between folate and the risks of CHD and colon cancer.

Folic acid fortification was instigated in order to reduce the risk of NTDs, but it is clear that there are many other health and economic considerations of such a far-reaching program. Future research needs to be done to assess fortification's effects on the population's health and economic well-being. It is our hope that the benefits are shown to far outweigh the costs, and that future research will provide further insight into means by which fortification can be employed to benefit the health of our society.

References

1. Centers for Disease Control and Prevention (CDC), Division of Birth Defects, Child-Development, Disability and Health. Folic Acid Now: Learn More About Folic Acid. Atlanta,GA: Centers for Disease Control and Prevention; 2001. Found at http://www.cdc.gov/ncbddd/folicacid/default.htm.
2. National Academy of Sciences, Institue of Medicine, Food and Nutrition Board. Dietary Reference Intakes: Thiamin, riboflavin, niacin, vitamin B6, folate, vitamin B12, pantothenic acid, biotin, and choline. 1998; National Academy Press. Washington, DC.
3. U.S. Food and Drug Administration (FDA), Office of Public Affairs. Folic Acid Fortification Fact Sheet. 1996. Found at http://vm.dfsan.fda.gov/~dms/wh-folic.html.
4. Williams, RD. Decreasing the Chance of Birth Defects. *FDA Consumer Magazine*. 1996; found at http://www.fda.gov/fdac/features/996_bd.html.
5. Centers for Disease Control and Prevention. Knowledge and Use of Folic Acid by Women of Childbearing Age - United States, 1997. MMWR 1997;46:721-23.
6. Centers for Disease Control and Prevention. Economic Costs of Birth Defects and Cerebral Palsy -- United States, 1992. MMWR. 1995;44(37);694-699.
7. Centers for Disease Control and Prevention (CDC). Recommendations for the use of folic acid to reduce the number of cases of spina bifida and other neural tube defects. *Morbidity and Mortality Weekly Report (MMWR)*. 1992;41(RR14):1.
8. Czeizel AE, Dudas J. Prevention of the First Occurrence of Neural Tube Defects by Peri-Conceptual Vitamin Supplementation. *New England Journal of Medicine*. 1992;327:1832.
9. MRC Vitamin Study Research Group. Prevention of Neural Tube Defects: Results of the Medical Research Council Vitamin Study. Lancet. 1991;338:131-137.

10. U. S. Food and Drug Administration, Center for Food Safety and Applied Nutrition. Illnesses and Injuries Associated With the Use of Selected Dietary Supplements. 1993; found at http://www.cfsan.fda.gov/~dms/ds-ill.html.
11. Willis, JL. All About Eating for Two. FDA Consumer. 1984 (rev. 1990);DHHS (FDA) pub no. 90-2183:found at http://www.cfsan.fda.gov/~dms/wh-preg1.html.
12. Merck & Co. The Merck Manual of Diagnosis and Therapy. 1995-2003; Merck & Co., Inc. NJ: found at http://www.merck.com/pubs/mmanual/section1/chapter3/3e.htm.
13. Tucker KL, Mahnken B, Wilson PW, Jacques P, Selhub J. Folic acid fortification of the food supply: potential benefits and risks for the elderly population. *Journal of the American Medical Association.* 1996;276:1879-1885.
14. Dickinson CJ. Does folic acid harm people with vitamin B12 deficiency? QJM. 1995;88:357-364.
15. Koehler KM, Pareo-Tubbeh SL, Romero LJ, Baumgartner RN, Garry PJ. Folate nutrition and older adults: challenges and opportunities. *Journal of the American Dietary Association.* 1997;97:167-173.
16. Bentley JR. Ferrini RL. Hill LL. American College of Preventive Medicine public policy statement. Folic acid fortification of grain products in the U.S. to prevent neural tube defects. *American Journal of Preventive Medicine.* 1999;16(3):264-7.
17. Abby SL, Harris IM, Harris KM. Homocysteine and Cardiovascular Disease. *Journal of American Board of Family Practice.* 1998;11(5):391-398.
18. Morris CD. Carson S. Routine vitamin supplementation to prevent cardiovascular disease: a summary of the evidence for the U.S. Preventive Services Task Force. *Annals of Internal Medicine.* 2003;139(1):56-70.
19. Rimm EB, Willett WC, Hu FB, Sampson L, Colditz GA, Manson JE, Hennekens C, Stampfer MJ. Folate and Vitamin B6 from Diet and Supplements in Relation to Risks of Coronary Heart Disease Among Women. *Journal of the American Medical Association.* 1998;279(5):359-364.
20. Freudenheim JL, Graham S, Marshall JR, Haughey BP, Cholewinski S, Wilkinson G. Folate Intake and Carcinogenesis of the Colon and Rectum. *International Journal of Epidemiology.* 1991;20:368-374.
21. Giovannucci E, Rimm EB, Ascherio A, Stampfer JF, Colditz GA, Willett WC. Alcohol, Low-Methionine-Low-Folate Diet, and Risk of Colon Cancer in Men. *Journal of the National Cancer Institute.* 1995;87:265-273.
22. Giovannucci E, Stampfer MJ, Colditz GA, Hunter DJ, Fuchs C, Rosner BA, Speizer FE, Willett WC. Multivitamin Use, Folate, and Colon Cancer in Women in the Nurses' Health Study. *Annals of Internal Medicine.* 1998;129:517-524.
23. Glynn SA, Albanes D, Pietinen P, Brown CC, Rautalahti M, Tangrea JA, Gunter EW, Barrett MJ, Virtamo J, Taylor PR. Colorectal Cancer and Folate Status: A Nested Case Control Study Among Male Smokers. Cancer Epidemiology, Biomarkers, and Prevention. 1996;5:487-494.
24. Kato I, Dnistrian AM, Schwartz M, Toniolo P, Koenig K, Shore RE, Akhmedkhanov A, Zeleniuch-Jacquotte A, Riboli E. Serum folate, homocysteine and colorectal cancer risk in women: a nested case-control study. *Br J Cancer* 1999a;79:1917-1921.
25. Su LJ, Arab L. Nutritional status of folate and colon cancer risk: evidence from NHANES I epidemiologic follow-up study. *Annals of Epidemiology.* 2001;11(1):65-72.
26. Thum M, Calle EE, Namboodiri MM, Flanders WD, Coates RJ, Byers T, Boffetta P, Garfinkel L, Heath CW Jr. Risk Factors for Fatal Colon Cancer in a Large Prospective Study. *Journal of the National Cancer Institute.* 1992; 84:1491-1500.
27. White E. Shannon JS. Patterson RE. Relationship between vitamin and calcium supplement use and colon cancer. *Cancer Epidemiology, Biomarkers & Prevention.* 1997;6(10):769-74.
28. Willett WC. Diet, Nutrition, and Avoidable Cancer. *Environmental Health Perspective.* 1995;103(8):165-170.
29. Daly S, Mills JL, Molloy AM, Conley M, Lee YJ, Kirke PN, Weir DG, Scott JM. Minimum Effective Dose of Folic Acid for Food Fortification to Prevent Neural Tube Defects. Lancet, 1997;350(9092):1666-1669.
30. Oakley GP Jr, Adams MJ, Dickinson CM. "More Folic Acid for Everyone, Now." *Journal of Nutrition.* 1996;126:751S-755S.
31. National Institutes of Health, Clinical Nutrition Service. "Facts About Dietary Supplements: Folate." Bethesda, MD: National Institutes of Health; 2001. Found at http://www.cc.nih.gov/ccc/supplements/folate.html.
32. Choumenkovitch SF, Selhub J, Wilson PWF, Rader JI, Rosenberg IH, Jacques PF. Folic Acid Intake from Fortification in United States Exceeds Predictions. *Journal of Nutrition.* 2002:132;2794-2798.
33. Cuskelly GJ, McNulty H, Scott JM. Fortification with low amounts of folic acid makes a significant difference in folate status in young women: implications for the prevention of neural tube defects. *American Journal of Clinical Nutrition.* 1999;70(2):234-9.
34. Jacques PF, Selhub J, Bostom AG, Wilson PWF, Rosenberg IH. The Effect of Folic Acid Fortification on Plasma Folate and Total Homocysteine Concentrations. *The New England Journal of Medicine.* 1999;340:1449-1454.
35. Lewis CJ, Crane NT, Wilson DB, Yetley EA. Estimated Folate Intakes: Data Updated to Reflect Food Fortification, Increased Bio-Availability, and Dietary Supplement Use. *American Journal of Clinical Nutrition.* 1999;70:198-207.
36. Rader JI, Weaver CM, Angyal G. Total folate in enriched cereal-grain products in the United States following fortification. *Food Chemistry.* 2000;70:275-289.
37. Honein MA, Paulozzi LJ, Mathews TJ, Erickson JD, Wong LY. Impact of folic acid fortification of the US food supply on the occurrence of neural tube defects. *JAMA.* 2001;285(23):2981-6.
38. Rader JI. Folic acid fortification, folate status and

plasma homocysteine. *The Journal of Nutrition.* 2002;132(8S):S2466-S2470.
39. Romano PS, Waitzman NJ, Scheffler RM, Pi RD. Folic acid fortification of grain. *American Journal of Public Health.* 1995;85:667-676.
40. Bialostosky K, Wright JD, Kennedy-Stephenson J, McDowell M, Johnson CL. Dietary intake of macronutrients, micronutrients and other dietary constituents: United States 1988-94. National Center for Health Statistics. *Vital Health Stat* 11(245). 2002.
41. Wright JD, Wang CY, Kennedy-Stephenson J, Ervin RB. Dietary intake of ten key nutrients for public health, United States: 1999-2000. Advance data from vital and health statistics; no. 334. Hyattsville, Maryland: National Center for Health Statistics. 2003.
42. Malinow MR, Duell PB, Hess DL, Anderson PH, Kruger WD, Phillipson BE, Gluckman RA, Block PC, Upson BM. Reduction of Plasma Homocysteine Levels by Breakfast Cereals Fortified with Folic Acid in Patients with Coronary Heart Disease. *New England Journal of Medicine.* 1998;338(15):1009-1015.
43. Pawlosky RJ, Flanagan VP. A quantitative stable-isotope LC-MS method for the determination of folic acid in fortified foods. *Journal of Agricultural & Food Chemistry.* 2001;49(3):1282-6.
44. Quinlivan EP, Gregory JFIII. Effect of food fortification on folic acid intake in the United States. *American Journal of Clinical Nutrition.* 2003;77:221-225.
45. Tice JA, Ross E, Coxson PG, Rosenberg I, Weinstein MC, Hunink MG, Goldman PA, Williams L, Goldman. Cost-effectiveness of vitamin therapy to lower plasma homocysteine levels for the prevention of coronary heart disease: effect of grain fortification and beyond. *JAMA.* 2001;286(8):936-43.
46. Lawrence JM, Petitti DB, Watkins M, Umekubo MA. Trends in serum folate after food fortification. *Lancet.* 1999;354:915-916.
47. Afman L, Bagley P, Selhub J. Determination of folic acid in fortified cereal products using the affinity/HPLC method. *FASEB Journal.* 1999;13:A891. abstract.
48. Bailey, Lynn B. "Dietary Reference Intakes for Folate: The Debut of Dietary Folate Equivalent." *Nutrition Review.* 1998; 56 (10):294-299.
49. Herbert V. Recommended dietary intakes (RDI) of folate in humans. *American Journal of Clinical Nutrition.* 1987;45(4):661-70.
50. Suitor W, Bailey LB. "Dietary Folate Equivalent: Interpretation and Application." *Journal of American Dietetic Association.* 2000; 100:88-94.
51. Erickson JD. Folic Acid and Prevention of Spina Bifida and Anencephaly. *Morbidity and Mortality Weekly Report.* 2002;51(RR13);1.
52. Mathews TJ, Honein MA, Erickson JD. Spina bifida and anencephaly prevalence–United States, 1991–2000. MMWR. 2002;51(RR13):8-10.
53. Meyer RE, Siega-Riz AM. Sociodemographic patterns in spina bifida birth prevalence trends–North Carolina, 1995–1999. MMWR. 2002;51(RR13):11-14
54. Williams LJ, Mai CT, Edmonds LD, et al. Prevalence of spina bifida and anencephaly during the transition to mandatory folic acid fortification in the Unites States. *Teratology.* 2002;66:33-9.
55. Wald NJ, Law MR, Morris JK, Wald DS. Quantifying the effect of folic acid. Lancet. 2001;358:2069-73.
56. Centers for Disease Control and Prevention. Folate status in women of childbearing age by race/ethnicity–United States, 1999–2000. *MMWR.* 2002;51(36);808-810.
57. Kelly AE, Haddix AC, Scanlon KS, Helmick CG, Mulinare J. Cost-effectiveness of strategies to prevent neural tube defects. In Gold MR, Siegel JE, Russell LB, Weinstein MC, (eds): ACost Effectiveness in Health and Medicine. 1996. New York: Oxford University Press, 313-348.
58. Mills JL, Von Kohorn I, Conley MR, Zeller JA, Cox C, Williamson RE, Dufour DR. Low vitamin B-12 concentrations in patients without anemia: the effect of folic acid fortification of grain. *Am J Clin Nutr.* 2003;77:1474-1477.
59. Federal Register. March 5, 1995;611:8793-8795.
60. Hirsch S, de la Maza P, Barrera G, Gattas V, Petermann M, Bunout D. The Chilean flour folic acid fortification program reduces serum homocysteine levels and masks vitamin B-12 deficiency in elderly people. *Journal of Nutrition.* 2002;132(2):289-91.