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Flour fortification with iron, folic acid, vitamin B_{12}, vitamin A, and zinc: Proceedings of the Second Technical Workshop on Wheat Flour Fortification

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The opportunity of flour fortification: Building on the evidence to move forward

Burden of vitamin and mineral deficiencies

The World Health Report 2000 [1] identified iron, vitamin A, and zinc deficiencies among the world's most serious health risk factors. Micronutrient malnutrition contributes to a vicious cycle of poor health and depressed productivity, trapping families in poverty and eroding economic security in dozens of countries worldwide. Ensuring adequate intake of these essential nutrients by vulnerable populations will offer enhanced protection from a range of disabilities and diseases, help children grow and learn, and improve health and productivity for adults.

Iron deficiency is the most prevalent nutrient deficiency in the world [2]. It is responsible for approximately 20,854 deaths and a reduction of 2 million disability-adjusted life years (DALYs) among children under 5 years of age [3]. In addition, iron-deficiency anemia in pregnancy is a risk factor for maternal mortality; 115,000 deaths per year from maternal causes and losses of 3.4 million DALYs among women of childbearing age have been attributed to iron deficiency [2, 3]. According to a World Health Organization (WHO) review of nationally representative surveys from 1993 to 2005, 42% of pregnant women and 47% of preschool children worldwide have anemia [3, 4]. Iron deficiency has its greatest impact on the health and physical and intellectual well-being of preschool children and women of childbearing age, though it may also affect other population groups. Although often more severe in poor and rural communities, iron deficiency also occurs in wealthier and urban populations.

Adequate folic acid intake by women before pregnancy and in the first weeks of gestation decreases the risk of neural tube defects (NTDs) [5–7], the world's leading preventable birth defect. The geographic distribution of NTD prevalence is based in part on dietary patterns. For example, in China folate deficiency is more severe and the prevalence of NTDs is decidedly higher among the predominantly wheat-eating populations of the northeast part of the country, where fresh vegetables are less available, than among the populations in the southern part of the country, where fresh vegetables are more available year-round [7]. In the United States and Canada, where a wide range is foods are accessible to the majority of the populations and vitamin and mineral deficiencies are much less common than in most developing countries, NTD rates were also significantly reduced following mandatory addition of folic acid to enriched flours and cereals [8, 9]. These findings suggest it is likely that other populations around the world could also substantially reduce NTDs by eating folic acid–fortified foods.

There is mounting evidence of widespread vitamin B_{12} depletion and deficiency in many population groups that consume low amounts of animal-source foods, the only natural source of vitamin B_{12}. Even in industrialized countries there is a high prevalence of vitamin B_{12} deficiency among the elderly, many of whom require synthetic sources of vitamin B_{12} because of their limited ability to release and absorb the vitamin from foods [10, 11]. Vitamin B_{12} deficiency has been linked to poor pregnancy outcomes and increased risk of NTDs, delayed child development, abnormal cognitive function and depression, anemia, and elevated plasma homocysteine concentrations.

Vitamin A deficiency is a widespread public health problem in developing nations, where it affects more than 130 million preschool children and is the leading preventable cause of childhood blindness [12] and a major underlying cause of child mortality [13]. Sufficient vitamin A intake is essential to maintain an adequate host response to infection. Vitamin A supplementation during early childhood appears to have its greatest impact in reducing case fatality from measles, diarrhea or dysentery, and malaria and other febrile

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illnesses [14]. Twenty million pregnant and lactating women also suffer from low vitamin A status [15], predisposing them to higher risks of night-blindness, anemia, morbidity, and mortality. Newborn vitamin A supplementation appears to be a promising way to reduce early infant mortality [13].

Zinc deficiency is responsible for approximately 4% of deaths and 16 million lost DALYs among children under five in lower-income countries [3] and can usually be found in populations that are iron deficient. Inadequate zinc intake in young children increases the rates of diarrhea and acute lower respiratory infections and reduces linear growth and physical development [16, 17]. Adequate zinc intake is also necessary for women of childbearing age to ensure normal pregnancy outcomes [18].

Worldwide, more than 450 million tons of wheat are used for human consumption each year. Most of the wheat is milled by commercial roller mills and consumed as noodles, breads, pasta, and other flour products by people in nearly every nation of the world. During the production of refined white flour, essential vitamins and minerals are removed by the milling process. As well as losing nutrients during milling, many cereal products also have elements such as phytates that block the absorption of iron and zinc.

Building on the past to gain consensus

Micronutrient fortification of wheat flour was introduced in the United States and Canada in the 1940s. In Latin America, Chile began to fortify wheat flour in the early 1950s [19]. During the 1960s, a number of Latin American countries passed legislation encouraging the addition of iron and B vitamins to flour; as a consequence, some millers began to fortify on a voluntary basis. In 1998, the United States and Canada required that enriched cereal grain products be additionally fortified with folic acid to reduce the prevalence of NTDs. In the late 1990s and early 2000s, public and private sector organizations organized a movement to promote mandatory wheat and maize fortification worldwide.

Among the organizations promoting fortification were WHO (especially the Pan American Health Organization and the Eastern Mediterranean Regional Office), UNICEF, the World Bank, the Asian Development Bank, the Micronutrient Initiative, the US Agency for International Development (USAID) the Centers for Disease Control and Prevention (CDC), SUSTAIN, the International Association of Operative Millers, and the Latin American Milling Association. The effort was also backed by leading public health and nutrition scientists, milling industry executives, and other industry and nongovernmental organization (NGO) leaders. Significant progress toward meeting fortification goals was made in the Americas, the Middle East, and Central Asia. The 2002 United Nations Special Session on Children marked the establishment of the Global Alliance for Improved Nutrition (GAIN) with support for food fortification from the Bill and Melinda Gates Foundation, the Canadian International Development Agency, and USAID. GAIN has since supported a number of countries in efforts to establish flour fortification programs and to further build national fortification alliances and has provided funds for infrastructure to help countries move toward nationwide flour fortification.

Following the 2004 International Grains Council, the Flour Fortification Initiative was formed to accelerate wheat flour fortification in roller mills throughout the world [20]. The Flour Fortification Initiative is a network of public, private, and civic sector leaders representing more than 50 organizations and drawing support from public health organizations and the wheat-growing, wheat-trading, wheat-milling, mill manufacturing, pharmaceutical, and vitamin/mineral premix industries and allied trades. Today flour fortification is increasingly being adopted as normal industrial milling practice in the production of quality flour. Flour Fortification Initiative network members are working with governments around the world to encourage and assist them to change food regulations and food control systems to meet mandatory flour fortification requirements. Disability sector and other civic sector organizations are also joining the cause.

The number of countries with mandatory wheat flour fortification programs rose from 33 in 2004 to 54 in 2007 [21]. Worldwide, 540 million more people gained access to wheat flour fortified with iron, folic acid, or both in 2007, an 8% increase from 3 years before. The number of women aged 15 to 60 years with access to fortified wheat flour increased by 167 million, and the number of births that potentially benefited from wheat flour fortification increased by 14 million. Yet despite these successes, more than two-thirds of the world's population, including millions of women of childbearing age, still lack access to fortified wheat flour and its benefits. Fortification standards and practices vary from country to country, as do the specifications for the type and quantity of the nutrients added [22].

As flour fortification programs gained momentum in the late 1990s and 2000s, WHO, USAID, SUSTAIN, and the Micronutrient Initiative engaged in a number of consultations with countries and regions to help establish guidelines for vitamin and mineral fortification of flour. Meanwhile, new studies suggested that the selection of the type of iron fortificant was complicated by significant differences in the bioavailability of various forms of iron powders and compounds. The Flour Fortification Initiative, in collaboration with the CDC and the Mexican Institute of Public Health, convened a Technical Workshop entitled "Wheat flour fortification: current knowledge and practical applications,"

in Cuernavaca, Mexico, in 2004 [23]. A key focus of the 2004 Workshop was to develop consensus recommendations for fortifying wheat flour with iron and folic acid. The recommendations were unique in that they called for fortification of low- and high-extraction flours with only bioavailable forms of iron fortificants (ferrous sulfate, ferrous fumarate, or electrolytic iron in low-extraction flour, and sodium-iron ethylenediaminetetraacetate [EDTA] in high-extraction flour), as well as folic acid. Recommendations from the Cuernavaca meeting are largely consistent with the recently published WHO/Food and Agriculture Organization (FAO) "Guidelines on food fortification with micronutrients" [24]. This is a key reference for countries considering food fortification to address the high public health burden of vitamin and mineral deficiencies.

Second Technical Workshop on Wheat Flour Fortification

Despite the established WHO/FAO guidelines and the specific call of experts convened in Cuernavaca, many countries where flour is fortified still use elemental iron fortificants (i.e., some forms of hydrogen-reduced iron and atomized iron) that are poorly absorbed. Also, in the past few years, consultants from different international organizations have given variable guidance and information to developers of fortification programs, resulting in confusion and slow progress toward effective flour fortification in a number of countries.

Other challenges include recently raised concerns by some experts about the high burden of vitamin B_{12} deficiency in populations around the world, as well as the growing awareness and understanding of zinc nutrition, which could affect fortification goals and programs. Furthermore, although fortification of flour with vitamin A has been initiated in a few countries, questions remain about the cost of adding vitamin A to flour, as well as the stability of the vitamin A fortificant in flour and flour products. Finally, because large populations in sub-Saharan Africa and Latin America consume maize (corn) flour products as staple foods, the organizers of the 2008 Workshop, the proceedings of which are published in this issue, considered it important to provide relevant guidance related to micronutrient fortification of maize flour.

Under the direction of the Flour Fortification Initiative, a Steering Committee was established for the Workshop. The Steering Committee was composed of internationally recognized nutrition scientists and a cereal chemist, representatives from United Nations agencies and NGOs active in flour fortification, and milling experts and staff from the CDC and the Flour Fortification Initiative (see list of participants on page S94).

The 4-day Second Technical Workshop on Wheat Flour Fortification in Stone Mountain, Georgia, USA, was supported by the CDC, GAIN, and Cargill, Inc. and brought together nutrition researchers, public health experts, specialists from regulatory agencies, international development, and NGOs, and representatives from the premix and milling sectors to develop consensus on "practical and feasible recommendations" for public health authorities, food regulators, and the milling sector to initiate flour fortification, as well as to improve the public health benefits of existing national flour fortification programs.

The purpose of the Workshop was to provide guidance on national fortification of wheat and maize flours, milled in industrial roller mills (i.e., with at least 20 metric tons (MT)/day milling capacity), with iron, zinc, folic acid, vitamin B_{12}, and vitamin A. The guidance was to follow reviews of the latest evidence of the effectiveness of flour fortification as well as new developments in premix products and costs since the 2004 Cuernavaca meeting. The primary aim of the Workshop was to develop consensus on formulations of premix based on the most common ranges of flour consumption. A secondary aim was to develop consensus around the best practices guidelines for premix manufacturers and millers.

Expert working groups prepared technical background documents and draft recommendations on fortification of low- and high-extraction wheat flour with iron, zinc, folic acid, vitamin A, and vitamin B_{12}, based on broad ranges of flour consumption. In addition, working groups prepared draft fortification guidelines for millers and background documents on special issues related to maize fortification and methodological issues in estimating wheat flour consumption.

These background documents served as the scientific and technical basis for discussions during the Workshop. The Workshop included plenary presentations based on the prepared technical background documents, breakout group discussions and debates to propose specific recommendations, followed by a second round of plenary discussions to finalize recommendations on fortification of wheat flour with iron, zinc, folic acid, vitamin B_{12}, and vitamin A, as well as to establish best practices guidelines for millers and premix manufacturers. The technical background documents were revised based on the discussions at the Workshop and are published in this special supplement of the *Food and Nutrition Bulletin*.

Disclaimer

The selection of the type and quantity of vitamins and minerals to add to flour, either as a voluntary standard or a mandatory requirement, lies with national decision makers in each country. This meeting fully recognizes this, and any guidance or recommendations should be

viewed in the context of each country's situation. In addition, the official normative-setting international organizations that guide countries on food standards are WHO and FAO, the Codex Alimentarius Commission, and the Joint FAO/WHO Expert Committee on Food Additives (JECFA).

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the organizations of individuals participating in the Workshop, including Centers for Disease Control and Prevention.

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Revised recommendations for iron fortification of wheat flour and an evaluation of the expected impact of current national wheat flour fortification programs

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Abstract

***Background**: Iron fortification of wheat flour is widely used as a strategy to combat iron deficiency.*

***Objective**: To review recent efficacy studies and update the guidelines for the iron fortification of wheat flour.*

***Methods**: Efficacy studies with a variety of iron-fortified foods were reviewed to determine the minimum daily amounts of additional iron that have been shown to meaningfully improve iron status in children, adolescents, and women of reproductive age. Recommendations were computed by determining the fortification levels needed to provide these additional quantities of iron each day in three different wheat flour consumption patterns. Current wheat flour iron fortification programs in 78 countries were evaluated.*

***Results**: When average daily consumption of low-extraction (≤ 0.8% ash) wheat flour is 150 to 300 g, it is recommended to add 20 ppm iron as NaFeEDTA, or 30 ppm as dried ferrous sulfate or ferrous fumarate. If sensory changes or cost limits the use of these compounds, electrolytic iron at 60 ppm is the second choice. Corresponding fortification levels were calculated for wheat flour intakes of < 150 g/day and > 300 g/day. Electrolytic iron is not recommended for flour intakes of < 150 g/day. Encapsulated ferrous sulfate or fumarate can be added at the same concentrations as the non-encapsulated compounds. For high-extraction wheat flour (> 0.8% ash), NaFeEDTA is the only iron compound recommended. Only nine national programs (Argentina, Chile, Egypt, Iran, Jordan, Lebanon, Syria, Turkmenistan, and Uruguay) were judged likely to have a significant positive impact on iron status if coverage is optimized. Most countries use non-recommended, low-bioavailability, atomized, reduced or hydrogen-reduced iron powders.*

***Conclusion**: Most current iron fortification programs are likely to be ineffective. Legislation needs updating in many countries so that flour is fortified with adequate levels of the recommended iron compounds.*

Introduction

The World Health Organization (WHO) estimates the global prevalence of anemia to be 47% in children under 5 years of age, 30% in nonpregnant women of childbearing age, and 42% in pregnant women [1]. Prevalence rates are highest in Africa and Asia. WHO does not report prevalence rates for iron deficiency; however, nutritional iron deficiency is the main etiologic factor responsible for anemia in industrialized countries and contributes to about 50% of the anemia in the developing countries of Africa and Asia [2]. Iron deficiency occurs when iron requirements cannot be met by absorption from the diet, such as during periods of rapid growth (infancy, adolescence), in pregnancy, and as a result of menstrual or pathological blood loss. Although physiologic mechanisms can up-regulate iron absorption more than 20-fold from single meals containing readily bioavailable iron [3], the plant-based diets that are characteristic of developing countries limit iron absorption because they are rich in phytate and polyphenols [4, 5]. They also contain little animal tissue, which is a source of highly bioavailable iron. The resultant imbalance between requirements and absorption leads to iron deficiency that, depending on severity, may or may not cause anemia.

The high prevalence of iron deficiency in developing

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countries has a significant adverse impact on the well-being and productivity of their citizens. Physical work capacity is reduced. Iron deficiency in pregnancy contributes to the risk of severe anemia, which is associated with higher maternal morbidity and mortality [6]. There is an increase in the risk of preterm delivery and low birthweight and a higher infant mortality rate [7]. Iron deficiency is also more likely to occur after 4 months of age in babies born to mothers with suboptimal iron status during pregnancy [8]. Iron deficiency in infants and young children is associated with delayed mental and motor development [9]. These children may experience emotional problems and fail to meet educational goals later in life, leading to a negative impact on earning capacity in adulthood. The median total annual productivity loss (physical and cognitive combined) has been estimated to be US$16.78 per capita or 4.05% of GDP [10]. The relationship between iron status and infectious diseases is complex and the subject of considerable debate. However, recent observations indicate that upper respiratory infections are more frequent and last longer and that the risk of severe morbidity related to falciparum malaria is increased in iron-deficient children [11, 12].

Four strategies for alleviating nutritional iron deficiency have been advocated. They are dietary diversification to improve iron bioavailability, selective plant breeding or genetic engineering to increase the iron content or to reduce absorption inhibitors in dietary staples, iron fortification of industrially manufactured foods, and iron supplementation with pharmacological doses, usually without food. Food fortification is regarded at the present time as the safest and most cost-effective approach for populations that consume significant quantities of industrially manufactured foods. Staple foods such as cereal flours and condiments are the most appropriate food vehicles for fortification.

Mass fortification is designed to improve the bioavailable iron intake of the whole population with the intention of eliminating iron deficiency in young children, adolescents, and menstruating women, without causing harm to men and postmenopausal women, who may consume more iron than they require. The efficiency of the physiologic mechanisms for preventing the absorption of unnecessary iron has been questioned, and mandatory wheat flour fortification programs were discontinued in two European countries, in part because of concern about possible adverse effects of iron fortification [13, 14].

The mechanisms controlling iron absorption and the central role of the hepcidin/ferroportin axis have been elucidated recently [15]. There are very few reports of iron overload resulting from the consumption of large quantities of iron, even large supplemental doses, over extended time periods by individuals with an apparently normal hepcidin/ferroportin axis. Systemic iron overload occurs in genetic disorders, such as hemochromatosis, that modify the function of hepcidin or ferroportin, or in diseases, such as the thalassemia syndromes, that reduce the efficiency with which these regulators prevent excessive iron accumulation [16]. Patients with phenotypically expressed iron loading conditions suffer the consequences of excessive iron absorption even if the diet is not fortified, although mass fortification would be expected to modestly increase their iron loads. These disorders are best managed by screening and treatment. Withholding iron fortification from the much larger population that is in need of extra iron would prolong the suffering and the negative health and economic consequences related to iron deficiency and have little impact on the clinical course of the iron overload diseases [17]. Iron overload does not occur in genetic carriers with normal phenotypes [18].

Effective fortification of staple foods or condiments with iron is thus expected to have significant benefits for large segments of the population, particularly in developing countries, with very little risk of adverse health effects. In this respect, wheat flour is the food vehicle most often fortified with iron. Fortification originally began in the United States and Europe in the 1940s as a way to combat iron deficiency by restoring the iron level of low-extraction wheat flour to that in the whole grain. Wheat flour fortification programs are in place or in the planning stages in 78 countries [19]. In 2004, a Centers for Disease Control and Prevention (CDC) expert group in Cuernavaca, Mexico, made global recommendations for the type and level of different iron compounds to be added to wheat flour [20]. WHO [2] recommended the same iron compounds but suggested that each country should estimate the level of fortification that would provide the required iron lacking in the traditional diet.

The first objective of this review was to evaluate and revise the guidelines for iron fortification of wheat flour that were formulated at the Cuernavaca Workshop [20]. This was achieved by reviewing all published efficacy trials of iron-fortified condiments and cereal staples in women and children. For each iron fortificant currently recommended for wheat flour fortification, the average increase in an individual's daily iron intake necessary to achieve a meaningful improvement in iron status was estimated. This information was used to calculate recommended fortification levels based on average per capita wheat flour consumption. The second objective was to evaluate to what extent the flour industry is following the Cuernavaca guidelines and to judge the potential impact of current national, regional, or planned wheat flour fortification programs on the iron status of the population.

Using iron efficacy studies to estimate iron fortification levels that will usefully improve iron status

The iron fortification levels recommended in the Cuernavaca guidelines [20] were largely derived from what was being practiced in the flour fortification industry and what was expected to be organoleptically acceptable. As wheat flour fortification has historically been based on restoration, the iron level recommended for ferrous sulfate fortification (30 ppm iron) was that needed to restore the iron level of low-extraction white wheat flour to that of the whole-grain wheat flour. This was increased to 45 ppm iron for countries where wheat flour consumption was less than 200 g per person per day. Isotopic iron absorption studies in adult humans have indicated that ferrous fumarate has a similar bioavailability to ferrous sulfate, so the Cuernavaca guidelines recommended that ferrous fumarate be added at the same level as ferrous sulfate.

Ferrous fumarate would be expected to have fewer sensory problems than ferrous sulfate. Encapsulation of ferrous sulfate or ferrous fumarate with hydrogenated vegetable oils may prevent lipid oxidation during wheat flour storage, and these compounds are useful alternatives; however, at the time of the Cuernavaca meeting the particle size of the commercially encapsulated compounds was too large, and it was concluded that, if added to flour, the compounds would be removed by the sieves commonly used at the end of the milling process. The Cuernavaca guidelines recommended that smaller particle-size encapsulated ferrous sulfate or encapsulated ferrous fumarate be developed for addition to wheat flour. Although this has been recently accomplished experimentally [21], the microcapsules need more complete sensory testing and scaling up for commercialization. Encapsulated ferrous sulfate and encapsulated ferrous fumarate are recommended for cereal flour fortification in the WHO guidelines [2].

Because elemental iron powders are organoleptically inert, they are widely used for wheat flour fortification. In 2002, a SUSTAIN task force evaluated the usefulness of the different elemental iron powders commonly employed in wheat flour fortification [22]. Based on *in vitro*, rat, and human studies, the task force recommended that electrolytic iron be the only elemental iron powder used and that it be added at twice the iron level of ferrous sulfate, since it is approximately half as well absorbed. They also recommended that carbon monoxide–reduced iron should not be used because of an unacceptably low absorption, and that more studies were needed of carbonyl and hydrogen-reduced iron powders before a recommendation could be made. It was subsequently found that another form of reduced iron (atomized iron powder) is widely used for wheat flour fortification because of its low cost. However, because of its low solubility in dilute acid under standardized conditions and its poor absorption in rat hemoglobin repletion studies and human iron tolerance tests [23], atomized reduced iron powder is not recommended for wheat flour fortification [2].

It has long been known that in the presence of phytate, the ethylenediaminetetraacetate (EDTA) component of NaFeEDTA enhances absorption of both the intrinsic food iron and the fortification iron. Additionally, NaFeEDTA does not promote lipid oxidation in stored wheat flour [24]. It has thus been recommended for the fortification of high-phytate flours (whole-grain and unleavened low-extraction). The level recommended for both whole-grain and unleavened low-extraction flours was 30 ppm iron [20], although it was realized that this level may be somewhat higher than that necessary for high-extraction flours which contain higher levels of (low-bioavailability) intrinsic iron.

The procedure used to determine the recommended iron levels at Cuernavaca was necessarily pragmatic. The preferred procedure would be the method recommended by WHO [2], in which each country must first measure the daily iron intake in the groups at risk for iron deficiency, estimate the iron bioavailability from the diet, compare estimated iron intake and bioavailability with iron requirements (based on dietary iron bioavailability), and calculate the amount of iron lacking in the diet. This amount of iron should then be added to the mean daily flour consumption of the targeted at-risk group(s) (e.g., women of childbearing age). Unfortunately, very few countries have the capability to use this procedure.

The approach used to develop the recommendations in the present document is a combination of the application of experimental evidence and pragmatism. This was made possible by the publication of a relatively large number of human efficacy trials, mostly after the Cuernavaca Workshop. We have reviewed these efficacy studies, in which different iron compounds and different food vehicles were employed. Studies in infants were not included, because this population group is not a primary target for mass fortification. Studies in which ascorbic acid was given together with the fortified food were also excluded, as this iron absorption enhancer is usually unstable to wheat flour storage and heat processing. We also excluded studies where the iron compound was not identified clearly or where the methodological details were inadequate. The duration of the intervention was taken into account. Hallberg et al. [25] estimated that it takes 2 to 3 years to stabilize the new iron balance and iron stores after changing the amount of bioavailable iron in the diet. However, 80% of the final impact is achieved in the first year. From this report, it can also be estimated that

efficacy studies carried out over 5 to 6 months should reach about 40% of final impact, whereas the final impact of studies lasting less than 5 months is too difficult to interpret. Based on this information, and based on the results of published efficacy studies in women and children, the daily amount of iron necessary to achieve an improvement in iron status was estimated for each recommended iron compound. Two efficacy studies in infants are referred to but are not part of the formal analysis. These studies indicate that relatively large quantities of electrolytic iron, especially in combination with ascorbic acid, can have a positive impact on iron status [26, 27].

It is proposed that iron fortification of wheat flour should be considered at the national or regional level only if there is laboratory evidence of a high prevalence of iron deficiency and iron-deficiency anemia in women or children in the country or region concerned (iron-deficiency anemia > 5%) and that the program should aim to decrease the prevalence of iron deficiency in the target at-risk populations to levels reported in industrialized countries (< 10% iron deficiency and < 5% iron-deficiency anemia [28]). These levels should be reached in 2 to 3 years after the start of the fortification program. For simplicity, we have based our evaluation of the published efficacy studies on the potential for these values to be attained. Trials that met these criteria were considered "highly efficacious." If one or more iron status parameters or hemoglobin improved significantly without satisfying these criteria, the trial was considered to be "moderately efficacious." When the hemoglobin or iron status parameters were not significantly changed, the fortification study was considered "not efficacious." Since the duration of most of the trials was less than 12 months, the maximal reduction in the percentage of iron deficiency and the percentage of iron-deficiency anemia would not have been reached. The model developed by Hallberg et al. [25] was thus used to modify the criteria for describing the study as efficacious based on study duration. A reduction in the percentage of iron deficiency and the percentage of iron-deficiency anemia to < 12.5% and < 6%, respectively, was required for studies lasting around 9 months to be considered highly efficacious. The corresponding values for studies lasting around 5 months were < 25% and < 12.5%. A major drawback of this approach is that iron status at the start of the intervention influences the final outcome, especially for short-term studies; however, with one exception, subject selection did not affect the ability to categorize study outcome.

Efficacy studies with NaFeEDTA

NaFeEDTA has been evaluated in nine efficacy studies employing a variety of fortified foods, including wheat and maize flour as well as condiments such as fish sauce, soy sauce, curry powder, and sugar (**table 1**). Although only two of these studies were conducted with wheat flour, two were conducted with maize flour and the condiments were added to maize-based and rice-based diets, all of which are moderately high in phytate. The studies with curry powder [29], sugar [30], and soy sauce [31] and one study with fish sauce

TABLE 1. Efficacy studies with NaFeEDTA

Dose (mg/day)	Subjects and vehicle	Length of study and country	Impact	Ref
7.1	Both sexes ≥ 10 yr Curry powder	24 mo South Africa	Highly efficacious	29
4.6	Both sexes ≥ 10 yr Sugar	32 mo Guatemala	Moderately efficacious	30
8.6	Women 17–44 yr Fish sauce	6 mo Vietnam	Moderately efficacious	33
7.5	Women 16–49 yr Fish sauce	18 mo Vietnam	Highly efficacious	32
4.9	Both sexes ≥ 3 yr Soy sauce	18 mo China	Highly efficacious	31
7	Both sexes 11–18 yr Wheat flour	6 mo China	Highly efficacious	34
7	Children 3-8 yr Maize porridge	5 mo Kenya	Highly efficacious	35
3.5	Children 3–8 yr Maize porridge	5 mo Kenya	Moderately efficacious	35
1.3	Children 6–11 yr Brown bread	8 mo South Africa	No effect on iron status	36

[32] were relatively long term, lasting from 18 to 32 months. One of the fish sauce studies [33] and the studies with maize flour or wheat flour lasted only 5 to 8 months [34–36]. Eight of the nine studies reported statistically improved iron status in women and children. Five trials that provided an additional 4.9 to 7.5 mg iron/day over 5 to 24 months were judged to be highly efficacious. Three studies [30, 33, 35] providing 3.5 to 8.6 mg additional iron per day were categorized as moderately efficacious. This was due in part to unavailability of data or study design in two of them. Viteri et al. [30] did not report the percentages of iron deficiency or of iron-deficiency anemia in the study subjects. Thuy et al. [33] preselected only anemic subjects, and there was still a 20% residual prevalence of iron-deficiency anemia at the end of this 6-month trial. It is possible that the intervention would have reached the criteria for being highly efficacious if the trial had continued for a longer time. It was assumed, therefore, that the interventions of Viteri et al. [30] and Thuy et al. [33] were misclassified as moderately efficacious rather than highly efficacious because of incomplete data in the former and unsuitable study design in the latter. NaFeEDTA was only moderately efficacious in children receiving 3.5 mg additional iron per day in fortified maize meal, whereas children given brown bread that provided 1.3 mg/day as NaFeEDTA showed no improvement in iron status [36].

The recommendation for the fortification of low-extraction wheat flour with NaFeEDTA is based on the lowest dose likely to be highly efficacious (4.6 mg in the study of Viteri et al. [30]). A daily dose of 3.5 mg was considered moderately efficacious, whereas 1.3 mg had no effect on iron status in children (**table 1**). Fortification levels supplying between 3.5 mg and 4.6 mg have not been tested, so it is possible that a daily iron intake from NaFeEDTA of somewhat less than 4.6 mg may suffice. Based on mean consumption rates, the required iron concentration is 13 ppm for low-extraction wheat flour consumption levels > 300 g/day and 20 ppm for levels of 150 to 300 g/day (**table 2**). These values are lower than the 30 ppm iron recommended at Cuernavaca for the same flour consumption rates. For a lower flour consumption level of 75 to 149 g/day, the required iron concentration should be increased to 40 ppm. When the daily flour consumption is < 75 g, 92 ppm would be necessary.

These recommendations for the fortification of wheat flour with NaFeEDTA would be expected to reduce national iron-deficiency anemia and iron deficiency prevalence rates to the ranges encountered in Western countries in 2 to 3 years. They are supported by a series of well-conducted studies. Although some studies were not conducted with iron-fortified wheat or maize flours, all the fortified condiments were used within cereal-based diets relatively high in phytic acid. We concluded, therefore, that these recommendations can be stated with greater confidence than the recommendations for ferrous sulfate and ferrous fumarate that are reported in the following sections of this review. Furthermore, the enhancing properties of EDTA on iron absorption in the presence of phytate would be expected to reduce the variability in iron status responses caused by differences in overall meal bioavailability.

TABLE 2. Required flour fortification levels based on the minimum iron dose that improved iron status in efficacy studies

Iron compound	Flour consumption (g/day)	Required level (ppm)	Cuernavaca recommendation (ppm)
NaFeEDTA	> 300	13	30
	150–300	20	30
	75–149	40	30
	< 75	92	30
Ferrous sulfate	> 300	20	30
	150–300	32	30
	75–149	63	45
	< 75	142	45
Electrolytic iron	> 300	29	60
	150–300	44	60
	75–149	89	90
	< 75	200	90

Efficacy studies with ferrous sulfate

Four efficacy studies with ferrous sulfate have been reported. Two studies fed foods fortified with encapsulated sulfate (**table 3**). Wheat flour or wheat flour biscuits were fortified in three trials [21, 34, 37], and salt was fortified in the fourth [38]. The iron-fortified salt was largely added to bread prior to baking. All trials reported statistically improved iron status in schoolchildren or young women consuming an additional 7.1 to 11.8 mg iron per day over 5.5 to 9 months. The two studies that supplied 10.3 and 11.8 mg additional iron per day were categorized as highly efficacious, and the two studies providing 7.1 and 11.0 mg iron per day were categorized as moderately efficacious. It should be noted that Biebinger et al. [21] evaluated a newly developed small-particle-size (d_{50} = 40 μm) encapsulated ferrous sulfate that is suitable for flour fortification and will be retained in the flour after the sifting process.

The minimum efficacious dose for ferrous sulfate was 7.1 mg/day. It was considered to be moderately efficacious. A somewhat higher dose (~ 11 mg) was highly efficacious in two studies, but only moderately efficacious in the third (**table 3**). It is likely that the efficacy of ferrous sulfate will depend to some extent on the other food items consumed in the meal containing the fortified wheat flour. When 7.1 mg iron/day is used as

TABLE 3. Efficacy studies with ferrous sulfate

Iron compound	Dose (mg/day)	Subjects and vehicle	Length of study and country	Impact	Ref
Encapsulated ferrous sulfate[a]	11.8	Children 6–15 yr Salt (bread, fava beans)	9 mo Morocco	Highly efficacious	38
Ferrous sulfate	10.3	Women 18–40 yr Wheat flour biscuits	9 mo Thailand	Highly efficacious	37
Ferrous sulfate	11	Students 11–18 yr Wheat flour	6 mo China	Moderately efficacious	34
Encapsulated ferrous sulfate[b]	7.1	Women 18–35 yr Wheat flour biscuits	5.5 mo Kuwait	Moderately efficacious	21

a. Encapsulated with partially hydrogenated vegetable oil (Balchem, NY, USA).
b. Encapsulated with hydrogenated palm oil; mean particle size ca. 40 μm.

the required iron dose of ferrous sulfate in wheat flour, the required fortification level for countries consuming > 300 g/day is 20 ppm, lower than the 30 ppm recommended at Cuernavaca; for countries consuming 150 to 300 g flour per day, the required level is 32 ppm (**table 2**). For the countries where wheat flour consumption is between 75 and 149 g/day, the estimated required iron fortification level for ferrous sulfate is 63 ppm, and for a flour consumption of < 75 g/day, the level is 142 ppm. These latter values are much higher than those recommended at Cuernavaca. In some settings, the recommended fortification levels may be too low to achieve optimal benefit.

We were unable to discover any field trials employing ferrous fumarate that met our criteria. However, isotopic studies suggest that the absorptions of ferrous sulfate and ferrous fumarate are equivalent. Our recommendations for ferrous fumarate are therefore the same as those for ferrous sulfate.

Efficacy studies with electrolytic iron

The results of six efficacy studies in women or children conducted with electrolytic iron are shown in **table 4**. Four studies reported no improvement in iron status or presence of anemia. Three of these studies were relatively short interventions that provided only 3.2 to 7 mg additional iron per day to children over a period of 5 to 8 months. The fourth study was that of Nestel et al. [39]. These workers provided 12.5 mg extra iron per day in wheat flour over 2 years to women and children in Sri Lanka and found no change in hemoglobin. Serum ferritin was not reported. A significant improvement in iron status was reported in two studies. Zimmermann et al. [37] fed electrolytic iron-fortified biscuits to young Thai women providing 10 mg additional iron per day over 9 months. The study was judged as moderately efficacious. The prevalence of iron deficiency decreased from 45% to 21%, although there was no change in hemoglobin. Sun et al. [34] provided 21 mg additional iron per day in wheat flour to schoolchildren over 6 months. The prevalence of iron-deficiency anemia decreased from 100% to 60%.

Two additional efficacy studies have been done in infants [26, 27]. These short-term studies also indicated that relatively large amounts of electrolytic iron can have a positive effect on iron status; however, both studies included ascorbic acid, which would be

TABLE 4. Efficacy studies with electrolytic iron

Iron compound (manufacturer)	Dose (mg/day)	Subjects and vehicle	Length of study and country	Impact	Ref
A131 (Höganäs)	12.5	Women 16–50 yr Wheat flour	24 mo Sri Lanka	No change in hemoglobin	39
A131 (Höganäs)	10	Women 18–50 yr Wheat flour biscuits	9 mo Thailand	Moderately efficacious No change in hemoglobin	37
Unknown	3.2	Children 6–11 yr Brown bread	7.5 mo South Africa	No change in iron status	57
Unknown	21	Children 11–18 yr Wheat flour	6 mo China	Moderately efficacious	34
IMP	7	Children 3–8 yr Maize porridge	5 mo Kenya	No change in iron status	35
Unknown	4.5	Children 6–11 yr Brown bread	8 mo South Africa	No change in iron status	36

expected to increase iron absorption and improve the impact on iron status. Walter et al. [26] provided 12 mg extra iron per day in rice cereal for 4 months and Lartey et al. [27] provided an extra 18 mg iron per day in a complementary food based on maize, soy, and groundnuts. Both studies demonstrated that relatively large doses of electrolytic iron can have a positive impact on iron status, suggesting that this form of iron can be used if the fortification level is high enough.

The lowest dose of electrolytic iron shown to have a significant impact on iron status is 10 mg. However, it is important to note that electrolytic iron was less efficacious than ferrous sulfate in reducing iron deficiency in the trial from which this value is derived [37] and that in this study there was no reduction in the percentage of subjects with anemia. Moreover, there was a 60% residual presence of iron-deficiency anemia among children in China after a 6-month trial using more than twice this 10-mg dose [34]. Because of the uncertainty about the lowest effective dose of electrolytic iron, we have not used the information summarized in **tables 2** and **4** to formulate the recommendations for electrolytic iron. It is suggested not to change the recommendation from the Cuernavaca Workshop, which was to add electrolytic iron at twice the concentration of ferrous sulfate.

Efficacy studies with hydrogen-reduced iron

Five efficacy studies have been reported with hydrogen-reduced iron (**table 5**). Only one of these studies [37] showed an improvement in iron status. This was the SUSTAIN study in Thailand, which provided 10 mg AC-325 hydrogen-reduced iron per day in wheat flour biscuits to young Thai women over a period of 9 months. This study showed a small reduction in the number of women with iron deficiency, but no change in of the percentage of women with anemia. Another study in Zambia [40] provided 14 mg iron per day as hydrogen-reduced iron (source not specified) in maize meal to refugees over 8 months. There were no changes in iron deficiency in children, adolescents, or women, although there was a small decrease in serum transferrin receptor concentration in adolescents. The percentage of children with anemia dropped from 48% to 24%. However, the study lacked a control group, making it impossible to determine whether iron fortification played any role.

Three other studies providing 3.6 to 14.3 mg hydrogen-reduced iron per day failed to demonstrate an impact on iron status or hemoglobin. It is perhaps not surprising that providing only 3.6 mg extra iron per day (source not specified) in a seasoning powder to Thai children over 7.5 months had no impact on iron status [41]; however, providing 12.5 mg iron (source not specified) per day in wheat flour to women and children in Sri Lanka over 24 months also resulted in no change in hemoglobin [39]. The most pertinent observations are those recently reported by Biebinger et al. [21]. In this study, young Kuwaiti women were fed 14.3 mg iron per day in the form of a newly developed hydrogen-reduced iron powder (Nutrafine RS, Höganäs AB, Sweden) in wheat flour biscuits over 5.5 months. There was no improvement in their iron status. This study is important because Nutrafine RS is now marketed for food fortification in place of AC-325 hydrogen-reduced iron. The other commercial product that is used widely is Atomet™ hydrogen-reduced iron (QMP, Canada). *In vitro* solubility studies, rat hemoglobin repletion tests, and human iron tolerance studies indicate that this iron powder is likely to be the least bioavailable of all commercial iron powders [23].

There is thus no new evidence to suggest that fortification with currently available reduced iron powders will have a significant beneficial effect on iron status. It is not recommended, therefore, to use any reduced iron powder for the fortification of wheat or maize flour.

TABLE 5. Efficacy studies with reduced iron powders

Iron compound (manufacturer)	Dose (mg/day)	Subjects and vehicle	Length of study and country	Impact	Ref
Unknown	12.5	Women 14–50 yr Wheat flour	24 mo Sri Lanka	No change in hemoglobin	39
Hydrogen-reduced iron AC-325 (Höganäs)	10	Women 18–40 yr Wheat flour biscuits	9 mo Thailand	Moderate efficacy, no change in hemoglobin	37
Hydrogen-reduced iron (unknown)[a]	3.6	Children 5–13 yr Seasoning powder	7.5 mo Thailand	No change in iron status	41
Reduced (unknown)[b]	14	Both sexes 10–59 yr Maize meal	8 mo Zambia	Small decrease in iron deficiency in adolescents only, no change in other groups	40
Hydrogen-reduced iron Nutrafine RS (Höganäs)	14.3	Women 18–35 yr Wheat flour biscuits	5.5 mo Kuwait	No change in iron status	21

a. Fortificant contained multiple micronutrients.
b. Fortificant contained vitamin A.

TABLE 6. Efficacy studies with micronized ground ferric pyrophosphate (2.5 μm)

Dose (mg/day)	Subjects and vehicle	Length of study and country	Impact	Ref
18	Children 6–15 yr Salt	10 mo Morocco	Highly efficacious	43
18.6	Children 6–14 yr Salt	10 mo Morocco	Highly efficacious	44
17	Children 6–13 yr Rice	7 mo India	Moderately efficacious	46
10.5	Children 5–15 yr Salt	6 mo Côte d'Ivoire	Moderately efficacious	45

Efficacy studies with ferric pyrophosphate

The efficacy studies conducted with ground ferric pyrophosphate (2.5 μm, Dr Lohmann, Germany) are summarized in **table 6**. Although this compound has never been used for flour fortification, it is organoleptically inert and, like electrolytic iron, would appear to be about half as well absorbed as ferrous sulfate in human subjects [42]. All four efficacy studies reported a significant improvement in iron status when schoolchildren consumed between 10.5 and 18.6 mg additional iron per day over 6 to 10 months. The two studies by Zimmermann et al. [43, 44] in Morocco fed 18 and 18.6 mg iron in salt to children over 10 months. The salt was largely added to home-cooked bread, and this fortification strategy was judged as highly efficacious. A third salt study [45] providing 10.5 mg iron per day took place in Côte D'Ivoire and was judged moderately efficacious, as was a study in India where schoolchildren were provided an extra 17 mg iron per day in extruded rice added to school meals [46].

Micronized ground ferric pyrophosphate may be a suitable iron compound for wheat flour fortification at concentrations similar to those suggested for electrolytic iron. However, because it is more expensive than electrolytic iron and has not been tested in wheat or maize flour, we have not made any recommendations for its use.

Revised recommendations for iron fortification of wheat flour

Table 7 gives the new recommendations for the iron fortification of wheat flour which are based on our review and discussions at this Workshop. Before deciding on a compound, countries should first test the recommended amounts of the specific compounds in both flour and final products made from fortified flour to ensure that no unacceptable sensory changes occur. The first choices as iron fortificants for wheat flour fortification are NaFeEDTA, ferrous sulfate, and ferrous fumarate. We have the greatest confidence in the recommendations for NaFeEDTA because of the larger database and because NaFeEDTA absorption is less likely to be affected by other components of the meals in which it is eaten. The higher iron bioavailability from wheat-based foods fortified with NaFeEDTA means that lower levels of fortification iron can be added. This in turn leads to less potential for sensory changes. Moreover, NaFeEDTA has been reported not to promote lipid oxidation in stored wheat flour.

These recommendations were discussed in the plenary session at the Workshop and are consensus recommendations. Four different daily wheat flour consumption ranges were agreed upon at the Workshop (> 300, 150 to 300, 75 to 149, and < 75 g/day), and mean daily consumption levels of 350, 225, 113, and 50 g, respectively, were used to compute the suggested flour fortification levels within each of these consumption bands. Recommended values (**table 7**) were rounded to the nearest 5 ppm interval. The reason for using the mean consumption, rather than the lower limit of consumption within a designated range, is that regulations customarily stipulate a minimum requirement for fortification levels or flour nutrient content.

TABLE 7. Recommended iron fortification levels (ppm) for wheat flour according to iron compound and daily flour consumption[a]

Flour consumption (g/day)	NaFeEDTA	Ferrous sulfate or ferrous fumarate	Electrolytic iron powder
> 300	15	20	40
150–300	20	30	60
75–149	40	60	Not recommended
< 75	40	60	Not recommended

a. These recommended levels are based on the calculated required levels presented in **table 2** but in some cases have been rounded off. For flour consumption < 75 g/day, lower levels have been recommended in order to cause no sensory changes.

It is standard procedure for producers to exceed this amount by a small margin (overage). It was therefore considered prudent to reduce the risk of excessive iron intake in individuals with high flour consumption by targeting the middle of the consumption range. The same concern applies to the risk of exceeding the acceptable daily intake (ADI) for EDTA in flour fortified with NaFeEDTA (discussed below).

It is recommended to add 15 ppm iron as NaFeEDTA for flour intakes > 300 g/day, 20 ppm iron for flour intakes of 150 to 300 g/day, and 40 ppm iron for flour intakes of 75 to 149 g/day. At these levels of iron fortification and consumption, the additional iron intake from the fortified flour would be expected to improve iron status significantly in women and children and reduce the prevalence of iron deficiency and iron-deficiency anemia to rates encountered in Western societies. A fortification level of 40 ppm is suggested for flour intakes < 75 g. At these low flour intakes, the extra iron intake from fortified flour consumption will make a useful contribution to improving iron status, but fortification of other food vehicles will be needed for an adequate iron intake to be attained. Levels of NaFeEDTA providing 15 and 20 ppm iron are considered unlikely to cause adverse sensory changes. Such changes are more likely with 40 ppm iron as NaFeEDTA. If they occur, encapsulated NaFeEDTA should be considered.

NaFeEDTA is the only iron compound that is recommended for the fortification of high-extraction (> 0.8% ash) wheat flour. The recommended fortification levels are the same as for low-extraction (≤ 0.8% ash) wheat flour: 15 ppm for flour consumption > 300 g/day, 20 ppm for 150 to 300 g/day, and 40 ppm for < 150 g/day. The higher phytate content in high-extraction wheat flour is expected to reduce the percent iron absorption, but it is anticipated that this will be offset by an enhancement in absorption of the native flour iron by the EDTA. NaFeEDTA is also recommended for wheat products, such as pasta, in which there is no fermentation process during manufacture. There are no published human efficacy studies to support the recommendations for the fortification of high-extraction flour or pasta.

The widespread use of NaFeEDTA will depend on clarification of the putative, but as yet unsubstantiated, potential risks of increasing the EDTA consumption of the whole population. The following recommendation [47] for the use of NaFeEDTA as a food additive was made at the 68th Meeting of the Joint FAO/WHO Expert Committee on Food Additives:

> Sodium iron EDTA is suitable as a source of iron for food fortification to fulfil nutritional iron requirements, provided that the total intake of iron from all food sources including contaminants does not exceed the Provisional Maximum Tolerable Daily Intake of 0.8 mg/kg body weight. Total intake of EDTA should not exceed acceptable levels, also taking into account the intake of EDTA from the food additive use of other EDTA compounds. An ADI of 0–2.5 mg/kg body weight was previously established for calcium disodium and disodium salts of EDTA, equivalent to up to 1.9 mg/kg body weight EDTA [47].

This specification was noted without revision at the 31st Session of the Joint FAO/WHO Food Standards Programme Codex Alimentarius Commission in Geneva, 30 June to 4 July 2008 [48].

The fortification levels proposed in this document would deliver approximately 4.5 mg/day of additional iron in the form of NaFeEDTA and 23 mg EDTA. This would amount to 0.42 mg EDTA/kg for a 55-kg woman, well below the ADI. However, EDTA consumption from mass fortification with NaFeEDTA may approach or exceed the ADI for relatively short periods of time in very young children when growth is rapid and caloric intake is high in relation to body weight. A 1-year-old child would be expected to weigh approximately 10 kg and have a caloric intake approximately half that of an adult woman. Under these circumstances, mean EDTA intake may exceed the ADI for EDTA of 1.9 mg/kg if wheat flour accounts for the same proportion of caloric intake in the child as in the adult. It will also be important for countries to evaluate EDTA intake from other sources, although this is likely to be low. These factors should be considered by countries planning to implement NaFeEDTA fortification of wheat flour or other food products. As indicated above, the desirable impact on iron status may be achievable with modestly lower levels of NaFeEDTA.

Ferrous sulfate has also consistently shown good efficacy in a variety of iron-fortified foods. It is widely used to fortify infant formulas and is the iron compound chosen by WHO for food fortification. It has been used in the highly successful wheat flour fortification program in Chile, where it provides about 6 mg additional iron per day in about 200 g wheat flour [2]. This amount is similar to the 7.1 mg/day minimum amount reported to be efficacious in the studies reviewed in this article. Ferrous fumarate is considered to be as efficacious as ferrous sulfate on the basis of isotopic experiments in human volunteers [49, 50]. However, there are no efficacy studies to support this assumption. Ferrous sulfate is preferred to ferrous fumarate but is more likely to lead to unacceptable sensory changes in some situations. Encapsulation of either compound will prevent lipid oxidation in stored flours, with no impact on bioavailability [51]. The recommended levels of fortification when using these compounds are 20 ppm iron for flour consumption > 300 g/day and 30 ppm iron for flour consumption between 150 and 300 g/day. For flour consumption < 150 g/day, sensory changes may result with the recommended level of 60 ppm unless

the iron compounds are encapsulated. Ferrous sulfate and ferrous fumarate are not recommended for the fortification of high-extraction (high-phytate) flours.

Electrolytic iron is the second-choice iron compound for wheat flour fortification. It should be considered when the first-choice compounds (NaFeEDTA, ferrous sulfate, and ferrous fumarate) cause sensory changes or are considered too expensive. Although one efficacy study suggested that 10 mg iron/day as electrolytic would be adequate, the results from other studies were not consistent. It is recommended, therefore, that the amount of electrolytic iron needed per day should be double the iron level recommended for ferrous sulfate, i.e., 14.2 mg/day. It would be helpful to have additional efficacy trials to confirm that this level of addition is adequate.

Another potential disadvantage of poorly soluble compounds such as electrolytic iron is that iron-deficient subjects up-regulate absorption from these compounds less efficiently than absorption from ferrous sulfate [52]. The advantages of electrolytic iron are that it causes few if any sensory changes and is less expensive. The recommended level of fortification for electrolytic iron is 40 ppm iron for flour consumption > 300 g/day and 60 ppm iron for flour consumption of 150 to 300 g/day. Electrolytic iron is not recommended when flour consumption is < 150 g/day because the high fortification levels required may cause sensory changes. Electrolytic iron also is not recommended for fortification of high-phytate flours.

There is no evidence to support the use of hydrogen-reduced iron powders or atomized reduced iron powders for wheat or maize flour fortification. These compounds are less well absorbed than electrolytic iron and are not recommended for wheat flour fortification. Although the newly developed Nutrafine RS hydrogen-reduced iron was not found to be efficacious, manufacturers are encouraged to continue the development of low-cost hydrogen-reduced iron powders. However, the efficacy of any new product should be tested in human volunteers and demonstrated to be equivalent to or better than that of electrolytic iron.

Recommendations for the iron fortification of maize flour

A detailed evaluation of maize flour fortification was not attempted in this review. There is much less experience with fortifying maize flours with iron than with fortifying wheat flours; however, similar considerations apply. Previous recommendations [53] can still be used. More research is needed to evaluate the best approach for maize flour fortification, especially the fortification of nixtamalized maize flour.

Predicted impact of current national programs of iron-fortified wheat flour

The marked reduction in the prevalence of iron deficiency among young children in the United States is attributed to the fortification of infant formulas and weaning foods with iron [54]. Similarly, the low prevalence of iron-deficiency anemia in female adolescents and women of childbearing age is attributed in part to the consumption of iron-fortified wheat flour [50]. Reports from Denmark and Sweden also provide indirect evidence of the impact of fortification. The withdrawal of mandatory iron fortification of wheat flour with carbonyl iron in Denmark in 1987 led to a decrease in serum ferritin levels among blood donors, a group that would be expected to have high iron requirements [13]. Mandatory fortification of wheat flour with carbonyl iron in Sweden was discontinued in 1994. Six years later, a 20% increase in the prevalence of iron deficiency was observed among 15- and 16-year-old girls [14]. Finally, the low prevalence of iron deficiency among women of childbearing age in Chile is attributed to the fortification of wheat flour with ferrous sulfate. The fortification level is 30 ppm, with an average daily intake of about 200 g per capita delivering an additional 6 mg iron [55].

Details of the current mandatory, voluntary, World Food Programme, and planned national and regional wheat flour fortification programs are summarized in **table 8**. The type of program, the iron compound used, and the level of iron added were taken from the Cereal Fortification Handbook compiled by the Micronutrient Initiative [19]. The wheat flour consumption data were based on Food and Agriculture Organization (FAO) wheat consumption data [56]. Most of the current wheat flour fortification programs would be expected to have little impact on iron status at the national level. The main reason is the failure to specify a recommended iron compound. Of the 78 programs listed in **table 8**, 47 do not stipulate a specific iron compound. These programs are understood to be using atomized or hydrogen-reduced iron powders because of their low cost and good sensory properties. Reduced iron is specified in Bangladesh, Fiji, and Qatar and permitted in the Philippines. A recommended iron compound (ferrous sulfate, ferrous fumarate, electrolytic iron, or NaFeEDTA) is specified in the remaining 27 countries. However, the average per capita wheat flour consumption for the whole country is < 75 g/person/day in 13 countries and 76 and 88 g/person/day in Costa Rica and the Dominican Republic, respectively. These consumption rates are too low for fortification of wheat flour alone to have an impact on iron deficiency based on national statistics, although it is important to note that average flour consumption may not reflect major

TABLE 8. National iron fortification programs with wheat flour

Country or region	Type of program	Flour consumption (g/day)	Iron compound	Iron fortification level (ppm)
Afghanistan	WFP	208	NS	37.5
Argentina	Mandatory	229	Sulfate	30
Azerbaijan	Voluntary	404	NS	40
Bahrain	Mandatory	200	NS	60
Bangladesh	WFP	49	Reduced	37.5
Barbados	Regional	111	NS	44
Belize	Regional	149	NS	60
Bolivia	Mandatory	63	Fumarate	35
Brazil	Mandatory	90	NS	42
Canada	Mandatory	159	NS	44
Caribbean	Regional	150	NS	29
Central African Republic	Planned	12	NS	45
Chile	Mandatory	215	Sulfate	30
China	Voluntary	115	FeEDTA	24
Colombia	Mandatory	50	NS	44
Congo DRC	WFP	11	Sulfate	45
Costa Rica	Mandatory	88	Fumarate	55
Côte d'Ivoire	Mandatory	29	Electrolytic	60
Cuba	Mandatory	76	Sulfate	45
Cyprus	Voluntary	193	NS	45
Dominican Republic	Voluntary	58	Fumarate	55
Ecuador	Mandatory	60	NS	55
Egypt	Planned	256	Sulfate	30
El Salvador	Mandatory	58	Fumarate	55
Fiji	Regional	233	Reduced	60
Georgia	Planned	179	NS	50
Ghana	Planned	39	Fumarate	45
Guatemala	Mandatory	60	Fumarate	55
Guinea	Mandatory	25	NS	54
Guyana	Voluntary	120	NS	29
Haiti	Regional	61	NS	44
Honduras	Mandatory	58	Fumarate	55
Indonesia	Mandatory	33	Electrolytic	50
Iran	Mandatory	354	Sulfate	30
Iraq	Voluntary	223	NS	30
Israel	Planned	221	NS	37.5
Jamaica	Voluntary	238	NS	44
Jordan	Mandatory	186	Sulfate	34
Kazakhstan	Voluntary	278	NS	40
Kuwait	Mandatory	209	NS	60
Kyrgyz Republic	Voluntary	380	NS	40

continued

TABLE 8. National iron fortification programs with wheat flour *(continued)*

Country or region	Type of program	Flour consumption (g/day)	Iron compound	Iron fortification level (ppm)
Lebanon	Regional	204	Sulfate	30
Lesotho	Voluntary	75	NS	35
Malawi	Planned	6	NS	30
Malaysia	Voluntary	102	NS	44
Mexico	Mandatory	60	Sulfate	40
Mongolia	Voluntary	202	NS	40
Morocco	Planned	366	NS	45
Nicaragua	Mandatory	55	Fumarate	55
Nigeria	Mandatory	36	NS	40.7
Oman	Mandatory	160	NS	30
Pakistan	Planned	248	FeEDTA	10
Palestine	Mandatory	213	Sulfate	25
Panama	Mandatory	74	NS	60
Paraguay	Mandatory	22	Sulfate	45
Peru	Mandatory	102	NS	28
Philippines	Mandatory	44	Sulfate, fumarate, reduced	70/Reduced, 50/sulfate, fumarate
Qatar	Mandatory	160	Reduced	60
Russia	Planned	267	NS	30
Saudi Arabia	Mandatory	206	NS	36.3
Sierra Leone	Voluntary	23	NS	30
South Africa	Mandatory	96	NS	35
St. Vincent	Voluntary	113	NS	44
Switzerland	Voluntary	158	NS	29
Syria	Mandatory	200	Sulfate	30
Tajikistan	Voluntary	302	NS	40
Trinidad and Tobago	Mandatory	166	NS	30
Turkmenistan	Mandatory	450	Sulfate	20
UAE	Mandatory	206	NS	30
Uganda	Planned	7	Fumarate	40
United Kingdom	Mandatory	191	NS	16.5
United States	Regional	182	NS	44
Uruguay	Mandatory	211	Sulfate	30
Uzbekistan	Regional	284	NS	40
Venezuela	Mandatory	85	NS	16
Vietnam	Planned	18	NS	60
Yemen	Mandatory	185	NS	30
Zambia	Voluntary	33	NS	28.9

FeEDTA, iron ethylenediaminetetraacetate; NS, iron compound not specified; WFP, World Food Programme
Source: Ranum and Wesley [19], FAO/WHO [56].

variations in consumption rates in different regions within a single country. If this is the case, fortification in the regions with higher consumption rates could have a significant impact. Specifications for levels of addition should be based on consumption rates in regions with intakes high enough to permit fortification to be effective.

In Bolivia, Costa Rica, the Dominican Republic, El Salvador, Guatemala, Honduras, and Nicaragua, wheat flour consumption is only 55 to 88 g/day, but it is fortified with 35 to 60 ppm iron as ferrous fumarate, providing some 2 to 4 mg extra iron per day. This amount of iron by itself would be judged as too low to have a positive impact on iron status, but it would make a useful positive contribution if combined with the fortification of other food vehicles such as maize.

NaFeEDTA is specified for voluntary programs in China and a planned program in Pakistan. Although an impact on iron status at the regional level might be anticipated in China, it might not be evident in a national database, because wheat is not a major staple in some parts of China. The addition level in Pakistan is lower than that recommended and may therefore be too low to allow confidence of a significant impact. Ferrous sulfate is specified in Palestine, but the addition level is inadequate (25 ppm).

The nine countries that can expect a positive impact from wheat flour fortification programs use ferrous sulfate. They are Argentina, Chile, Egypt, Iran, Jordan, Lebanon, Syria, Turkmenistan, and Uruguay. They could provide an average of 5.4 to 9.6 mg additional iron per day via fortified flour with optimal coverage.

The way forward

Despite a strong interest by flour millers and national governments in the use of wheat flour fortification to combat iron deficiency and iron-deficiency anemia, it would appear that only 9 of the 78 national wheat flour programs could expect to have the desired nutritional impact. Most millers do not follow the Cuernavaca (2004) [20] or WHO (2006) [2] guidelines for wheat flour fortification. In many countries, wheat flour is still fortified with atomized and hydrogen-reduced elemental iron powders. These iron powders are not recommended for food fortification because of poor absorption, but they are commonly used because they cost less and cause few if any sensory changes. Other national wheat flour fortification programs appear to use fortification levels that are too low in relation to the wheat flour consumption patterns, or have too little coverage. It seems unlikely, therefore, that a meaningful reduction in the worldwide prevalence of iron deficiency will be achieved via wheat flour fortification unless current practices are changed. The first step is to modify national regulations for wheat flour fortification so that only recommended iron compounds are added at concentrations necessary to achieve a satisfactory impact. There is an also an urgent need for further efforts to resolve the regulatory issues that have limited the use of NaFeEDTA. Once the millers have clear guidelines for the efficacious fortification of wheat flour with iron, the small extra cost will be a price worth paying for the meaningful health benefit to women and children.

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Fortification of flour with folic acid

Folic Acid Working Group: Robert J. Berry, Lynn Bailey, Joe Mulinare, Carol Bower, and Omar Dary

Abstract

***Background**: After randomized, controlled trials established that consumption of folic acid before pregnancy and during the early weeks of gestation reduces the risk of a neural tube defect (NTD)-affected pregnancy, the United States Public Health Service recommended in 1992 that all women capable of becoming pregnant consume 400 μg folic acid daily. In 1998, folic acid fortification of all enriched cereal grain product flour was fully implemented in the United States and Canada.*

***Objective**: To provide guidance on national fortification of wheat and maize flours to prevent 50 to 70% of the estimated 300,000 NTD-affected pregnancies worldwide.*

***Methods**: An expert workgroup reviewed the latest evidence of effectiveness of folic acid flour fortification and the safety of folic acid.*

***Results**: Recent estimates show that in the United States and Canada, the additional intake of about 100 to 150 μg/day of folic acid through food fortification has been effective in reducing the prevalence of NTDs at birth and increasing blood folate concentrations in both countries. Most potential adverse effects associated with folic acid are associated with extra supplement use not mandatory fortification. Fortification of wheat flour has a proven record of prevention in other developed countries. In 2009, 51 countries had regulations written for mandatory wheat flour fortification programs that included folic acid.*

***Conclusions**: NTDs remain an important cause of perinatal mortality and infantile paralysis worldwide. Mandatory fortification of flour with folic acid has proved to be one of the most successful public health interventions in reducing the prevalence of NTD-affected pregnancies. Most developing countries have few, if any, common sources of folic acid, unlike many developed countries, which have folic acid available from ready-to-eat cereals and supplements. Expanding the number of developed and developing countries with folic acid flour fortification has tremendous potential to safely eliminate most folic acid-preventable NTDs.*

Key words: Deficiency, flour, folic acid, fortification

Introduction and background

It is widely recognized that adequate consumption of folic acid before pregnancy and during the early weeks of gestation protects fetuses from developing neural tube defects (NTDs) [1–3]. In response, many countries have developed recommendations for the prevention of NTDs. Most recommend that women take a supplement of folic acid periconceptionally (usually for at least 1 month prior to conception and during the first 3 months of pregnancy). In addition, advice to increase dietary intake of naturally occurring folate is often included. Finally, many countries have permitted manufacturers to voluntarily fortify certain foodstuffs, such as breakfast cereals, and/or introduced mandatory fortification of a staple food with folic acid for the prevention of NTDs.

Recommending women to take a daily periconceptional supplement containing 400 μg folic acid beginning at least 1 month before conception through early pregnancy has been the mainstay of public health measures for the primary prevention of NTDs in many

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developed countries [4]. Although education campaigns can increase the use of supplements, their effectiveness is limited because the maximum level of use is usually much less than 50% and supplemental use does not reach all segments of the population equally [5, 6]. In addition, because approximately 50% of pregnancies in the United States are unplanned [7] and NTDs are more prevalent among women in lower social strata [8], the women at greatest risk for having an affected infant are those least likely to be taking supplements. Closure of the neural tube occurs within the first 28 days after conception, and therefore an NTD occurs before most women are aware of their pregnancy. Even in the United States, where substantial efforts have been made to promote the use of supplements, only 30% to 40% of women of childbearing age report adhering to the daily supplementation guidelines [9, 10]. In addition, educational activities to promote the use of supplements will have to be designed to reach women of childbearing age in perpetuity, because new young women are continuously joining the pool of reproductive-age women.

Although recommending improvement of intake of natural folate has appeal—permanent increases in dietary intake of food folate might provide some protection for unplanned pregnancies—efforts to date to achieve dietary change on a population basis have had limited success [11, 12] As well as requiring a behavior change to be effective, dietary increase in natural folates needs to be accessible, affordable, and sustainable if it is to work on a population basis.

There have been studies examining trends in NTD prevalence in relation to recommendations for maternal use of periconceptional folic acid in the absence of concomitant food fortification. Data from the European network of population-based registries for congenital anomalies (EUROCAT) (excluding the United Kingdom and Ireland) showed that among countries that by 1999 had recommended a policy of increasing supplementation or dietary intake of folate to increase periconceptional folate status, there was virtually no decline in the total prevalence of NTDs (2% reduction; 95% confidence interval, 28% reduction to 32% increase) compared with countries with no such policy in place by 1999 (8% reduction; 95% CI, 26% reduction to 16% increase) [13]. Based on data from registries that are members of the International Clearinghouse for Birth Defects Surveillance and Research (some of which were also included in the EUROCAT study), no significant changes in NTD trends were seen in areas with supplementation recommendations alone. The authors concluded that recommendations alone remain an ineffective approach in translating the known protective effect of folic acid into population-wide declines in NTD prevalence [14].

Prior to the knowledge that folic acid prevented NTD-affected pregnancies, there was a long history of permitting voluntary fortification of food products, e.g., breakfast cereals, with folic acid in many countries, including the United States and the United Kingdom. In other countries, e.g., Australia and New Zealand, voluntary fortification of food products was permitted only after folic acid was proven efficacious for preventing NTD-affected pregnancies. In order to have a population-wide effect, voluntary fortification requires that women know about and choose specially fortified foods, or that foods that most women commonly eat are fortified and affordable, so that most women consume them whether or not they are aware that they are fortified [15].

Potential issues with voluntary fortification include the difficulty in knowing whether a particular food, over time, is consistently fortified with the same amount of folic acid. In addition, it might be difficult to monitor overall intake from consumption of voluntarily fortified foods in the population. Such estimates require knowledge of brand names and timing of consumption by individuals to calculate the amount of folic acid provided by voluntarily fortified foods such as breakfast cereals. There is also the potential for exposure to higher intakes of folic acid for individuals who might consume large quantities of certain highly fortified foods such as breakfast cereals. On the other hand, mandatory fortification of a staple food with folic acid has the potential to overcome many of the disadvantages of promotion of an increase in dietary folate, periconceptional supplement use, and/or voluntary fortification. Most women will consume the fortified food whether or not their pregnancy was planned, and the socioeconomic disparities seen with voluntary programs can be minimized.

In March 1996, the United States Food and Drug Administration (FDA) mandated that by January 1, 1998, all products made from enriched cereal grain flours be fortified with folic acid at the level of 140 μg/100 g of flour (1.4 mg/kg) to prevent NTDs [16]. This effort was designed to be a program that was—first and foremost—safe, and secondly, effective. By 2009, 59 countries had documented national regulations for mandatory wheat flour fortification, 51 of which require folic acid [17].

During the past 5 years, food safety agencies from several countries that are considering mandatory folic acid fortification programs have conducted systematic reviews of the potential beneficial and adverse effects associated with folic acid. Among these agencies are the Food Standards Australia New Zealand (FSANZ) [18], the Food Safety Authority of Ireland [19], the Scientific Advisory Committee on Nutrition (SACN) for the UK Food Standards Agency [20], and the Health Council of The Netherlands [21]. All of these agencies have written final reports that recommend approval of folic acid fortification programs in their respective countries.

This report will only describe the relevant proven

beneficial effects of periconceptional consumption of folic acid and will not discuss other potential beneficial effects (reduction in the occurrence of other birth defects, other reproductive outcomes, childhood and adult cancer, and adult chronic disease) that have been positively associated with folic acid intake. Those interested in more information about these potential beneficial effects of folic acid are encouraged to refer to the systematic reviews conducted by the food safety agencies discussed earlier [18–21].

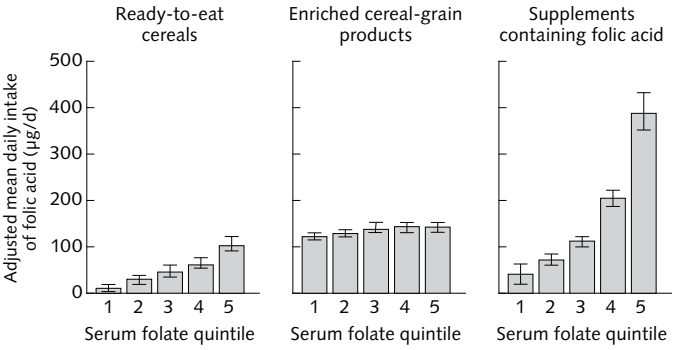

FIG. 1. Estimated daily intake of folic acid by serum folate quintile. National Health and Nutrition Examination Survey (NHANES) 2001–04 [27] for 8,655 nonpregnant adults aged 19 years and older. (Error bars indicate 95% confidence intervals)

This report will concentrate on selected issues that have arisen since the Cuernavaca Technical Workshop in December 2004 [22]. These issues are likely to be important when countries consider the implementation of folic acid fortification programs. This report will provide the basic findings, information about study design, the population studied, and the generalizability of the findings for the studies that have raised these issues. Given the proven beneficial effect of folic acid in preventing NTD-affected pregnancies, although other potential benefits of folic acid fortification might prove to be important, the primary basis for implementation of any folic acid fortification program is prevention of NTD-affected pregnancies. Finally, most of the questions raised in the recent literature about potential adverse effects of folic acid are associated with higher blood folate concentrations or reported higher intakes of folic acid that probably result from excessive supplement use, rather than consumption of fortified flour products alone [23, 24]. During the present Workshop, it was agreed that when flour is fortified with appropriate amounts of folic acid, based on appropriate estimates of the consumption profile of flour by the human population, the intervention is designed to reduce any likelihood of a public health risk [25].

Global patterns: Sources of folic acid and concentrations of blood folates

Sources of dietary folic acid in the United States

The three main sources of folate and/or folic acid intake in the United States are naturally occurring food folate, synthetic folic acid added to food (fortified foods), and supplements containing folic acid. In the United States, folic acid (to prevent NTDs) is added to food through two US FDA regulations: mandatory fortification of enriched cereal-grain products (ECGP) through a standards of identity regulation at 1.4 mg/kg flour or cereal-grain product [16] and optional fortification of ready-to-eat products such as breakfast cereals through a food additive regulation that allows the voluntary addition of folic acid [26].

Figure 1 summarizes a recent analysis of sources of folic acid among nonpregnant participants 19 years of age or older in the National Health and Nutrition Examination Survey (NHANES) 2001–04. In the lowest and highest serum folate quintiles, the mean daily folic acid intake was 181 and 643 μg, respectively, and the mean serum folate concentration was 14 nmol/L (6 ng/mL) and 55 nmol/L (24 ng/mL), respectively. In the lowest and highest quintiles, 11% and 75% of participants, respectively, used supplements containing folic acid. In the highest quintile, use of supplements containing folic acid contributed, on average, 61% of the total daily folic acid intake, and ECGP contributed 22% of total daily intake. A strong correlation existed between serum folate concentration and daily use of supplements containing folic acid [24], indicating that higher serum folate concentrations are primarily associated with the use of supplements containing folic acid.

Definitions of folate deficiency/insufficiency

Clinical folate deficiency is usually defined as a serum folate concentration < 7 nmol/L (~ 3 ng/mL) or a red blood cell folate concentration < 315 nmol/L (~140 ng/mL) [27]. Selhub et al. have proposed that the observed decrease in concentration of homocysteine in relation to increasing blood folate concentrations be used as a new functional definition of folate deficiency [28]. The blood folate concentrations below which serum homocysteine concentration begins to increase are 10 nmol/L (~ 4.4 ng/mL) for serum folate and 340 nmol/L (~150 ng/mL) for red blood cell folate.

Daly et al. [29] found in a cohort study of the Irish population that NTD risk decreased as blood folate increased and that the risk of NTDs continued to decrease in women with blood folate concentrations above the classical cutoffs for deficiency. From these

data one should assume that folate deficiency among women of childbearing age should not be based solely on levels associated with classic folate deficiency. NTD-affected pregnancies may occur among women who are not classified as folate deficient. The minimum effective blood folate concentration required for the optimal prevention of NTDs is not known, and "sufficient" blood folate concentrations for the reduction in risk of a folate-sensitive NTD-affected pregnancy still need to be determined. Raising blood folate levels to higher concentrations observed to be associated with NTD risk reduction would probably require that women consume folic acid from supplements and/or fortified foods in addition to natural folates from the usual diet [30].

Global patterns of folate deficiency/insufficiency and NTDs

One recent review has summarized what is known about estimates of available blood folate concentrations worldwide [31]. In 2006, the March of Dimes published the Global Burden of Birth Defects, which estimated yearly numbers of NTDs for every country in the world (almost 324,000 worldwide) [32]. Bell and Oakley, using these March of Dimes estimates, calculated that of the more than 300,000 NTD-affected pregnancies worldwide, the majority (more than 200,000) are probably preventable with folic acid [33]. When interpreting NTD prevalences, it is important to assess the completeness of the birth defects surveillance system. NTD-affected pregnancies may be underreported, especially when they end in fetal death or elective terminations are not consistently available.

Chemistry and stability of folic acid and naturally occurring food folate

Chemistry

Folate is the generic name for the water-soluble vitamin, which includes a family of compounds containing a pteridine ring joined with p-aminobenzoic acid, and one or more glutamic acid residues [34]. The form of naturally occurring folate depends on the side chain composition, in terms of the number of glutamic acids, as well as the specific position of attachment of the one-carbon moieties on the vitamin. Naturally occurring folates (food folate) are derived from tetrahydrofolate (THF), which is the fully reduced form. The term "folic acid" specifically refers to the fully oxidized monoglutamate form of the vitamin that is used in supplements and fortified foods and rarely occurs in nature.

Stability of storage and cooking conditions

A proportion of dietary folate is lost during cooking and preparation of foods as a result of a combination of thermal degradation and leaching of the vitamin into the cooking water [35]. Folic acid is more stable than dietary folates [36–38]. The retention of folic acid in fortified breakfast cereals and vitamin–mineral premixes has been examined, and it has been shown that little or no loss occurs during storage of up to 6 months at ambient temperatures [39, 40]. Likewise, folic acid stability during baking of bread products and following storage of fortified flour and grains has been found to be high [40–43].

Analytical techniques for monitoring food folate/folic acid

Pfeiffer et al. recently reviewed the analytical techniques for monitoring food folate/folic acid [44]. Among the methods used for food folate analysis, the microbiologic assay is considered to be the best and most versatile [45, 46]. *Lactobacillus casei* subspecies *rhamnosus* (ATCC 7469) has been the most widely used microorganism, since it responds almost equally to the widest variety of folate derivatives [45]. Key studies have confirmed that the trienzyme microbiologic assay results in significantly higher folate values than conjugase treatment alone, and that the higher values are not an artifact of the assay procedure [47]. Food folate can also be measured by high-performance liquid chromatographic (HPLC) methods [47], and procedures are also available to allow the identification of specific one-carbon derivatives of folate and/or to characterize the length of the polyglutamic chain [48]. Nevertheless, HPLC analysis of folate in food has been reported to result in lower values than does the microbiologic analysis [49–51], which is coupled with trienzyme extraction resulting in comparable values for select foods [52]. The microbiologic assay with trienzyme extraction has been accepted as an official Association of Official Analytical Chemists (AOAC) method.

It is important to recognize how modifications of the accepted AOAC microbiologic assay might significantly affect total folate values, as illustrated by findings from two interlaboratory studies. Koontz et al. [53] reported large variability in values for food folate between different commercial laboratories in the United States that routinely analyze total folate by the microbiologic assay for monitoring purposes. The inconsistencies between laboratories might be attributed to modifications of the AOAC microbiologic method. The effects of different assay conditions on total folate determination might depend on characteristics of the specific food matrix; thus, if control materials assayed in parallel do not share these same characteristics, incorrect values might go undetected. In an international interlaboratory study of

food folate [54], only a small percentage of the participating laboratories used the AOAC-accepted trienzyme extraction technique, and there were numerous other modifications of specific aspects of the AOAC microbiologic method for food folate analysis that could have a major impact on interlaboratory variability.

In summary, for monitoring purposes, any laboratory conducting total folate analysis on foods should use the official AOAC method [55]. However, other HPLC methods work reasonably well if the interest is only to determine the presence of added folic acid.

Analytical techniques for monitoring blood folate/folic acid

Methodology for monitoring blood folate or folic acid has been recently reviewed [44]. The serum concentration of folate is an important marker of nutritional status and has been measured for more than 30 years as part of the NHANES [56]. The Bio-Rad QuantaPhase radioassay (BR), which was used from 1988 to 2006 to measure serum folate, has been discontinued, and beginning in 2007 the primary assay for NHANES is the *L. casei* microbiologic assay (MA) [57, 58]. In addition, the new isotope-dilution liquid chromatography-tandem mass spectrometry method (LC-MS/MS) will be used for a subset of samples [59, 60]. The good correspondence between the sum of folate species determined by LC-MS/MS and folate measured by MA makes these two assays interchangeable. The BR produces much lower concentrations, probably because of under-recovery of 5-methyl-THF. The lower response of the BR than of the MA has been known for many years [61]. Multiple linear regression models yielded a good fit for converting BR data to MA or LC-MS/MS data, and these conversion equations could be used for future NHANES time trends. In summary, the MA is the currently preferred method for monitoring blood folate [57, 58]. The National Institute of Standards and Technology (NIST) standard reference material for folate in human serum, SRM 1955 [62], should be used in monitoring assays in addition to internal controls.

When comparing blood folate concentrations among studies worldwide, it is important to remember two points. First, measurements of blood folate concentrations using the BR radioimmunoassay underestimate the serum folate concentrations measured by the microbiologic assay by about 30%, so comparisons between these assays should be made with caution. Second, blood folate concentrations can be expressed in either ng/mL or nmol/L (1 ng/mL = 2.266 nmol/L).

Bioavailability of folic acid and folates

When considering folic acid fortification, it is important to understand how the intake of folic acid from fortified foods will affect folate status when it is consumed chronically as a component of a mixed diet, compared with naturally occurring food folate. The bioavailability of folic acid and food folate is defined as the portion of the vitamin that is physiologically absorbed and utilized in the organism. Bioavailability might be influenced by many factors, including the chemical form of folate, the food matrix, the chemical environment in the intestinal tract, and factors affecting the metabolic utilization of folate postabsorption [63].

The bioavailability of folic acid (relative to food folate) consumed as part of a mixed diet has been evaluated in a series of "long-term" studies (3 to 14 weeks) with differing protocols and different status indicators [30, 49, 64–67]. The Dietary Folate Equivalent (DFE) is a common way to express total folate coming from both food folate and synthetic folic acid. The quantity of synthetic folic acid is multiplied by 1.7, and this quantity is added to the micrograms of food folate. The conversion factor (1.7) is based on the observation that when folic acid is consumed with a meal, it is approximately 85% bioavailable [47], whereas food folate is approximately 50% bioavailable [64]; thus, the ratio 85/50 yielded the multiplier of 1.7 in the DFE calculation.

Dietary Reference Intakes/Dietary Folate Equivalents/NTD recommendation

The Dietary Reference Intakes (DRIs) established by the Institute of Medicine are a set of reference values that are expressed in Dietary Folate Equivalents (DFEs) (except the tolerable Upper Intake Level [UL], which is expressed as micrograms of folic acid/day). DFEs are units that account for the higher bioavailability of synthetic folic acid compared with naturally occurring food folate. The DRIs include the Estimated Average Requirement (EAR), Recommended Dietary Allowance (RDA), Adequate Intake (AI), and UL [27]. The RDA for all males and nonpregnant females 14 years of age or older is 400 μg DFE/day. For pregnant and lactating women, the RDA increases to 600 and 500 μg DFE/day, respectively. RDAs for children are extrapolated from those of adults based on relative body weight for age.

In 1998, the Institute of Medicine reviewed potential adverse effects due to excessive intakes of certain vitamins and minerals [27]. The Institute of Medicine determined that for the US population, the lowest observed adverse effect level (LOAEL) for consumption of folic acid was 5,000 μg/day among those adults with pernicious anemia. The Institute of Medicine divided the LOAEL by 5 to create a UL of 1,000 μg/day for the adult US population (for details, see "Potential adverse effects of folic acid," below). Despite the fact that pernicious anemia is rare in children, the Institute of Medicine created lower UL values for children based on body size.

To reduce the risk of NTDs, the Institute of Medicine also established a separate recommendation specifically targeted to women capable of becoming pregnant. This recommendation was similar to that made by the United States Public Health Service in 1992 and stated, "It is recommended that women capable of becoming pregnant consume 400 μg of folic acid daily from supplements, fortified foods, or both in addition to consuming food folate from a varied diet" [27].

Unmetabolized folic acid in blood

When folic acid intakes exceed a specific physiologic threshold, the ability of intestinal enzymes to convert the vitamin to the reduced form is exceeded, resulting in "unmetabolized" folic acid in the circulation. Following uptake by the liver, folic acid is normally reduced to THF [34]. Unreduced folic acid will appear in the systemic circulation when the capacity of both the intestinal and the hepatic enzymes required for folic acid reduction is exceeded.

A number of experimental studies have estimated the threshold intake above which unmetabolized folic acid would appear in the blood and found that individuals who consume folic acid from fortified food, breakfast cereals, or folic acid–containing supplements have varying concentrations of unmetabolized folic acid in their blood [68–70]. In the United States, individuals who consume vitamin supplements containing 400 μg or more of folic acid [71] will likely have unmetabolized folic acid in their blood. In addition, unmetabolized folic acid can be found among individuals who are not consuming supplements but who are likely to be consuming folic acid from ready-to-eat cereals [72]. The consequences of these findings are uncertain. It is clear that more research is needed to confirm any potential beneficial or adverse effects.

Known public health benefits of folic acid

As noted earlier, this report will describe only the relevant proven beneficial effects of periconceptional consumption of folic acid and will not discuss other known or potential beneficial effects.

Scientific evidence of the effectiveness of folic acid flour (wheat and maize) fortification programs

The effectiveness of mandatory folic acid fortification programs has been documented by a decline in the prevalence of NTDs. **Figure 2** shows that programs in the United States, Canada, Costa Rica, Chile, and South Africa have resulted in significant declines in the occurrence of NTD-affected pregnancies [73–79]. The declines range from 28% in the United States to 46% in Canada. All these countries, at a minimum, are fortifying wheat flour and wheat products. Costa Rica also fortifies milk, corn flour, and rice [73]. South Africa fortifies both wheat and maize flours [79].

Changes in blood folate concentration

Since 1998, mandatory fortification of cereal grain products has more than doubled the median serum folate concentration in the US population from 12 nmol/L (5.5 ng/mL) in the prefortification period (1988–94) to 29.7 nmol/L (~ 13.1 ng/mL) in the early postfortification period (1999–2000). The prevalence of clinical folate deficiency (< 7 nmol/L or ~ 3 ng/mL) also decreased from 21% to <1% during this period

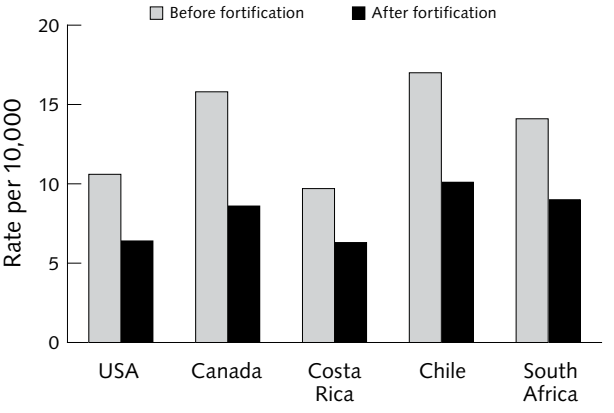

FIG. 2. Changes in the prevalence of neural tube defects in five countries after the introduction of folic acid fortification

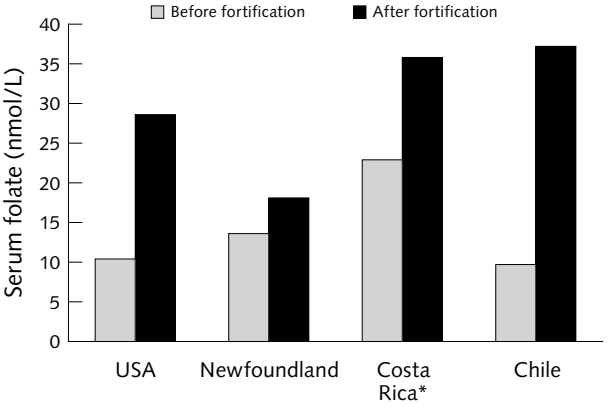

FIG. 3. Changes in serum folate levels among women of reproductive age in four countries in the Americas after the introduction of folic acid fortification

* Costa Rica data are from the total population

[80]. All the countries in **Figure 3** had documented increases in blood folate concentrations after the start of their fortification programs [73–75, 81].

Potential adverse effects of folic acid

In 1998, the Institute of Medicine reviewed the scientific literature to evaluate the general toxicity of folic acid, its reproductive effects, and the occurrence of neurologic manifestations among patients with pernicious anemia who were taking high-dosage folic acid. The Institute of Medicine found little evidence of direct toxicity from folic acid itself, even at dosages of more than 100,000 μg/day. Sleep disturbances, gastrointestinal effects, and mental changes have only been observed in one uncontrolled, nonblinded trial in which oral doses of folate of 15,000 μg/day were taken for 1 month [82]. "However," the Institute of Medicine report [27] continues, "studies using comparable or higher dosages, longer durations, or both, failed to confirm these findings" [83–87]. The Institute of Medicine concluded that there was no evidence of direct toxicity of folic acid and no evidence of adverse reproductive effects from folic acid [27].

The only potential adverse effect of folic acid that the Institute of Medicine identified at that time occurred among patients with diagnosed pernicious anemia whose existing therapy was replaced by high-dosage folic acid (described in 23 articles, which contained 284 case reports). Of these 284 cases, 155 were reported to have neurologic complications when the underlying pernicious anemia was not treated appropriately, and folic acid was inappropriately given in high dosages for many months. Most of these reports involved four or fewer patients; in the larger reports (20 or more patients), dosages of more than 5,000 μg/day were typically given for 2 years or more. Using these case reports, the Institute of Medicine determined that for the US population, the LOAEL for consumption of folic acid was 5,000 μg/day and that folic acid dosages below the LOAEL were not associated with reported neurologic damage; however, the uncertainty of establishing a UL such that any person in the US population with undiagnosed vitamin B_{12} deficiency would have *no* risk of developing neurologic manifestations led the Institute of Medicine to divide the LOAEL by 5 to create a UL of 1,000 μg/day for the adult US population. The Institute of Medicine concluded that there was "no clear evidence of folate-induced neurotoxicity in humans" [27]

In the past several years, many articles have been written describing potential adverse effects associated with consumption of folic acid, and the strength of these findings varies greatly. These articles have prompted many reviews and commentaries that interpret the strength of these findings differently [88–96]. Observational and ecologic studies, hypotheses, and opinions should not be used as evidence of cause and effect. Among the best sources of detailed, balanced information about the potential risks and benefits of folic acid fortification are several systematic reviews recently completed by national food safety agencies (FSANZ Australia and New Zealand [18], FSA Ireland [19], FSA United Kingdom [20], and the Health Council of The Netherlands [21]).

Interactions between higher blood folate concentrations and vitamin B_{12} deficiency

In the 1998 Institute of Medicine report, early concerns were that folic acid would mask anemia of vitamin B_{12} deficiency or exacerbate the neuropathy associated with vitamin B_{12} deficiency. Mills et al. failed to find evidence that folic acid fortification was associated with masking anemia of vitamin B_{12} deficiency [97]. Since the publication of the DRIs by the Institute of Medicine in 1998, there has been no clinical or research evidence that currently recommended intakes of folic acid exacerbate the neuropathy of vitamin B_{12} deficiency. One study failed to find a relation between mean corpuscular volume (MCV) at different concentrations of serum folate [98]. Another study suggests lower values of MCV postfortification [99].

Recently, an observational study proposed that among pregnant women in India with vitamin B_{12} deficiency, higher red blood cell folate concentrations, which are probably the result of consumption of daily prenatal supplements containing 500 μg folic acid, might be associated with the development of insulin resistance in their offspring later in childhood [100]. In contrast, in 2009, follow-up of 6- to 8-year-old children in Nepal whose mothers participated in a randomized, controlled trial that tested the effect of different prenatal micronutrients on pregnancy outcome found no evidence that prenatal use of micronutrients increased insulin resistance in their children. The study did find a borderline reduction in the risk of the metabolic syndrome among those mothers who were in the folic acid group [101].

Cancer

Findings from studies for most cancers are equivocal, according to extensive reviews from FSANZ [18] and FSA (Scientific Advisory Committee on Nutrition) [20]. Nevertheless, some articles on colorectal cancer have received attention because of the concern that folic acid might be associated with increases in colorectal cancer incidence.

In 2007, an ecologic study hypothesized that a temporary increase in the incidence of colorectal cancer from 1996 to 1999 might be associated with the start of folic acid fortification in the United States and Canada in 1998 [102]. The authors' hypothesis was generated from

examination of changes in colorectal cancer incidence rates in the National Cancer Institute's Surveillance and Epidemiology and End Results (SEER) database [103]. The authors proposed that the start of the increase in incidence of colorectal cancer coincided with the introduction of folic acid fortification, although in fact, the increase in colorectal cancer appears to have begun in 1996, despite the fact that blood folate concentrations in California did not begin increasing until 1997 [104]. The FDA did not publish the final rule to fortify flour until March 1996 [16]. Such an early increase in the incidence of colorectal cancer would imply a nearly instantaneous biologic response following the FDA action [91]. In their summary, the authors of the colorectal cancer hypothesis did conclude that further work was needed to definitively establish the nature of the relation between folic acid fortification and the incidence of colorectal cancer.

In 2009, an ecologic study of Chilean hospital discharge records for colorectal cancer reported higher rates of discharge after Chile began to fortify bread flour in 2000 [105]. Hospital discharges for 3 years (1992, 1993, and 1996) before 2000 were compared with those for the years from 2002 to 2004. Donoso et al., using vital records from 1990 through 2003, found that colorectal cancer mortality in Chile had increased by 68%, with an average yearly increase of 4.3% [106]. It is difficult to attribute the increase in hospital discharges for colorectal cancer to a folic acid fortification program that began in 2000 when the observed colorectal cancer mortality rate in Chile had already increased 50% in the 10 years before the fortification program began.

In 2007, a randomized, controlled trial tested whether supplements containing 1,000 μg folic acid prevented the recurrence of colorectal adenomas [107]; the investigators reported that folic acid did not prevent the recurrence of colorectal adenomas. The authors concluded that the evidence for increased or decreased risk of recurrence of adenomas was equivocal and required further research. However, a secondary finding that the folic acid group had an increased recurrence risk of having three or more adenomas (but not one or two adenomas) raised concern. In 2008, two other published randomized, controlled trials of folic acid to prevent recurrence of colorectal adenomas found evidence that folic acid did not result in an increase in the incidence of colorectal adenomas [108, 109]. However, these trials had other findings and conclusions. One trial found that 500 μg folic acid daily did not prevent the recurrence of colorectal adenomas [108], and the other found that 5,000 μg folic acid daily did prevent the recurrence of colorectal adenomas [109].

Cognition

In 2005, a cohort study of seniors found that those who were in the highest quintile of folate intake (97% of whom reported using multivitamins) had more rapid decline in cognitive function than those in lower quintiles. The authors attributed this more rapid decline to higher folic acid intake [110]. An alternative explanation for the more rapid decline in cognition among those seniors in the highest folic acid quintile is that their baseline level of cognition, which was more than four times higher than the level of cognition in the lowest folic acid quintile, was artificially high. The more rapid decline might have occurred because the level of cognition was returning to baseline, an example of regression to the mean.

In 2007, a cross-sectional study of eligible senior participants 60 years of age or older from NHANES 1999–2002 (67% of whom reported using dietary supplements) reported that among those who were in the highest serum folate quintile, the 97% who had adequate vitamin B_{12} status were protected from cognitive impairment, whereas the 3% who had low vitamin B_{12} status had evidence of cognitive impairment. The authors attributed this cognitive impairment among those with low vitamin B_{12} status to higher serum folate concentrations in the age of folic acid fortification [111]. An alternative explanation for this apparent cognitive impairment is that seniors who would have been having problems with cognition because of vitamin B_{12} deficiency were more likely to be consuming multivitamins containing folic acid, which would increase their blood folate concentrations enough to place them in the highest quintile of serum folate and create an apparent association between higher blood folate concentrations and cognitive impairment [23].

Other studies have failed to confirm these potential adverse effects. One randomized, controlled trial [112] found that consuming supplements containing folic acid for 3 years significantly improved domains of cognitive function that tend to decline with age. In this study, lower red blood cell folate concentrations were associated with poorer cognitive performance. However, another randomized, controlled trial found that lowering homocysteine concentrations with B vitamins did not improve cognitive performance [113].

Twinning

Findings from Sweden and Hungary have associated maternal use of folic acid with an increase in the occurrence of twinning [114–117]. However, these studies are probably confounded by the frequent use of assisted reproductive technologies in those countries [118, 119]. One large cohort study in China found no evidence that maternal use of 400 μg/day increased twinning [120], and five studies in the United States evaluating secular trends in twinning before and after fortification began in 1998 failed to find any evidence that folic acid fortification had changed the rate of

twinning [121–125].

More recently, Haggarty et al. [126] studied women using assisted reproductive technologies and found that higher concentrations of blood folate were associated with an increase in dizygotic twinning. The authors recommended that women using assisted reproductive technologies restrict their intake of folic acid to prevent increased twinning. However, in this study, virtually all women had two zygotes inserted into their uterus; therefore, essentially all pregnancies began as dizygotic twins. An alternative conclusion is that higher daily intakes of folic acid increased zygote survival, which would result in a greater number of dizygotic twins surviving to birth.

Spontaneous first-trimester abortion (miscarriage)

In 1997, it was proposed that folic acid increased the occurrence of miscarriage [127]. Subsequently, findings from a large-scale folic acid intervention study conducted in China provided strong evidence that maternal use of periconceptional folic acid does not increase miscarriage rates [128]. Additionally, a Swedish population-based, matched, study of case women with spontaneous abortion and control women found that women with low serum folate concentrations had a higher risk of early spontaneous abortion than women with normal serum folate concentrations [129]. This study and a more recent study, which found that self-reported vitamin supplementation in early pregnancy reduced the risk of miscarriage [130], support the findings from earlier studies that suggested a low folate status increases the occurrence of miscarriages [131, 132].

Natural killer cell activity

Troen et al. [133] described an association between higher concentrations of unmetabolized folic acid in serum and decreased natural killer cell activity in a select population of older, obese, postmenopausal women. This finding was one of many results in a panel of immunologic tests done to evaluate an exercise program among these women. Fifty-seven percent of these women were taking multivitamins that provided on average approximately 255 μg/day of supplemental folic acid in addition to folic acid consumed as a part of a mixed diet containing folic acid-fortified foods. Unmetabolized folic acid was detected in 78% of plasma samples collected from these women. Other studies have associated vitamin deficiency with decreased natural killer cell activity [134, 135]. The clinical importance of the association between unmetabolized folic acid and decreased natural killer cell activity is unclear [94]

Recommendations on the level of folic acid added to flours

A recent analysis of empirical data from 20 developing countries suggested that wheat flour consumption varies widely among and within countries [25]. **Table 1** shows the highest average folic acid fortification levels that should maintain daily usual intake of folic acid below the UL for four different patterns of consumption of fortified products [136]. The fortification levels mentioned in **Table 1** should be adjusted downwards if more than one folic acid intervention is being implemented for the general population [136]. If women of reproductive age consume 100 g/day of flour and it is fortified at a level of 2.6 mg folic acid per kilogram of flour, then the estimated folic acid intake of those women from fortified flour products would be 260 μg/day.

Cost of folic acid fortification

The price of folic acid is likely to continue increasing along with the price of the fortificant. However, despite this increase, it remains one of the less expensive fortification interventions. **Table 1** shows the calculated cost of folic acid for the four different levels of flour consumption.

Research recommendations

Engage in public health activities and research:

» Improve monitoring of NTDs and other birth defects;

» Monitor other potential adverse health outcomes,

TABLE 1. Highest average level of folic acid added to flour and cost of fortificants for different ranges of usual daily intake of flour

	Mean usual daily flour intake (g/day)			
Variable	Low (< 75)	Medium (75–149)	High (150–300)	Very high[a] (> 300)
Highest average level of folic acid added to flour (mg/kg)	5.0	2.6	1.3	1.0
Cost[b] (US$/MT flour)	≤ 1.08	≤ 0.56	≤ 0.28	≤ 0.22

a. Few countries have per capita consumption > 300 g/day.

b. The current (April 2008) worldwide price of folic acid is US$195/kg for a product that is 90% folic acid.

such as cancer and adult chronic diseases;
» Establish support for countries preparing to develop plans to fortify flour with folic acid to enable them to simultaneously implement flour fortification and to collect baseline blood folate concentrations with which to compare pre- and postfortification blood folate.
» Conduct a workshop to assess possibilities for defining blood folate concentrations that estimate the concentrations at which folic acid–preventable NTDs rarely, if ever, would occur.
» Standardize the different assays used to measure blood folate concentrations to the microbiologic assay (see "Analytical techniques for monitoring blood folate/folic acid," above) so that results from these different assays can be converted into uniform concentrations that can be compared easily.

Summary

The global public health burden of NTDs is great. NTDs are important preventable causes of infant mortality and permanent disability, affecting an estimated 300,000 newborns worldwide each year [137]. NTDs are known to occur widely, having been reported on every continent and among diverse populations of different geographic areas and varying levels of economic development [138]. Preventing NTD occurrence should be a priority worldwide, given the link between many of these severe birth defects and insufficient folate intake among reproductive-age women.

Fortification of wheat flour with folic acid has a proven record of prevention of NTDs in developed countries. In 2009, 51 countries had regulations written for mandatory fortification of wheat flour with folic acid. Most developing countries have few, if any, sources of folic acid, unlike developed countries, which have folic acid available from ready-to-eat cereals, voluntarily fortified foods, and supplements. By continuing to increase the number of countries with programs to fortify flour with folic acid, we have the opportunity to deliver a proven prevention effort for families and to help tens of thousands of babies around the world to be born healthy instead of paralyzed or dying from NTDs.

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Considering the case for vitamin B_{12} fortification of flour

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Abstract

Reasons to fortify flour with vitamin B_{12} are considered, including the high prevalence of depletion and deficiency of this vitamin that occurs in persons of all ages in resource-poor countries and in the elderly in wealthier countries, and the adverse functional consequences of poor vitamin B_{12} status. From a global perspective, the main cause of inadequate intake and status is a low intake of animal-source foods; even lacto-ovo vegetarians have lower serum vitamin B_{12} concentrations than omnivores, and for various reasons many populations have limited consumption of animal-source foods. Infants are vitamin B_{12}-depleted from early infancy if their mothers' vitamin B_{12} status and intake are poor during pregnancy and lactation. Even in the United States, more than 20% of the elderly have serum vitamin B_{12} concentrations that indicate depletion, and an additional 6% have deficiency, primarily due to gastric atrophy, which impairs the absorption of the vitamin from food but usually not from supplements or fortified foods. Although the evidence is limited, it shows that fortified flour, consumed as bread, can improve vitamin B_{12} status. Where vitamin B_{12} fortification is implemented, the recommendation is to add 20 µg/kg flour, assuming consumption of 75 to 100 g flour per day, to provide 75% to 100% of the Estimated Average Requirement; the amount of the vitamin that can be added is limited by its cost. The effectiveness of this level of addition for improving vitamin B_{12} status in programs needs to be determined and monitored. In addition, further research should evaluate the bioavailability of the vitamin from fortified flour by elderly people with food cobalamin malabsorption and gastric atrophy.

Key words: Deficiency, flour, fortification, vitamin B_{12}

Introduction

There are compelling reasons to consider vitamin B_{12} fortification of flour. These include mounting evidence of widespread depletion and deficiency in the many population groups that consume low amounts of animal-source foods, which are the only natural source of the vitamin; the high prevalence of deficiency in the elderly, even in industrialized countries, many of whom require synthetic sources of the vitamin because of their limited ability to release and absorb vitamin B_{12} from foods; new evidence of an association between poorer vitamin B_{12} status and a higher risk of neural tube defects (NTDs); and persistent debate about the potential, although perhaps unlikely, exacerbation of the symptoms of vitamin B_{12} deficiency by folic acid fortification of flour. There is no upper limit to safe intake of the vitamin, so that flour fortification should be safe as well as efficacious.

The Estimated Average Requirement (EAR) for the vitamin increases from 0.7 µg/day in early childhood to 2.0 µg/day for adults [1]. This amount will meet the requirements of 50% of the population. The Recommended Dietary Allowance (RDA) increases from 0.9 to 2.4 µg/day across the life span and meets the needs of 97.5% of the population. Although requirements are not higher in the elderly, it is recommended that they obtain a higher proportion of their requirement from fortified foods or supplements because they are at greater risk for being unable to absorb the vitamin

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from food [1]. The goal in fortification programs is that the EAR should be met for approximately 95% of the individuals in specific population groups; fortification should fill the gap between the amount of a nutrient consumed in the usual diet and the EAR [2].

Indicators of the vitamin B_{12} status of a population

The cutoffs recommended by the Institute of Medicine for diagnosing vitamin B_{12} depletion and deficiency are < 148 pmol/L (200 pg/mL) and 148 to 221 pmol/L (200 to 300 pg/mL), respectively, in plasma or serum [1, 2]. The 148 pmol/L cutoff is the concentration below which clinical symptoms of deficiency (e.g., neurologic, cognitive, and hematologic) may occur. Below 221 pmol/L, biochemical signs of inadequacy start to appear, including elevated serum and urinary methylmalonic acid (MMA) and plasma total homocysteine (tHcy). Serum is preferred over plasma for the detection of vitamin B_{12} deficiency, because improper centrifugation of plasma, which sometimes occurs under field conditions, can result in falsely high measures of the vitamin (Allen et al., unpublished data), although in most situations the samples give similar values.

The emerging standard for detection of vitamin B_{12} deficiency is elevated serum MMA. Although there is debate about the cutoff for defining "elevated," > 210 μmol/L is the upper value of the range found in vitamin B_{12}-replete subjects with normal serum creatinine [3]. In the US population, the lowest serum MMA concentration is found at a serum vitamin B_{12} concentration > 150 pmol/L [4]. Some investigators have classified deficiency on the basis of a combination of serum vitamin B_{12} in the deficient or depleted range and elevated MMA [5] . One limitation of MMA is that it can be elevated by intestinal bacterial overgrowth [6] and poor renal function [7]; ideally, the latter cases should be detected by measurement of serum creatinine and eliminated from estimates of deficiency prevalence. MMA analysis has the limitations of requiring a mass spectrometer and being relatively expensive.

Vitamin B_{12} deficiency causes elevated plasma tHcy, but it is inappropriate to estimate the prevalence of vitamin B_{12} deficiency based solely on tHcy unless there is normal status of all other vitamins for which deficiency can elevate this metabolite (i.e., folate, riboflavin, vitamin B_6). Plasma tHcy is also elevated in individuals with poor renal function and in hypothyroidism. In a folate-replete population, the lowest plasma tHcy concentration (< 10 μmol/L) is found when the serum vitamin B_{12} concentration is > 300 pmol/L [4].

There is increasing interest in the diagnostic value of plasma holotranscobalamin (holoTC, previously termed holoTC II) concentrations. A cutoff of ≤ 40 pmol/L has been suggested to represent deficiency [8]. HoloTC comprises only about 20% of the total vitamin B_{12} in plasma [8], but its importance lies in the fact that it is the only form in which vitamin B_{12} is transported from the intestine and taken up by the tissues. The other 80% of vitamin B_{12} in plasma is bound to haptocorrin, the function and importance of which are not well understood. HoloTC is gaining increasing attention from a functional perspective. For example, low plasma holoTC (and not low total plasma vitamin B_{12}) predicted increased risk of NTDs in Canada, once the population was folate-replete after fortification of flour with folic acid [9]. The ratio of holoTC to total plasma vitamin B_{12} predicted poorer cognitive function in an elderly population with depressive symptoms, whereas total plasma vitamin B_{12} did not [10]. However, there is usually a strong correlation between total plasma vitamin B_{12} and holoTC in populations [7, 11], and holoTC may be only slightly superior to serum vitamin B_{12} in its ability to detect vitamin B_{12} deficiency diagnosed on the basis of elevated MMA [7]. The higher cost of the holoTC assay (about twice that of serum vitamin B_{12}) means that in most situations it may be more practical to rely solely on plasma vitamin B_{12} to estimate the prevalence of deficiency in a population. If functional outcomes of fortification are assessed, then the inclusion of holoTC should be considered. A new assay requires 100 μL plasma (Axis-Shield, Dundee, Scotland).

Dietary intake

There is relatively little information on the vitamin B_{12} intakes of populations, especially in developing countries, which often lack data on the vitamin B_{12} content of foods. Methods for vitamin B_{12} analysis in foods are quite complex and need to exclude vitamin B_{12} analogues that are not utilized by humans. The main dietary sources of vitamin B_{12}, with their approximate contents of the vitamin in micrograms per serving, are red meat 2.4, chicken 0.3, fish 1 to 5, milk 0.9, yogurt 1.4, eggs 0.6, cheese 0.3, and liver 48. The bioavailability of vitamin B_{12} from these sources is assumed to be about 50% of that of crystalline vitamin B_{12} [1], although there is some evidence that the efficiency of absorption from food and synthetic sources might be similar [12]. In nonusers of supplements aged 26 to 83 years in the United States, plasma vitamin B_{12} increased by 34 pmol/L for each doubling of intake in the range of 0 to 10 μg/day [13]. The increase was 28, 24, and 19 pmol/L with each doubling of intake from supplements, fortified cereal, and other foods, respectively, but the magnitude of the difference among the sources was not statistically significant. The increase in plasma vitamin B_{12} by log intake was lower for meat than for dairy products and fortified cereals, possibly reflecting poorer absorption from meat.

Importantly, the intestinal mechanisms for active absorption of the vitamin are saturated above a dose of about 2 μg (70% is absorbed from 0.5 μg of the crystalline vitamin, 50% from 1 μg, 20% from 5 μg, 5% from 25 μg, and < 1% from ≥ 25 μg) [1, 14], so only about 5% to 10% is absorbed from a serving of liver [14]. In adults without pernicious anemia, plasma vitamin B_{12} concentrations tend to plateau at intakes of about 10 μg/day in both users and nonusers of supplements [13]. Since about 1% is absorbed from high doses by passive absorption, large oral intakes can improve vitamin B_{12} status even in persons with limited capacity for active absorption. More research is needed on the efficiency of absorption of different forms and doses of vitamin B_{12}. Lack of information about the efficiency of absorption of the vitamin from fortified foods, especially by the elderly, is another strong reason that the effects of vitamin B_{12} fortification programs on vitamin B_{12} status need to be monitored carefully.

Because the vitamin is present only in animal-source foods, including fish, lower intake of these foods is a proxy for risk of vitamin B_{12} deficiency. For example, in a group of Kenyan schoolchildren with a high prevalence of vitamin B_{12} deficiency, animal-source foods provided only 4% of total dietary energy on average, mostly from milk [15]. The serum vitamin B_{12} concentration of about 70% of the children was < 221 pmol/L. Even with these low intakes of animal-source foods, the odds ratio for plasma vitamin B_{12} levels < 148 pmol/L was 6.3 times higher for children in the lowest tertile of intake of animal-source foods (0% to 1.3% of energy), and 1.6 times higher in the second tertile of intake (1.4% to 4.2% of energy), than in the highest tertile (4.2% to 37.1% of energy). Increasing the children's vitamin B_{12} intake by about 1 μg/day with supplemental meat (60 to 85 g/day) or milk (200 to 250 mL/day) increased plasma vitamin B_{12} by about 40 pmol/L over the course of a year. In a generally well-nourished US population group aged 26 to 83 years who did not take supplements, a higher proportion of those with low or deficient plasma vitamin B_{12} concentrations were in the lowest than in the highest tertile of dairy product and meat intake [13].

Many investigators have reported poorer vitamin B_{12} status in lacto-ovo vegetarians than in omnivores (see below). Although low intake of animal-source foods is probably the strongest risk factor for vitamin B_{12} deficiency at the population level, some elderly people with gastric atrophy cannot absorb enough of the vitamin from natural food sources, and infections such as bacterial overgrowth can also reduce its absorption from food. Thus, vitamin B_{12} status can be poor even when intakes appear to be adequate, especially in the elderly.

Global prevalence of low vitamin B_{12} status

In 2005, the World Health Organization (WHO) created a global data bank on vitamin B_{12} status, based on available data on plasma or serum vitamin B_{12} concentrations. The criteria for inclusion of data in the data bank are the following: study published since 1995; subjects from the general population (i.e., generally healthy); at least 50 subjects in the study; and preferably national-level survey data, which, if available would replace smaller studies from the same country. Of the 118 eligible studies, 75% were in the Americas or the European region, but there were no data from the Eastern Mediterranean region [16]. National data were available for seven countries (Costa Rica, Germany, New Zealand, Norway, the United Kingdom, the United States, and Venezuela), and provincial data were available for Canada, Senegal, Spain, and Sweden. However, most countries are represented by small studies of population groups that were not selected to be representative.

The prevalence of vitamin B_{12} deficiency (defined as < 148 pmol/L) in adults was highest in India (nearly 50%) and about 5% to 30% in most other countries. WHO did not calculate the prevalence of vitamin B_{12} depletion, which is much higher than that of deficiency. For example, a review of nine smaller studies in Latin America revealed that the plasma vitamin B_{12} concentration of approximately 40% of individuals from infants to adults was ≤ 221 pmol/L and that 15% to 20% of individuals were in the range of deficiency [17].

Causes of vitamin B_{12} deficiency

Vitamin B_{12} deficiency has three main causes: a low intake of animal-source foods; food-bound vitamin B_{12} malabsorption, which is seen primarily in the elderly and in some gastrointestinal tract abnormalities; and much less commonly, pernicious anemia.

Pernicious anemia is a condition in which there is loss of gastric parietal cells and subsequent inability to synthesize intrinsic factor, as well as impaired function of intrinsic factor, which is required for the active intestinal transport of vitamin B_{12}. Thus, pernicious anemia is a disease and is not caused by a low intake of vitamin B_{12}. A study of undiagnosed pernicious anemia in 729 persons 60 years of age or older in the United States showed the prevalence to be 1% to 4% among different sex and ethnic groups [18]. Thus, in any study, a small proportion of low serum vitamin B_{12} concentrations could be explained by undiagnosed pernicious anemia.

There is substantial evidence that a low intake of animal-source foods is the main cause of the widespread low plasma vitamin B_{12} concentrations across the world [19]. It is commonly assumed that only strict

vegetarians develop dietary vitamin B_{12} deficiency, but data from Australia [19], Germany [20, 21], Slovakia [22], The Netherlands [23], and India [24] reveal higher prevalences of deficiency and depletion among lacto-ovo vegetarians than among omnivores. It is clear that strict avoidance of animal-source foods is not necessary for individuals or population groups to develop vitamin B_{12} deficiency.

After assuming a lower efficiency of absorption of vitamin B_{12} from liver than from other foods (on account of its high content of the vitamin), a significant correlation between usual vitamin B_{12} intake and serum vitamin B_{12} was observed in lactating Guatemalan women [25], Guatemalan infants [26], and Kenyan schoolchildren [15]. In the latter group, supplementation with meat (60 g/day) or milk (200 mL/day) at school for 1 or 2 years significantly reduced the prevalence of vitamin B_{12} deficiency [27]. In the 1999 Mexican national nutrition survey, vitamin B_{12} intake predicted plasma vitamin B_{12} concentrations in both women and their children under 5 years of age (M. Anaya et al., unpublished data). Low plasma vitamin B_{12} concentrations may be very common in infancy because of maternal deficiency and/or inadequate intake, resulting in low stores in the infant at birth [28], followed by low concentrations in human milk [29]. Among Guatemalan infants, for example, about 60% were deficient or had marginal status by 7 months of age, with breastfeeding the main predictor of poor infant status [26]. Those who consumed more cow's milk, which is higher in vitamin B_{12} than breastmilk, had significantly higher plasma vitamin B_{12}. Similar observations have been reported from India, where plasma vitamin B_{12} concentrations were much lower (median 183 pmol/L, with 36% < 150 pmol/L) in breastfed than in nonbreastfed (median 334 pmol/L, 9% < 150 pmol/L) low-to-middle-income children aged 6 to 11 months [30]. Once an infant becomes vitamin B_{12} deficient in a population with low intake of animal-source foods, the low plasma vitamin B_{12} persists for at least a year [26].

With aging, some individuals gradually lose their ability to absorb vitamin B_{12} from food. The cause of this "food-bound cobalamin malabsorption" is thought to be loss of gastric acid resulting from gastric atrophy and subsequent inability to release the vitamin from proteins in animal-source foods [31]. In the elderly, gastric atrophy is most likely to be caused by chronic, long-term infection with *Helicobacter pylori*; associations among plasma vitamin B_{12}, *H. pylori* infection, and gastric function have been reported in this group [32, 33]. There has been no study of the effect of treatment for *H. pylori* on the reversal of vitamin B_{12} malabsorption. In Californian Hispanics aged 60 years of age or older, of whom 16% were vitamin B_{12}-depleted and an additional 6% had deficiency, 48% of those with a plasma vitamin B_{12} concentration indicating deficiency had elevated serum gastrin, a symptom of gastric corpus atrophy [34]. However, the global prevalence of food cobalamin malabsorption is uncertain because of lack of representative surveys. This malabsorption explained a high proportion of low serum vitamin B_{12} concentrations in American (40%) [35] and French [36] patients referred for vitamin B_{12} deficiency. Based on the high prevalence and earlier age of onset of *H. pylori* infection in developing countries, it is probable that the prevalence of food-bound cobalamin malabsorption in these locations is even higher, but this has not been studied. Moreover, almost no data are available on vitamin B_{12} status of the elderly in developing countries, except for nonrepresentative samples from Chile (28% with < 165 pmol/L) [37] and Guatemala (38% with < 150 pmol/L) [38].

It is assumed that most persons with food-bound cobalamin malabsorption are still able to absorb the free vitamin provided in fortified foods, although this has not been tested. In a population of Californian elderly, similar relationships were found between plasma vitamin B_{12} and intake of the vitamin from supplements, fortified beverages, and fortified cereals, suggesting that absorption from these sources is similar [34]. Similarly, the relationship between plasma vitamin B_{12} and intake of the vitamin from fortified cereals or dairy products was similar in 26- to 83-year-old healthy Americans and was not associated with age [13]. The availability of vitamin B_{12} as a fortificant in flour, which is processed into cooked foods, may or may not be the same as that coated onto fortified breakfast cereals. Further research is needed on the bioavailability of vitamin B_{12} from various sources, including fortified products, especially in persons with food-bound cobalamin malabsorption.

Adverse effects of vitamin B_{12} deficiency

The consequences of vitamin B_{12} deficiency have been reviewed and described in greater detail in a series of articles from the 2005 WHO Technical Consultation on Folate and Vitamin B_{12} Deficiencies [39]. The participants summarized current knowledge of the adverse functional effects of deficiency as follows.

Anemia. It is commonly assumed that populations with vitamin B_{12} deficiency or depletion are at greater risk for anemia. However, the existing studies show that widespread deficiency at the population level is not associated with higher risk of anemia, either in the elderly or among people with low consumption of animal-source foods [40]. Vitamin B_{12} supplementation of deficient and depleted adult Mexican women [11] and preschoolers [41] did not affect any hematologic measure in a complete blood count. Megaloblastic anemia is common, however, when vitamin B_{12} deficiency is more severe, such as in infants breastfed by mothers

who are strict vegetarians [42].

Neurologic disorders. One of the classical signs of severe vitamin B_{12} deficiency is demyelinating neurologic impairment, including subacute combined degeneration of the spinal cord. Symptoms include loss of posterior column functions and memory and cognitive impairment [1]. More recently, reports from different countries have emphasized neurologic presentation of vitamin B_{12} deficiency over hematologic signs. The mechanisms underlying the neurologic effects of vitamin B_{12} deficiency are poorly understood; the rapid neurologic improvement after severely deficient individuals are given intramuscular injections of the vitamin, and changes in tumor necrosis factor α (TNF-α) and epidermal growth factor in animal studies, suggest that demyelination is not the only cause [42].

Cognitive impairment. There has been a recent focus on cognitive impairment resulting from vitamin B_{12} insufficiency, especially when deficiency is identified by elevated MMA and tHcy, which are more sensitive metabolic indicators of vitamin B_{12} status in aging and elderly populations. Vitamin B_{12} is second to folate as a determinant of circulating tHcy, and elevated tHcy is associated with cerebrovascular disease, impairment of cognitive function in healthy aging populations, and higher risk of incident dementia in prospective studies [43]. Moreover, since the fortification of flour with folic acid, vitamin B_{12} status is the main nutritional determinant of plasma tHcy in US elderly [43]. Reports of associations between plasma vitamin B_{12} and/or transcobalamin concentrations and cognitive function in the elderly have been inconsistent [44]. A recent longitudinal study revealed that elderly people with low holotranscobalamin, or with plasma vitamin B_{12} in the lowest tertile (< 308 pmol/L) versus the highest tertile, had a sixfold higher risk of brain volume loss over a 5-year period, which was not explained by higher plasma tHcy [45]. Intervention studies with vitamin B_{12} have not been well designed, and improvement in cognitive function has been inconsistent except for the rare study of the effect of vitamin B_{12} injections in populations with low vitamin B_{12} status [44]. Vitamin B_{12} deficiency was a risk factor for depression in several studies [46].

Neural tube defects and pregnancy outcomes. The WHO Technical Consultation concluded that there was moderately convincing evidence for an association between vitamin B_{12} deficiency and increased risk of NTDs. Studies at that time had occurred prior to folic acid fortification and/or did not adjust for folate status. A population-based case–control study in Canada, after folic acid fortification of flour, found that the risk of NTDs increased inversely with serum holoTC concentrations, was 2.9-fold higher for women in the lowest (≤ 55 pmol/L) than for those in the highest quartile of holoTC, and could explain 34% of the NTDs in Canada [9]. An intervention with vitamin B_{12} supplements or fortification is needed to confirm these associations, as are studies of the association between NTD risk and holoTC in countries with a high prevalence of vitamin B_{12} deficiency in women of childbearing age—which is not the situation in Canada. The WHO Technical Consultation concluded that there was insufficient information to conclude that vitamin B_{12} deficiency increases the risks of other birth defects or affects other aspects of pregnancy outcome, although women with pernicious anemia are at higher risk for recurrent spontaneous abortions [47]. Maternal vitamin B_{12} status during pregnancy affects the vitamin B_{12} status of the newborn, and low maternal intake and/or status during lactation reduces the concentration of the vitamin in breastmilk [28, 42, 47]. As discussed above, infants exclusively breastfed in vitamin B_{12}-depleted populations are at high risk for developing vitamin B_{12} deficiency. Intriguingly, normal to high folate status combined with low serum vitamin B_{12} during pregnancy predicted higher adiposity and insulin resistance in the offspring of Indian women, raising the question of a potential effect of maternal vitamin B_{12} status on fetal programming [48].

Bone health. Several epidemiologic studies identified an association between low serum vitamin B_{12} concentrations and low bone mineral concentration [49, 50], higher risk of osteoporosis [51], and higher rate of bone mineral loss during aging [52]. Elevated plasma tHcy is associated with similar outcomes. For example, in the third National Health and Nutrition Examination Survey (NHANES) in the United States, based on data from 737 men and 813 women with an average age of 68 years, bone mineral density was lower and osteoporosis increased significantly with each higher quartile of serum MMA [51]. Impressively, those in the highest MMA quartile had a 7.2-fold greater risk of osteoporosis than those in the lowest MMA quartile. Serum vitamin B_{12} was related to bone mineral density in a concentration-related manner up to approximately 200 pmol/L, and those with tHcy > 20 μmol/L had significantly lower bone mineral density than those with values < 10 μmol/L. Since poor bone mineralization occurs in pernicious anemia, in which condition it can be prevented by supplementation [53], and the vitamin B_{12} content of bone cells in culture affects bone formation [54], it is suspected that vitamin B_{12} deficiency plays a causal role in poor bone mineralization, although this has not been confirmed by randomized, controlled interventions to increase vitamin B_{12} intake. The mechanism could be a direct effect of vitamin B_{12} deficiency on bone-forming cells, elevated plasma tHcy, or increased production of the inflammatory marker TNF-α, which has been reported to occur in vitamin B_{12} deficiency [55]. Randomized, controlled trials are needed to confirm the extent to which vitamin B_{12} deficiency affects bone mineralization and the mechanisms involved.

Who would benefit most from vitamin B_{12} fortification of flour?

In contrast to the situation with some nutrients, such as iron, individuals across the life span, both male and female, are at risk for vitamin B_{12} deficiency in populations with a low intake of animal-source foods. In addition, the elderly are at even greater risk for deficiency and need to consume vitamin B_{12} in fortified foods or supplements to obtain sufficient amounts of the vitamin.

Within populations consuming low amounts of animal-source foods, pregnant and lactating women, their infants, and young children have both an especially high prevalence of deficiency and the greatest risk of adverse consequences, including birth defects and impaired child development. In wealthier countries, the main target group for fortification is probably the elderly, and this age group is likely to have an even higher prevalence of deficiency in poor regions of the world.

Fortification is intended not to treat existing nutrient deficiencies but rather to protect populations against becoming deficient. Patients diagnosed with vitamin B_{12} deficiency or depletion (plasma vitamin B_{12} < 150 pmol/L and/or plasma MMA ≥ 210 μmol/L) in a clinical setting should be treated appropriately by a physician. A common treatment regimen is high-dose oral supplements (e.g., 1,000 μg/day for 1 month followed by 125 to 500 μg/day maintenance) [56] or intramuscular doses (1 mg every 2 months), as the improvement in status due to a flour fortification program will be gradual, and it may take years for a deficient population's status to normalize.

There are no known adverse consequences of vitamin B_{12} fortification and no known adverse effects of high intakes of the vitamin [1], although some potential, as yet unproven, adverse effects were suggested in a recent review [57].

Inter-relationships between vitamin B_{12} and folate

In population studies, combined supplementation with folic acid plus vitamins B_{12} and B_6 is only marginally more effective than folic acid alone in lowering plasma tHcy. However, after flour fortification in the United States and Canada, vitamin B_{12} status became the main nutritional determinant of circulating tHcy concentrations [58].

Earlier literature tended to attribute the adverse effects of administering folic acid in the face of vitamin B_{12} deficiency to "masking" or delaying of the diagnosis of vitamin B_{12} deficiency anemia while neurologic degeneration proceeded. Reports, mostly from the 1940s and 1950s, of high-dose folic acid treatment of patients with undiagnosed pernicious anemia suggested exacerbation of neurologic deficits independently of delaying the diagnosis and treatment of the pernicious anemia-induced vitamin B_{12} deficiency [1], although whether this exacerbation truly occurs has been questioned [59]. More recent reports have emphasized adverse associations between cognitive impairment and high folic acid and folate intake accompanied by low vitamin B_{12} intake in a Chicago cohort [60], and in the NHANES, elderly people with high serum folate (arbitrarily defined as > 59 nmol/L) and low vitamin B_{12} status (defined as serum vitamin B_{12} < 148 pmol/L or serum MMA > 210 nmol/L) had the highest risk of anemia and poorest performance on memory tests [5]. The combination of high serum folate and low serum vitamin B_{12} is also associated with the highest tHcy and MMA levels [61], suggesting that the clinical associations with anemia and poor memory may have additional metabolic correlates. An alternative explanation, speculated by Berry et al. based on the fact that such high serum folate concentrations usually occur only after the consumption of supplements, and the fact that these commonly contain both folic acid and vitamin B_{12}, is that the persistent low vitamin B_{12} status in such elderly persons is caused by preclinical pernicious anemia due to severe gastric atrophy, which could explain the higher prevalence of anemia and poorer cognitive performance [62].

In Indian mothers, two-thirds of whom had serum vitamin B_{12} < 150 pmol/L and 90% of whom had elevated MMA, higher maternal erythrocyte folate concentrations at 28 weeks of pregnancy predicted greater fatness and insulin resistance in their offspring at 6 years of age [48]. Only 1 of the 700 women had low erythrocyte folate, and the median concentration was 874 nmol/L. The women were supposed to have taken 500 μg/day folic acid from 18 weeks of gestation (although actual intakes were not recorded), but erythrocyte folate levels were already normal at this stage of gestation. These observations call for greater examination of potential adverse interactions between higher intakes of folic acid and poorer vitamin B_{12} status in such populations, and determination of whether changes would need to be made to supplements for pregnant women if flour is also fortified with folic acid. Vitamin B_{12} fortification might alleviate such adverse interactions, but this remains to be proven. There is also need for more information on whether there is an interaction between free, unmetabolized circulating folic acid and vitamin B_{12}–requiring systems in cells [61, 63].

Overview of existing guidelines and ongoing programs for vitamin B_{12} fortification of wheat flour

There has been very little experience with vitamin B_{12} fortification. A few efficacy trials on healthy elderly people have shown that the vitamin can be absorbed and improves vitamin B_{12} status when added to flour

and consumed as bread. In The Netherlands, men and women aged 50 to 65 years were randomly assigned to bread fortified to provide 138 μg folic acid and 9.6 μg vitamin B_{12} per day (n = 72), or unfortified bread (n = 70), for 12 weeks [64]. In the fortified group, serum folate increased by 45% and serum vitamin B_{12} by 49%, and the proportion of vitamin B_{12}–deficient individuals (serum vitamin B_{12} < 133 pmol/L) fell from 8% to 0%. The generalizability of this study is limited by the relatively high level of vitamin B_{12} fortification and the exclusion of elderly subjects with serum vitamin B_{12} < 118 pmol/L, who might have had more severe gastric atrophy and/or preclinical pernicious anemia. No trials have been conducted to assess the impact of fortification on clinical or functional outcomes.

Monitoring the effects of vitamin B_{12} fortification

There is little information with which to predict the extent or timing of changes in serum vitamin B_{12} or MMA in populations after vitamin B_{12} fortification of flour. In the Netherlands study of fortified bread, there was a substantial increase in serum vitamin B_{12} in 12 weeks (by 102 pmol/L on average) when the bread provided 9.6 μg/day [64]. This intake from bread was almost five times higher than the proposed fortification level would provide on the assumption of a medium intake of flour (75 to 100 g/day, see below). Feeding breakfast cereal to healthy volunteers in the United States increased vitamin B_{12} intake by 3.4 μg/day and serum vitamin B_{12} by 58 pmol/L after 14 weeks of daily feeding [65]. Only 9.7% had serum vitamin B_{12} < 185 pmol/L at baseline, but the prevalence fell to 3.3% during the study. Where there is a high prevalence of deficiency or depletion at baseline, it might take some years for flour fortification to move a population's median serum vitamin B_{12} concentrations into the normal range of adequacy. In Guatemalan communities with a high prevalence of vitamin B_{12} depletion, daily supplementation with the RDA from the ages of 7 to 13 months did not change serum concentrations of the vitamin, nor did 9 months of supplementation with the RDA as a crystalline supplement or as beef from age to 12 to 21 months (L. H. Allen, unpublished data). In rural Kenya, schoolchildren with a high prevalence of deficient and marginal plasma vitamin B_{12} concentrations were supplemented with approximately 1 μg/day in milk or 0.85 [μg]/day in beef for approximately half the days in the school year [27]. At the end of the first year of supplementation, the median plasma vitamin B_{12} concentration had increased from 131 to 189 pmol/L in the meat-supplemented group (with the prevalence of values < 125 pmol/L reduced from 47% to 21%) and from 164 pmol/L to 236 pmol/L in the milk-supplemented group (with the prevalence of values < 125 pmol/L reduced from 31% to 10%). In another flour fortification study in which 1 μg vitamin B_{12} was added to 100 g flour (total vitamin B_{12} intake not reported) and served as bread, Israeli women with normal vitamin B_{12} status had a very small but significant increase in serum vitamin B_{12} concentration after the 6-week intervention (S. Gabriel-Levy et al., unpublished data).

Clearly, if fortification is undertaken, it is essential to monitor baseline parameters and then changes in population status after fortification. Values to be monitored (means or medians and prevalence of abnormal values) include serum vitamin B_{12} and MMA, if possible.

Recommendations on the level of vitamin B_{12} addition for countries choosing to fortify flour

For countries that elect to fortify foods based on their public health priorities, WHO/Food and Agriculture Organization (FAO) has recommended a procedure for calculating the amount of a nutrient that should be added as a fortificant [2]. Basically this requires knowledge of the distribution of intakes of the nutrient by specific population groups of special concern, the distribution of intakes of the food vehicle proposed to be fortified (e.g., wheat flour), and the calculated effect that different levels of fortification would have on intake — particularly on the proportion of the population consuming less than the EAR and/or more than the tolerable upper level (if one has been established). This detailed information might be less necessary in the case of vitamin B_{12} than for other micronutrients, for the following reasons: there is no upper level of intake known to cause adverse effects on health; for those (notably the elderly) with food-bound cobalamin malabsorption, the amount consumed in nonfortified foods may be less relevant than the amount absorbed; and estimates of mean or median intakes of vitamin B_{12} from foods with a high content of the vitamin, such as liver, can be misleading, since the percentage absorbed from such foods is low, as described above. Therefore, we have made recommendations based on the usual intake of wheat flour (**table 1**), and the following assumptions [66]: there is no need to establish an upper level because of concern about possible toxicity; plant-source foods, such as cereals, do not contain vitamin B_{12}, so there is no need to consider the intrinsic content of the fortified flour; there is no technological constraint to the addition of vitamin B_{12} to food in the range of relevant concentrations, i.e., there are no adverse effects on color, sensory qualities, etc.; and cost is the biggest constraint to the amount of vitamin B_{12} that can be added.

Table 1 shows the suggested levels of fortification of wheat flour to supply 2 μg vitamin B_{12}/day to the

TABLE 1. Suggested levels of vitamin B_{12} addition for different usual daily intakes of wheat flour to supply 2 μg vitamin B_{12}/day to the consumer

	Level of refined wheat flour consumption			
Variable	Low	Medium	High	Very high
Adjusted mean per capita intake (g/day)	< 75	75–149	150–300	> 300
Vitamin B_{12}, average addition (mg/kg, 0.1% water-soluble)[a]	0.04	0.02	0.01	0.008

Source: Dary [66].

a. To supply 100% of the Estimated Average Requirement (EAR) in the medium consumption range. EAR = 2 μg/day for adults.

consumer [66]. The vitamin should be purchased in a diluted form (0.1%) with 100% active particles (i.e., all spray-coated with vitamin B_{12}) and diluted 1:15 to 1:25 in a premix. If iron is also included in the premix at a known ratio relative to vitamin B_{12}, analysis of the iron content can provide an approximate estimate of the vitamin B_{12} content. The average coefficient of variation of vitamin B_{12} content in fortified flour is assumed to be ± 35% of the mean value, so the minimum amount present should be the mean ± 45% (35% × 1.28 for 80% of the expected values). Thus, if the average content is 20 μg/kg, the expected allowable range is from 10 to 30 μg/kg. Both the minimum and the maximum values should be enforced. Loss of the vitamin during storage on the floor is assumed to be 10%. Bread has a short shelf-life, so loss in the prepared product is assumed to be negligible.

Recommended form of fortificant, stability, and bioavailability

The only recommended form of vitamin B_{12} for fortification is cyanocobalamin, which has been stabilized by the addition of cyanide and is also the form used in supplements. Cyanocobalamin is relatively stable to light, moisture, and heat. A recent study found 77% recovery of the vitamin from fortified flour made into bread [64]. The American Institute of Baking found that adding vitamin B_{12} up to 1,000 μg/100 g flour did not impact dough handling or fermentation rates of white pan breads; subjective ratings of external and internal characteristics of breads made with the addition of vitamin B_{12} were slightly higher than in breads without added vitamin B_{12}; and addition of vitamin B_{12} up to 10,000 μg/100 g flour did not produce a noticeable red or pink crumb color [67].

No adjustment is needed for the extraction rate of flour. There is no vitamin B_{12} present in cereals, so restoration is not an issue. Absorption of cyanocobalamin will not be affected by substances present in higher amounts in low-extraction flours, such as fiber and phytate.

Cost of fortification

The cost of adding vitamin B_{12} at 20 μg/kg will be US$0.85/MT. This will add 0.21% to the cost of wheat flour, assuming that flour costs US$0.40/kg, or one-tenth of the 2% increase in the final cost of fortified products that is generally considered acceptable to producers and the public.

Research recommendations

Although much remains to be learned about the benefits of vitamin B_{12} fortification of flour [57, 68], the scale of the deficiency problem is tremendous, affecting the elderly wordwide and infants, young children, and pregnant and lactating women, among others, in poor countries. This argues for the addition of vitamin B_{12} to flour as part of the Flour Fortification Initiative.

The most important information needed is the bioavailability of the vitamin from fortified flour and bread, especially for the elderly and others with severe food-bound vitamin B_{12} malabsorption. Given that cost has limited the current recommended level of addition of vitamin B_{12} to flour, it is important to monitor serum vitamin B_{12} concentrations before and at intervals during fortification with different levels of addition in specific population groups; these might include women and children in populations with a low intake of animal-source foods and a known high prevalence of depletion, and those aged 50 years and over. Monitoring and evaluation should ideally include a plan to assess the benefits of vitamin B_{12} fortification on functional outcomes, such as NTD prevalence; breastmilk concentrations of the vitamin; infant development; and strokes, cognitive performance, bone mineralization, and anemia in the elderly.

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Vitamin A fortification of wheat flour: Considerations and current recommendations

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Abstract

***Background**: Vitamin A deficiency is a major public health nutrition problem, affecting an estimated 190 million preschool-aged children and 19 million pregnant and lactating women globally, and 83 million adolescents in Southeast Asia alone. Its consequences (disorders) include xerophthalmia (the leading cause of early childhood blindness), increased severity of infection, anemia, and death. Because vitamin A deficiency is largely due to chronic dietary insufficiency of preformed vitamin A and proactive carotenoids, food fortification can offer an effective approach to prevention.*

***Objective**: To provide guidance on fortifying wheat and maize flour milled in industrial rollers for national fortification programs in countries where vitamin A deficiency is considered a public health problem.*

***Methods**: Critical review of the literature on the dietary gap in vitamin A intake and levels of wheat flour intake among risk groups as a basis for determining vitamin A fortificant levels. Additional review of efficacy evidence, safety and cost considerations, and country experiences related to wheat-flour fortification with vitamin A.*

***Results**: Mill-rolled wheat flour is a technically fortifiable, centrally processed food vehicle that, where routinely and adequately consumed by target groups, should be considered a candidate for fortification. Vitamin A can be stable in flour under typical, ambient conditions, with processing losses estimated at approximately 30%, depending on source and premix conditions.*

***Conclusions**: Factors to guide a decision to fortify flour with vitamin A include the extent of deficiency, availability of other food vehicle options, the centrality of milling, market reach and population intake distributions of the flour products, the dietary vitamin A intake required, and associated costs. Large gaps persist in knowledge of these factors, which are needed to enable evidence-based fortification in most countries, leaving most decisions to fortify guided by assumptions. Where flour can and should be fortified, guidelines are given for providing nearly 25% of the Recommended Dietary Allowance for vitamin A to vulnerable groups consuming varying ranges of flour products. The costs will vary according to the level of fortification.*

Key words: Dietary intake, food fortification, vitamin A deficiency, wheat flour

Introduction

Wheat consumption is rising on a per capita basis in many developing countries where vitamin A deficiency remains a public health problem. Wheat flour fortification, therefore, may provide a growing opportunity to improve vitamin A intake of the poor [1]. Many low-income countries import wheat, either through commercial channels or as food aid; the latter route tends to self-target the poor and, therefore, presumably the most vitamin A deficient [2, 3]. Imported wheat is often milled at a limited number of private (e.g., Philippines and Indonesia) or government (e.g., Sri Lanka, Egypt) mills prior to national distribution, making centralized wheat flour fortification feasible. On the other hand,

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fortification of wheat flour may be less feasible in countries with large numbers of dispersed small mills, or where home-based milling is commonly practiced, because of challenges such decentralized milling poses to maintaining quality control.

The goal of a vitamin A fortification program is to prevent vitamin A deficiency. Its objectives are to increase vitamin A intake and to improve vitamin A status among population groups whose daily dietary needs for vitamin A are not routinely met, while minimizing the risk of overconsumption among groups whose vitamin A status is normal. For wheat flour to be a suitable vehicle, however, target groups should routinely consume a minimum amount of centrally processed wheat flour, preferably within a definable intake range. This requirement is not met in many low-income countries, particularly by poorer groups for whom foods other than wheat flour may serve as staples (e.g., maize, rice, sorghum, millet, and roots and tubers). In many developing countries, wheat-based foods are consumed primarily by the upper and upper middle socioeconomic groups, who tend to be vitamin A sufficient [4]. In some countries, such as the Philippines, there is also wide geographical and urban–rural variability in wheat flour consumption, even among poor communities [5], posing challenges to setting effective and safe fortification ratios.

Vitamin A deficiency: Magnitude of the problem

On a global scale, countries with per capita annual incomes under US$15,000 per year are considered to harbor most populations at risk for vitamin A deficiency [6]. Within low-income countries, the groups at greatest risk, and thus the primary target groups for prevention, are preschool-aged children, school-aged children through adolescence, and women of reproductive age, especially during pregnancy and lactation. These groups should, therefore, be given careful consideration in the design and evaluation of the public health impact of vitamin A fortification initiatives.

Preschool-aged children

An estimated 190 million children, or approximately 33% of all children under 5 years of age in low-income countries, are vitamin A deficient (**table 1**) [6], with the prevalence being highest among children in Southern Asia (50%) and sub-Saharan Africa (44%) and lower in the Region of the Americas (16%), based on distributions of serum retinol concentrations below 0.70 μmol/L (< 20 μg/dL) [6]. These figures are based on data to 2005, although the results of periodic, more recent studies are consistent with the extent of risk across regions [7–10]. Nearly 1% of preschoolers, or approximately 5 million, have xerophthalmia [6], which in its severe form (keratomalacia) remains the leading preventable cause of childhood blindness [1]. Approximately 11% to 14% of a typical low-income population is composed of children under 5 years of age [11], providing a target of considerable risk and size for intervention, such that adequately reaching this group with vitamin A can have a substantial public health impact. Supplementation trials conducted over the past 20 years, including one that tested the impact of vitamin A–fortified monosodium glutamate (MSG) in Indonesia, have reported reductions in preschool child mortality of, on average, 23% to 34% [12, 13], attributed to reductions in severity and fatality from infections such as measles, diarrhea, malaria, and other febrile illnesses [14]. Although control is largely achieved in young children through direct, high-potency vitamin A supplementation [1], the demonstrated effectiveness of vitamin A fortification of some food items, such as sugar [15, 16] or MSG [17, 18], in improving vitamin A status and reducing xeropthhalmia, anemia,

TABLE 1. Prevalence and numbers of cases of preschool-aged child and antenatal vitamin A deficiency and xerophthalmia by region

	Children < 5 yr				Pregnant women			
	Serum retinol < 0.70 μmol/L		Xerophthalmia		Serum retinol < 1.05 μmol/L		Night-blindness	
Region	%	No. (millions)	%	No. (millions)	%	No. (millions)	%	No. (millions)
Africa	44.4	56.4	2.0	2.55	13.5	4.2	9.8	3.02
The Americas	15.6	8.7	0.6	0.36	2.0	0.2	4.4	0.50
South/Southeast Asia	49.9	91.5	0.5	1.01	17.3	6.7	9.9	3.84
European Region	19.7	5.8	0.8	0.24	11.6	0.7	3.5	0.22
Eastern Mediterranean	20.4	13.2	1.2	0.77	16.1	2.4	7.2	1.09
Western Pacific	12.9	14.3	0.2	0.26	21.5	4.9	4.8	1.09
Total	33.3	190	0.9	5.17	15.3	19.1	7.8	9.75

Source: WHO [6].

or mortality reflects the potential that can be achieved when food items regularly consumed by this age group are fortified with vitamin A.

School-aged children

Data remain scant on the burden of vitamin A deficiency in school-aged children and adolescents, although the few reports available suggest mild-to-moderate vitamin A deficiency to be widespread in this age group in low-income countries. Based on the measurement of hyporetinolemia (< 0.70 μmol/L) and liberal extrapolation, 83 million children (~ 23%) 5 to 15 years of age in Southeast Asia are estimated to be vitamin A deficient, of whom 2.6% or 9 million have mild xerophthalmia (night-blindness or Bitot's spots). Corneal xerophthalmia in this age group appears to be negligible [19]. Sporadic reports from South Asia [20, 21], Africa [22, 23], and Latin America [24] are consistent with a 10% to 15% prevalence of vitamin A deficiency among school-aged children and adolescents. Although the health consequences are largely unreported, vitamin A deficiency in this age group could predispose young women to vitamin A deficiency during pregnancy and lactation [25, 26]. Since vitamin A supplementation programs do not extend beyond the preschool years, school-aged children and adolescents in undernourished societies represent large target groups to reach with food fortified with vitamin A. In the Philippines, a pilot trial revealed increased apparent liver stores of vitamin A (by a modified relative dose–response test) among school-aged children consuming vitamin A–fortified wheat flour used in making local bread (*pandesal*) [27], demonstrating proof of principle in this target-aged population.

Women of reproductive age

Recent updated figures from the World Health Organization (WHO) suggest that approximately 19 million pregnant and lactating women (15%) in low-income countries are vitamin A deficient, based on serum retinol concentrations < 0.70 μmol/L, of whom nearly 10 million (~ 8%) have night-blindness. According to the WHO report, the highest-risk regions for maternal deficiency exist in the Western Pacific (21%), South and Southeast Asia (17%), and the Eastern Mediterranean (16%), with a far lower prevalence estimate, at present, given for the Region of the Americas (~ 2%) [6]. Subnational reports from Southern Asia [28–30] and Africa [23, 31–33] tend to support the persistence of maternal vitamin A deficiency in these major regions, while representative data for this group in Latin America remain sparse. The health consequences of vitamin A deficiency during pregnancy include night-blindness [25, 34], anemia [25, 28], and, in some remote and undernourished settings, increased morbidity and mortality [35, 36]. Many countries have policies to reduce maternal vitamin A deficiency through one-time, high-potency, postpartum vitamin A supplementation, but coverage tends to be low. Further, this approach is not designed to raise the overall vitamin A status in a population, leaving a need for more sustainable, broader approaches such as can be afforded by fortification, when feasible. Breastmilk vitamin A has been shown to increase after fortification of sugar [16] and MSG [17] with vitamin A in Central America and Indonesia, respectively, providing evidence of public health potential among women of reproductive age with this intervention.

The dietary gap in vitamin A

Low serum vitamin A distributions (< 0.70 μmol/L) can be assumed to reflect chronic dietary inadequacy of vitamin A from preformed and proactive carotenoid sources. However, status data do not provide information about the size of the dietary deficit, or gap, in requirements to meet via fortification or other dietary strategies. One set of indices that can be used to assess dietary adequacy are the Dietary Reference Intakes (DRIs), including the Estimated Average Requirement (EAR), Recommended Dietary Allowance (RDA), and, for safety purposes, the Tolerable Upper Intake Level (UL), that have been developed by the Institute of Medicine in the United States with appropriate caveats about their applicability to undernourished populations [37]. One estimate of dietary gap is the added amount of vitamin A (in micrograms of retinol activity equivalents [μg RAE]) required to shift the intake distribution to the right of the EAR so that only approximately 3% remain below that level in an age group. A second approach is to estimate the amount of vitamin A required to bring the mean of the population to the level of the RDA. Either estimate serves to represent the extent to which fortification should increase vitamin A intake to minimize the risk of deficiency. Both require quantified dietary intake data, preferably collected by repeated 24-hour recalls from a representative sample of a target population, assumptions of normality of usual intake distributions, and an adequate food composition database. Few data of this nature, caliber, and specificity exist.

Table 2 illustrates the kinds of dietary data from which estimates of vitamin A intake and prevalence of dietary inadequacy could be constructed for the above three high-risk groups: preschool-aged children, school-aged and young adolescent children, and women of reproductive age. For example, in India in the mid-1990s, the National Nutrition Monitoring Bureau collected single 24-hour dietary recalls from rural adolescents across nine states, enabling the estimation of mean vitamin A intakes [38]. Weighting these estimates with population-based dietary studies in other

TABLE 2. Average daily dietary vitamin A intakes in children and adults[a]

Region/country	*N*	Vitamin A intake (μg RAE[b])		
		Preschool[c]	School-age[c]	Women[c]
EAR [41]		242	445	500
RDA [41]		350	600	700
Southern Asia[d]				
India[e]				
Average vitamin A intake (μg RAE)		158	147	184
Average % of EAR[f]		65%	33%	37%
Prevalence of inadequacy[g]		72%	87%	85%
Dietary gap (vs RDA, μg RAE)		−193	−453	−516
Andhra Pradesh [68]	220	ND	ND	233
Bihar [69]	1,847	150	ND	ND
Delhi [70]	1,328	208	ND	ND
Gujarat [39]	60	ND	ND	124
Kerala [39]	60	ND	ND	61
Rajasthan [71]	209	ND	216	187
Haryana [40]	117	114	ND	ND
Meghalaya [72]	650	ND	ND	137
9 Indian states [38]	2,579	ND	139	224
Indonesia				
Average vitamin A intake (μg RAE)		331	ND	778
Average % of EAR		137%	ND	156%
Prevalence of inadequacy		27%	ND	18%
Dietary gap (vs RDA, μg RAE)		−19.25	ND	78
Central Java [73]	450		ND	1,232
S. Sulawesi [74]	1,500	329	ND	609
S. Kalimantan [74]	2,112	333	ND	494
Philippines				
Average vitamin A intake (μg RAE)		82	ND	459
Average % of EAR		34%	ND	92%
Prevalence of inadequacy		86%	ND	55%
Dietary gap (vs RDA, μg RAE)		−268	ND	−241
National [75]	3,405	82	ND	ND
National [76]	589	ND	ND	494
National [76]	1,215	ND	ND	425
Africa[h]				
Egypt [42]				
Average vitamin A intake (μg RAE)		465	ND	796
Average % of EAR		192%	ND	159%
Prevalence of inadequacy		6%	ND	16%
Dietary gap (vs RDA, μg RAE)		115	ND	96
South Africa				
Average vitamin A intake (μg RAE)		245	ND	363
Average % of EAR		101%	ND	73%
Prevalence of inadequacy		49%	ND	68%
Dietary gap (vs RDA, μg RAE)		−105	ND	−337
National [43]	2,391	245	ND	ND
Northwest Province [44]	99	ND	ND	661
Limpopo Province [45]	46	ND	ND	318
KwaZulu—Natal [77]	475	328	ND	ND
KwaZulu—Natal [46]	291	94	ND	111

TABLE 2. Average daily dietary vitamin A intakes in children and adults[a] *(continued)*

Region/country	*N*	Vitamin A intake (μg RAE[b])		
		Preschool[c]	School-age[c]	Women[c]
Other countries in Eastern and Southern Africa				
Average vitamin A intake (μg RAE)		227	175	575
Average % of EAR		94%	39%	115%
Prevalence of inadequacy		54%	84%	40%
Dietary gap (vs RDA, μg RAE)		−123	−425	−125
Kenya—Marsabit [78]	300	ND	ND	243
Kenya—Nakuru [79]	716	ND	ND	712
Malawi—Mangochi District [80]	281	526	ND	ND
Malawi—Balaka [81]	144	94	ND	ND
Mozambique—Zambezia [82]	243	181	ND	ND
Namibia—Kaokoland [83]	53	ND	147	ND
Tanzania—Dar-es-Salaam [84]	271	ND	203	195
Zambia—Lusaka [85, 86]	34	107	ND	ND

EAR, Estimated Average Requirement; ND, no data; RAE, retinol activity equivalent; RDA, Recommended Dietary Allowance; RE, retinol equivalent

a. Intakes represent reported mean intakes of vitamin A by 24-hour recall or food records from population studies or surveys.

b. Data reported as micrograms RE have been assumed to have used a 6:1 conversion factor for provitamin A carotenoids. Intakes were converted to micrograms RAE using study- or region-specific estimates for the proportion of vitamin A intake from carotenoids, multiplying that proportion of overall intake by the previous conversion factor, and then dividing by the current bioconversion factor of 12 μg provitamin A carotenoids to 1 μg RAE [41].

c. Ages reported are for any interval of years up through age 5 years for preschool-aged children, 6 to 15 years for school-aged children, and 16 years and above to provide provisional estimates for "adults."

d. Assumes that 80% of vitamin A intake in South Asia [39, 40, 68, 87] and 40% of vitamin A intake in South East Asia is from provitamin A carotenoids [70–74].

e. An average intake for a country represents the mean of the mean estimates from surveys or states sampled; for preschoolers in India, only one survey is represented, and is taken as a provisional national average; for school-aged children, the nine-state survey estimate of 265 μg RAE was multiplied by 9, to which the two other state estimates were added, and the sum was divided by 11 = 280 μg RAE; the same approach was taken for estimating the average intake for adult women and men.

f. Percentage of the age-gender EAR represented by the mean vitamin A intake level; EAR values used are (as micrograms RAE): 242 for children < 5 years, 445 for school-aged children, and 500 for women of reproductive age [41].

g. Based on an estimate that the percent coefficient of variation (%CV) is 60% of the mean from studies using six to eight repeated 24-hour recalls of dietary intakes that were carried out to reduce sampling variation [39, 40], and calculating the probability area below the EAR cutoff assuming intakes follow a normal Gaussian distribution.

h. Assumes that 80% of vitamin A intake in Eastern and Southern Africa is from provitamin A carotenoids [73, 77, 85].

states (see footnotes to **table 2**), it is possible to derive crude average vitamin A intakes of 158, 147, and 184 μg RAE per day for these three age groups, respectively, representing 65%, 33%, and 37% of the EAR values for preschoolers (using 242 μg RAE as a mid-value for children 1 to 5 years of age), school-aged children and young adolescents (using 445 μg RAE as a mid-value), and women of reproductive age (using 500 μg RAE as a mid-value). Data from studies that collected multiple 24-hour recalls can be used to estimate the variance in usual vitamin A intake: the coefficient of variation is approximately 60% [39, 40]. Applying this estimate to the above average intakes under assumptions of normality, the prevalence of dietary vitamin A inadequacy in these three high-risk groups in rural India is estimated to be 72%, 87%, and 85%, respectively, far in excess of the approximately 3% recommended by the Institute of Medicine [37, 41]. The same calculations suggest that in Indonesia 27% of preschoolers and 18% of reproductive-age women have inadequate vitamin A intakes; for the Philippines, 86% and 55% of individuals in these high-risk groups, respectively, are not meeting the recommended intake levels. These estimates for the prevalence of dietary inadequacy correspond in magnitude, from an ecologic perspective, with the known extent of preschool, adolescent, and maternal vitamin A deficiency across South and Southeast Asia (**table 1**).

If the distribution of dietary vitamin A intake were well-characterized with respect to spread and shape, it should be possible to estimate the amount of vitamin A needed to add via fortification in order to shift the lower intakes of a population above the EAR. In the near-universal lack of such data, an alternative is to estimate the increment to be added as the difference between the mean or median vitamin A intake and the RDA. Thus, in India, for example, this calculation leads to average deficits in the RDA and amounts needed to be delivered via fortification to be 193, 453, and 516 μg

RAE for preschoolers, older children and young adolescents, and women of reproductive age, respectively (using a mid-point RDA estimate of 350 for 1–8-year olds, and 600 and 700 μg RAE for the other two groups, respectively).

Far fewer representative 24-hour dietary recall studies exist in African countries (though **table 2** is not exhaustive) from which to derive estimates of the dietary gap. According to several surveys in Egypt [42], the prevalence rates of inadequate intake in preschoolers and women are 6% and 16%, respectively, based on the EAR, suggesting little need for fortification, which is discordant with estimates of low-to-deficient serum retinol distributions in large subnational groups in the country [42]. Data on vitamin A intakes and status suggest a greater need for added vitamin A in sub-Saharan Africa. For example, nationally representative data from South Africa suggest that 49% of preschool-aged children have inadequate vitamin A intakes [43]. The estimate is 68% among women of reproductive age, based on surveys in selected provinces [44–46]. Published data from other eastern and southern African countries suggest levels of dietary vitamin A inadequacy comparable in magnitude to those in South Africa (**table 1**).

The forgoing provides an approach for estimating a dietary gap to consider in planning a vitamin A fortification initiative. The examples suggest a role for fortification in correcting dietary gaps in vitamin A, although a scarcity of reliable intake distributions makes it difficult to estimate the amount of vitamin A needed to be delivered via food vehicles. If dietary data are in hand, programs exist for calculating the amounts of vitamin A needed to be delivered via fortification that consider requirements and intakes across targeted groups in a population (O. Dary, personal communication, 2008). To date, concentrations of delivered fortificant have been based on ranges of food vehicle intake, efficacy in improving vitamin A status, the possibility that multiple fortified foods could be consumed, and concerns for safety [47, 48]. Most fortification projects and the few national programs that exist have sought to deliver 30% to 60% of an RDA via fortification to specific population groups [47].

Wheat flour as a vehicle for vitamin A fortification

Vitamin A is virtually absent in whole-grain cereals and flours. Nonetheless, flour from cereal grains can be fortified with a dry, powdered form of vitamin A.

Intakes and fortification levels

In considering whether to fortify a food supply with vitamin A, wheat flour should be considered a candidate. Wheat flour is technically fortifiable and is gradually being consumed more over time in low-income populations, although variation is high among countries and among populations (markets) within countries [49]. Thus, countries should first consider whether wheat flour is consumed on a regular basis, within a manageable range, and in amounts by members of different age and societal groups that would allow it to deliver nutritionally significant levels of vitamin A. Too little or irregular intake patterns by the target population can render a product ineffective. Nationally representative, individual wheat flour intake data are rarely available, revealing a fundamental inadequacy in dietary data for planning fortification of flour, and most other potential food vehicles, in low-income countries. Next most useful are per capita consumption estimates of food vehicles derived from national household income and food expenditure survey data that may be available from selected countries in major regions [49], to which intrahousehold weights can be applied to estimate average intakes within age groups. Least useful for estimating individual intake distributions, but most readily available, are country estimates of per capita wheat (or other food) availability based on food balance sheets maintained by the Food and Agriculture Organization (FAO) [50].

On the assumption that ranges of usual wheat flour intake are in hand, **table 3** shows the additional vitamin A intake expressed as micrograms of RAE that could be achieved for a referent adult male and each target group, assuming average fortificant intakes based on relative energy requirements (i.e., weights of 1.00, 0.37, 0.60, and 0.80 units for adult men, preschool children, school-aged children, and adult women, respectively) [49], across the range of wheat flour fortification levels. The estimates assume 30% losses due to transport, storage, and cooking (H. Cori, personal communication, 2009). **Table 3** also displays the corresponding incremental vitamin A intakes expressed as a percentage of the RDA [41] for each group. For example, fortifying wheat flour at 5.9 ppm will provide an additional 207 to 1,652 μg RAE/day, depending on whether average daily flour consumption is very low (< 75 g or ~ 50 g) or very high (> 300 g or ~ 400 g). These values represent 23% and 184%, respectively, of the RDA for adult men. Applying the fractional weights to estimate intakes for other groups, the corresponding ranges of additional intake are 76 to 611, 124 to 991, and 165 to 1,322 for preschoolers, school-aged children, and adult women, respectively, representing 22% to 175%, 21% to 165%, and 24% to 189% of the RDA for each group, respectively. Lower concentrations of vitamin A in wheat flour will deliver corresponding less vitamin A and lower percentages of the RDA, so that for low consumers of wheat (< 75 g/day), negligible amounts of the vitamin are delivered when the fortification level is at 1.5 ppm or lower. Thus, in settings where wheat intake is low

TABLE 3. Intake of vitamin A and %RDA provided at different levels of fortification of wheat flour at the mill and flour intake for adult men (per capita), preschoolers, school-aged children, and adult women[a]

Fortification level—ppm (µg RAE/ g)[c]	Target group	Flour intake (g/day)[b]								Fortificant cost (US$/MT)[f]
		< 75		75–149		150–300		> 300		
		RAE consumed (µg)[d]	%RDA[e]	RAE consumed (µg)	%RDA	RAE consumed (µg)	%RDA	RAE consumed (µg)	%RDA	
5.9 (4.13)	Adult man	**207**	**23**	413	46	826	92	1,652	184	
	Adult woman	**165**	**24**	330	47	661	94	1,322	189	8.62
	School-aged	**124**	**21**	248	41	496	83	991	165	
	Preschool	**76**	**22**	153	44	306	87	611	175	
3.0 (2.10)	Adult man	105	12	**210**	**23**	420	47	840	93	
	Adult woman	84	12	**168**	**24**	336	48	672	96	4.38
	School-aged	63	11	**126**	**21**	252	42	504	84	
	Preschool	39	11	**78**	**22**	155	44	311	89	
1.5 (1.05)	Adult man	53	6	105	12	**210**	**23**	420	47	
	Adult woman	42	6	84	12	**168**	**24**	336	48	2.19
	School-aged	32	5	63	11	**126**	**21**	252	42	
	Preschool	16	5	39	11	**78**	**22**	155	44	
0.75 (0.525)	Adult man	26	3	53	6	105	12	**210**	**23**	
	Adult woman	21	3	42	6	84	12	**168**	**24**	
	School-aged	16	3	32	5	63	11	**126**	**21**	1.10
	Preschool	10	3	19	6	39	11	**78**	**22**	

RAE, retinol activity equivalent; RDA, Recommended Dietary Allowance

a. Bolded numbers represent recommended fortification levels for population vulnerable groups consuming wheat flour within specified ranges, providing nearly 25% of the RDA, assuming cooking losses of approximately 30%.

b. Micrograms of RAE consumed and %RDA calculations based on daily flour intake levels of 50, 100, 200, and 400 g considered as adult equivalent intake midpoints within each of the following ranges of intake, respectively: < 75, 75–149, 150–300, and > 300 g.

c. Parts per million (ppm) = µg RAE/g. Figure in parentheses assumes 30% losses due to transport, storage, and cooking and is the value from which micrograms of RAE consumed is calculated. For example, 5.9 × 0.70 × 50 g = 207 µg RAE by the referent adult man in the first cell.

d. Based on adult equivalent units (AEU) for which the AEU equals 1.0, 0.8, 0.6, and 0.37 for adult men, women of reproductive age, school-aged children, and preschoolers, respectively [49].

e. RDA = 900 µg RAE for an adult man. A midrange RDA estimate of 350 µg RAE is used for preschoolers, and 600 and 700 µg RAE are used for school-aged children and adult women, respectively [41].

f. Cost based on an estimate of US$1.46/MT of flour fortified at a level of 1,000 ppm, equivalent to 1,000 µg RAE/kg, at the mill (i.e., before deducting 30% losses).

and the estimated dietary gap is high, wheat flour may not be the vehicle of choice to correct vitamin A deficiency. Alternatively, fortifying wheat flour at, for example, 1.5 ppm can deliver nutritionally effective amounts of vitamin A where flour consumption is moderate (150 to 300 g/day or ~ 200 g/day). The bolded figures in **table 3** show the recommended fortification levels, assuming 30% cooking and preparation losses, required to deliver approximate 25% of an RDA to target groups consuming wheat flour within the specified ranges. The costs of fortification will vary according to the level of the fortificant. The calculations illustrate the utility of incorporating population estimates of daily flour and vitamin A intake into planning a wheat flour fortification initiative with vitamin A.

Efficacy in improving vitamin A status

Pursuing wheat flour fortification with vitamin A for public health purposes presumes prophylactic efficacy, that is, the ability of a selected concentration of vitamin A in wheat flour to protect populations from deficiency at a given level of intake. To date, two efficacy trials have published findings on the efficacy of vitamin A–fortified wheat flour to raise vitamin status (**table 4**). In the Philippines, wheat flour used in making a popular bun called *pandesal* was fortified with vitamin A at a level of 4.5 mg/kg to produce a pandesal product with 2.8 mg vitamin A/kg [27]. Each school day for 6 months, children received a 60-g piece of fortified pandesal, intended to provide 133 μg RAE per bun, or a nonfortified product of identical size and appearance. Daily pandesal intake per child averaged 53.4 g ± 6.4 (SD) in the experimental group, providing 121 μg RAE, and 53.6 ± 6.1 g in the control group, providing no additional vitamin A. Among all children whose vitamin A status was below the median at the outset, those assigned to the fortified bread showed a 0.07 ± 0.03 μmol/L increment in serum retinol over controls after 6 months ($p = .02$). Apparent liver storage of vitamin A was assessed at the 6-month follow-up by a *modified-relative-dose-response* (MRDR) test in children who had exhibited the lowest vitamin A status at baseline (the lowest 20%) in each group. Nearly half of lowest-status children assigned to fortified pandesal had follow-up MRDR values above the ratio cutoff of 0.06 (15.3%), reflecting low liver stores, compared with controls (28.6%, $p = .05$).

In an unpublished second randomized, controlled trial in Bangladesh, vitamin A–fortified wheat flour (at a concentration of 3,000 μg RAE/kg) was made into chapattis providing approximately 212 μg RAE per two-piece serving [51] plus six other nutrients. Children were fed two chapattis daily for 6 months. The mean serum retinol concentration and percentage of children with values < 0.70 μmol/L at 6 months were higher (1.05 vs 0.94 μmol/L, $p < .05$) and lower (7.4% vs 22.5%, $p < .05$), respectively, in children assigned to fortified than in those assigned to unfortified chapattis. The findings from both trials suggest that vitamin A status can be improved by regular consumption of breads baked with vitamin A–fortified wheat flour; however, to date there have been no studies evaluating the effectiveness of this intervention as a routine program.

Safety considerations

Guidelines exist for gauging the likely safety limits at which foods can be fortified with vitamin A. Life-stage- and sex-specific ULs, published by the Institute of Medicine, are intended to serve as the highest levels of usual dietary intake of a nutrient from all sources that is likely to pose no risk of adverse health to almost all individuals in a healthy, North American population [37]. The UL is specifically not intended to guide vitamin A supplementation or fortification in undernourished populations [41] and should not deter food fortification interventions in countries or regions at risk for vitamin A deficiency [52]. ULs for vitamin A were established on the basis of minimizing the risks of teratogenicity in women of reproductive age, liver abnormalities in all other adults 19 years of age and older (extrapolated to children and adolescent boys), and hypervitaminosis A in infants [37]. The UL for vitamin A set by the Institute of Medicine for adults, including pregnant and lactating women over 18 years of age, is 3,000 μg RAE/day; it has also been set at 2,800 μg RAE/day for adolescents, including young pregnant and lactating women; 1,300 μg RAE/day for 9- to 13-year-olds; 900 μg RAE/day for 4- to 8-year-olds; and 600 μg RAE/day for infants and children under 4 years of age [41]. As another reference, the UK Expert Group on Vitamins and Minerals has set a lower Guidance Level—considered less firm than an Upper Limit—for vitamin A at 1,500 μg RAE/day (half of the UL for adults) in light of observational and animal research linking chronic preformed vitamin A intakes above this level with risk of reduced bone mineral density and fracture [53, 54]. Upper limits can best be avoided by aiming to deliver minimally effective fractions of either an EAR or an RDA to targeted population groups that will also minimize the collateral risks of excessive intake by high consumers of a food vehicle. Sometimes this can also be achieved, in part, by fortifying specific milled streams of wheat flour that self-target the poor or other groups with known narrower intake distributions. Since dietary sources of vitamin A in most regions of the developing world consist of provitamin A carotenoids, it is less likely that individuals in most target populations will exceed the UL on a regular basis.

Stability of vitamin A in premix and flour products

Stable forms of vitamin A palmitate have existed for

at least 30 years and have significantly increased the number and kind of foods that can be fortified with vitamin A, particularly cereal grain products [55]. Commercial forms of dry vitamin A as palmitate or acetate are available embedded in a water-soluble matrix (e.g., gelatin, gum acacia, starch) and stabilized with antioxidants. The most common form of vitamin A used to fortify cereal flours is dry stabilized powder-form vitamin A palmitate, generically referred to as Type 250-SD (75,000 μg RAE/g) [56]. This form of vitamin A added to wheat flour to form a premix can remain stable for approximately 15 days, even under hot, humid conditions [57]. Under routinely tested conditions of 30° C and 60% humidity, retention is repeatedly observed to be approximately 90% (H. Cori, unpublished data, 2009), although stability thereafter can vary considerably [57]. Retention studies done to date conclude that a primary factor in vitamin A loss can be premix moisture content (i.e., humidity), but that the different qualities of vitamin A compounds may also underlie wide variability in the vitamin A content of the premix.

Once premix is added at intended ratio concentrations to wheat flour, the stability of vitamin A continues to vary according to temperature, humidity, duration of storage, and other conditions of storage. In the Philippines, approximately 81% of original vitamin A content (500 μg RAE/100 g flour) was retained in fortified wheat flour after 1 month of storage under ambient conditions [58]. Other studies have shown retention rates of fortified, low-extraction wheat flour to exceed 95% for up to a year at temperatures of 40° C [59]. Cort et al. found vitamin A losses to be higher in flour stored at 45° C than in flour stored at room temperature, reaching approximately 30% if the flour was stored for 3 months at higher temperatures [60]. High moisture content, however, may markedly increase losses of stored wheat flour [61].

After baking, vitamin A retention

TABLE 4. Summary of efficacy trials on the impact of vitamin A–fortified wheat flour

Country	Design and study subjects	Sample size		Food vehicle	Nutrient level	Target daily vitamin A intake from fortified flour product	Results
		Fortified flour	Unfortified flour				
Philippines [27]	Individually randomized, controlled trial Schoolchildren 6–13 yr	396	439	Pandesal	6,000 μg RAE/kg	133 μg (33% RDA)	Mean (± SD) serum retinol (μmol/L) fortified vs nonfortified: Baseline: 1.17 ± 0.33 vs 1.18 ± 0.30 30 wk: 1.32 ± 0.37 vs 1.31 ± 0.40 % low liver stores based on MRDR[a] ≥ 0.06 among subsample of children with lowest baseline serum retinol concentration (n = 72 and n = 77 for vitamin A and no vitamin A) at 30 wk: 29 vs 15*
Bangladesh [51]	Cluster-randomized, controlled trial Schoolchildren 6–15 yr	191	143	Chapatti	3,000 μg RAE/kg plus other nutrients	212 μg (35–55% RDA)	Mean (± SD) serum retinol (μmol/L) fortified vs nonfortified: Baseline: 0.96 ± 0.26 vs 0.98 ± 0.29 3 mo: 1.07 vs 1.04 6 mo: 1.05 vs 0.94* % < 0.70 μmol/L: vitamin A vs no vitamin A Baseline: 13.6 vs 15.4 3 mo: 7.9 vs 16.2* 6 mo: 7.4 vs 22.5*

MRDR, modified relative dose response; RAE, retinol activity equivalent; RDA, Recommended Dietary Allowance

* Between-group differences are statistically significant at $p < .05$.

a. MRDR is an indirect assessment of liver stores that tests the relative responsiveness of serum retinol following receipt of a standard, small dose of vitamin A.

in fortified flour used in traditional Persian breads has been shown to be approximately 70%, alone or when mixed with other nutrients [62, 63]. Cort et al. found no losses of vitamin A when bread was baked or after 5 days of storage compared with the level declared on the product label [60]. Other studies in the past have observed losses of vitamin A of 10% to 20% during baking of bread and 13% and 17% after drying and cooking of long durum wheat pasta, respectively [64].

Vitamin A loss in wheat flour products may vary with inclusion of other nutrients. In the Philippines, vitamin A retention remained greater than 70% after a month of storage in a premix that included iron (45 mg/kg flour) [65]. Further vitamin A losses from premixes with different forms of added iron ranged from 3% to 46% in products such as baked loaves of bread, raw noodles (prepared from hard flour), and biscuits (prepared from soft flour). Losses were higher for loaf bread and noodles (40% and 46%, respectively) when the iron fortificant was ferrous fumarate versus either ferrous sulfate (21% and 28%) or reduced iron (3% and 21%). Vitamin A losses, however, were 20% to 30% in biscuits, irrespective of the type of iron fortificant. Rubin et al. investigated the stability of vitamin A in bread made from flour enriched by six vitamins and four minerals, among which added calcium and magnesium appeared to adversely affect retention during the baking process [66]. The paucity of data on the stability of vitamin A under a myriad of (often adverse) storage and baking conditions, in diverse products without and with many kinds of nutritive and non-nutritive additives, makes it difficult to generalize about retention of vitamin A in premix and finished wheat flour products, and how the available evidence can guide overages. The data available, however, suggest that premix vitamin A retention of about 80% to 90% can be expected within a month of its use, assuming reputable supplies and reasonable protection from high temperature and humidity, and that additional losses of up to 30% can be expected across a range of baked products and conditions.

Organoleptic qualities

Sensory tests have been conducted on flour and wheat flour-based products prepared with vitamin A. Solon et al. [65] found no detectable differences in color or odor of flour fortified with 490 μg RAE/kg until 3 months after fortification. No differences were found in the flavor of pandesal with fortified flour, stored for up to 3 months, and baked under laboratory testing conditions. Neither were there detectable differences in color, odor, flavor, or texture of the flour or the fortified food products when vitamin A–fortified flour had any of three different forms of iron (each added at 45 mg/kg) [65]. School-based programs delivering fortified cookies in Guatemala have added up to 10 mg/kg of vitamin A in flours without sensorial changes (O. Dary, personal communication, 2008). Thus, the data to date suggest that vitamin A fortification of wheat flour has little effect on the organoleptic qualities of the final product.

Country program experiences

Historically, vitamin A has not been added to cereal flours in most industrialized countries, because margarine and milk are the preferred food vehicles and today vitamin A deficiency is no longer a problem of public health significance in such societies. Since 1969, however, cereal flour–based food aid commodities, such as wheat–soy and corn–soy blends, have been fortified with 7 and 10 mg vitamin A/kg, respectively, providing an estimated 80% to 90% of the RDA for school-aged children consuming approximately 75 g/day [3]. Currently 10 low- and low-middle-income countries and two upper-middle-income countries are fortifying or proposing to fortify wheat flour with vitamin A (**table 5**). In the Philippines, wheat flour was selected as a preferred vehicle for fortification because there is relatively high penetration of wheat flour products, even among the poor, and there is no local wheat production, so that all wheat is imported and is milled centrally by 12 millers in the country [58].

TABLE 5. Countries with voluntary or mandatory vitamin A fortification of wheat flour

Country	Product	Mandated level—μg RAE/g (IU/g)
Nigeria	Wheat flour	9.0 (30)
South Africa	Wheat flour (white)	1.68 (5.36)
	Wheat flour (brown)	1.414 (4.712)
	Wheat bread (white)	0.8 (2.664)
	Wheat bread (brown)	0.700 (2.331)
Lesotho	Wheat flour	1.784 (5.947)
Indonesia	Noodles	
Palestine	Wheat flour	1.0 (3.333)
Philippines	Enriched wheat flour	3.0–6.5 (10.0–21.7)
Afghanistan[a]	Wheat Flour	7.078 (23.594)
Bangladesh[a]	Wheat flour	3.3 (11.0)
Venezuela	Wheat flour	2.85 (9.5)
Jordan	Wheat flour	1.5 (5.0)
Ghana	Wheat flour	2.0 (6.666)
Uganda[b]	Wheat flour	2.52 (8.4)

RAE, retinol activity equivalent
Source: Nutriview [88].
a. Managed by the World Food Programme.
b. Voluntary except for World Food Programme–purchased flour

The government passed a law mandating fortification of hard wheat flour with vitamin A in 2000 (Republic Act 8976, 2000), which started to be applied nationally in 2004. Wheat flour is now fortified with SD-250 at a level of 4.5 mg/kg to produce bread products with a vitamin A content of 2.2 μg RAE/g [47]. At an average bread intake of approximately 40 g/day by school-aged children, this level of fortification meets approximately 33% of the Recommended Nutrient Intake (RNI, comparable to the RDA) for this age group [67].

In 1999, Bangladesh initiated a trial program of vitamin A wheat flour fortification with assistance from the US Agency for International Development and the World Food Programme (WFP). Vulnerable group families were targeted with fortified *atta* in lieu of the usual monthly ration of 30 kg of whole-grain wheat [51]. Program activities included studies to assess organoleptic changes, efficacy, utilization, acceptability, and cost. The findings were favorable with respect to the taste, texture, and appearance of vitamin A–fortified flour; consumer acceptability and utilization relative to the previous whole-grain ration; efficacy in improving vitamin A status (**table 4**); and cost (~ US$5/MT or ~ 1.6% of the retail price of commercial white flour sold in plastic bags) [51]. Efforts are under way in Bangladesh to promote this intervention more broadly, although a national program does not yet exist, except through WFP commodity importation.

In South Africa, fortification of white and brown wheat flour and white and brown bread is mandated. Nigeria has mandated adding vitamin A to wheat flour, as have Jordan and the Palestinian territories. Vitamin A is currently added to the wheat flour provided through the WFP in Afghanistan. Finally, Egypt is considering adding vitamin A to the iron and folic acid premix that is currently being used to fortify its subsidized *baladi* bread flour (82% extraction) [24].

Cost considerations

Cost and commercial viability, rather than public health benefit, can often determine whether vitamin A fortification is a feasible and sustainable option for producers and potential beneficiaries. The benefits of vitamin A fortification need to be convincingly sold to private producers, who face research and development costs as well as potential marketing losses in modifying existing food products. Similarly, the benefits must be marketed to the public to promote the use of fortified products.

Dary and Mora [47] compared the cost of fortifying different foods with vitamin A, considering food consumption patterns and losses of vitamin A during storage, transport, and cooking (**table 6**). A comparison of the costs of vitamin A fortification of oil, cereal flours (including wheat flour), sugar, and MSG shows that vitamin A programs for each of these four food vehicles have the potential to be cost-effective, with annual per person costs ranging from US$0.008 for edible oils to US$0.121 for sugar [47]. On a per person basis, and under a set of comparable consumption and stability assumptions, Dary estimates that vitamin A fortification of wheat flour costs approximately 11 times more than oil fortification (personal communication, 2008). Another important cost consideration is the relative price increase of the fortified food vehicle compared with its unfortified version, because this price will determine the feasibility of production, trade, enforcement, and affordability among the lower-income groups who are often the main targets for food fortification. More comparative cost data are required to establish a reliable database across diverse food production and marketing systems that can adequately inform decisions on candidate food vehicles for vitamin A fortification.

TABLE 6. Comparative cost of vitamin A fortification to supply 180 μg RAE (30% of RDI) with different food vehicles

Food vehicle	Typical consumption (g/day)	Level at households[a] (mg/kg)	Level at stores[b] (mg/kg)	Overage for production[c] (%)	Cost (US$/MT)	% of purchasing price	Annual cost/person (US$)
Oil or margarine	15	12	15	20	1.87	0.37	0.008
Cereal flours	200	1	1.25	40	1.25	0.26	0.091
Sugar	50	3.5	4.5	100	6.65	1.39	0.121
MSG[d]	0.25	720	900	100	1266	25.32[d]	0.116

MSG, monosodium glutamate; RAE, retinol activity equivalent; RDI, Recommended Daily Intake
Source: Dary and Mora [47].
a. Level = dietary goal (μg RAE/consumption pattern [g/day]).
b. Assuming 25% additional amount to compensate for any losses.
c. Theoretical estimate based on reported stability information and length of product marketing life.
d. The cost of MSG is assumed to be US$5/kg

Recommendations for fortifying wheat flour with vitamin A

Fortification of foods with vitamin A is a potentially effective intervention to prevent or control vitamin A deficiency in low-income countries where undernutrition and poverty coexist. The following recommendations are offered to guide fortification of wheat flour or other potential food vehicles with vitamin A:

» Vitamin A fortification should be motivated and guided by evidence of deficiency as a public health problem. This evidence should be derived from population-based findings of deficient vitamin A status and dietary inadequacy of the vitamin or its food sources.
» Vitamin A deficiency is a public health concern in preschool-aged children, women of reproductive age, and school-aged children and young adolescents.
» Fortification of food with vitamin A should be designed to correct estimated dietary inadequacy in one or more vulnerable groups, that is, to fill a dietary gap.
» Wheat flour is a suitable candidate for vitamin A fortification. Its selection as a vehicle of choice should be guided by estimates of intakes of vitamin A and wheat flour by intended beneficiaries, levels of fortificant required to meet dietary corrective and safety goals, stability under ambient conditions, stability under usual conditions of product preparation (e.g., high temperature and humidity during cooking or baking) and product storage conditions, and comparative costs.
» The form of vitamin A and premix to be used in fortification should be the highest grade, appropriate for the intended food vehicle, stable under ambient conditions and for the duration of expected use, and introduced into the food supply in accordance with industry standards.
» In general, provision of 15% to 50% of the RDA can be expected to meet both nutritional and safety goals. **Table 3** displays the recommended fortificant levels to meet roughly 25% of the RDA for adult women, preschoolers, and school-aged children (using adult men as the referent weight), at mid-range wheat flour intake levels of 50, 100, 200, and 400 g/day.

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Zinc fortification of cereal flours: Current recommendations and research needs

Kenneth H. Brown, K. Michael Hambidge, Peter Ranum, and the Zinc Fortification Working Group*

Abstract

***Background**: Zinc fortification is recommended as an appropriate strategy to enhance population zinc status, but guidelines are needed on the appropriate types and levels of zinc fortification of cereal flours for mass fortification programs.*

***Objective**: To review available information on the scientific rationale, efficacy, and effectiveness of zinc fortification programs, and to develop guidelines on appropriate levels of fortification of cereal flours, based on simulations of the amount of zinc absorbed under different dietary conditions and information on possible adverse effects.*

***Methods**: Systematic review of scientific literature and application of an existing prediction equation to estimate zinc absorption.*

***Results**: Previously completed research demonstrates that zinc intake and absorption are increased when zinc-fortified foods are consumed, but little information is, as yet, available on the biologic impact of large-scale fortification programs. Studies suggest that there are no disadvantages of the recommended ranges of zinc fortification with regard to the sensory properties of zinc-fortified foods, and most research indicates that there are no adverse effects of zinc fortification on the utilization of other minerals.*

***Conclusions**: Zinc fortification of cereal flour is a safe and appropriate strategy for enhancing the zinc status of population subgroups who consume adequate amounts of fortified cereal flour, although additional information is needed to confirm the efficacy and effectiveness of large-scale zinc fortification programs to control zinc deficiency. The appropriate level of fortification depends on the population subgroup, their usual amount of flour intake, the degree of milling and fermentation that is practiced, and the usual intakes of zinc and phytate from other food sources. Fortification recommendations are presented for different dietary scenarios.*

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Introduction

Public health benefits of zinc fortification

Adequate zinc nutrition is necessary for optimal child health and physical growth and for normal pregnancy outcomes [1]. Community-based intervention trials among young children in multiple settings have found that zinc supplementation decreases their rates of diarrhea and acute lower respiratory infections [2, 3]. Several sets of investigators have also reported that children who received supplemental zinc have significantly reduced mortality rates [4–8], and the authors of the recently published *Lancet* series on maternal and childhood undernutrition estimated that zinc deficiency is responsible for approximately 4% of deaths among children under 5 years of age in lower-income countries [9]. This places zinc intervention programs among the key strategies for ensuring greater child survival through improved nutrition. In addition to the effects of zinc on morbidity and mortality from common childhood infections, a considerable number of studies indicate that preventive zinc supplementation increases the linear growth and weight gain of stunted or underweight children [8, 10]. Recent trials in Peru, Nepal, and Bangladesh also found that maternal zinc supplementation during pregnancy increased children's postnatal growth [11, 12] and/or resistance

to infection [13, 14].

Despite these remarkable effects of zinc supplementation, public health planners have been slow to embrace zinc-related interventions, possibly because there is very little information available on the global prevalence of zinc deficiency. However, the International Zinc Nutrition Consultative Group (IZiNCG) estimates that approximately one-third of the global population lives in countries with an elevated risk of zinc deficiency [15]. The public health response is also restrained by the scarcity of well-documented programmatic experiences regarding the effectiveness of particular public health platforms for delivering additional zinc. With regard to zinc fortification programs, in particular, very few rigorous evaluations of ongoing programs have been reported.

Current status of zinc fortification programs

Zinc fortification of cereal food staples (wheat flour, maize flour, or rice) is not yet widely practiced, but many new flour fortification programs are beginning to include zinc (**table 1**). Currently, four countries—Indonesia, Mexico, Jordan, and South Africa—require zinc fortification of wheat flour. Thirteen countries include zinc in voluntary wheat flour fortification programs, and five countries have recently proposed new programs that would include zinc. The presently recommended levels of zinc fortification of wheat flour for national fortification programs range from 14 to 33 mg zinc/kg flour (14 to 33 ppm). Four countries (Mexico, South Africa, Uganda, and Zambia) also include zinc in currently operating or recently proposed maize fortification programs, with recommended zinc fortification levels ranging from 10 to 25 ppm.

All national mass fortification programs use zinc oxide as the chemical form of added zinc, presumably because of its low cost, demonstrated bioavailability, and lack of undesirable organoleptic effects. The cost of adding 30 ppm zinc as zinc oxide is approximately US$0.25/MT fortified flour.

TABLE 1. Current levels of zinc fortification recommended in national flour fortification programs

Country	Type of flour	Type of program	Fortification level (mg zinc/kg flour)
Azerbaijan	Wheat	Voluntary	18
Bangladesh	Wheat	Voluntary	33
China	Wheat	Voluntary	25
Fiji	Wheat	Voluntary	30
Ghana	Wheat	Voluntary	20
Guinea	Wheat	Voluntary	14
Indonesia	Wheat	Mandatory	30
Jordan	Wheat	Mandatory	20
Kazakhstan	Wheat	Voluntary	18
Kenya	Wheat	Voluntary	30
Kyrgyzstan	Wheat	Voluntary	18
Lesotho	Wheat	Voluntary	15
Mexico	Wheat and maize	Mandatory	16
Mongolia	Wheat	Voluntary	18
Palestine	Wheat	Voluntary	15
South Africa	Wheat and maize	Mandatory	15
Tajikistan	Wheat	Voluntary	18
Tanzania	Wheat	Voluntary	30
Uganda	Wheat and maize	Voluntary	30
Uzbekistan	Wheat	Voluntary	18
Vietnam	Wheat	Voluntary	30
Zambia	Wheat and maize	Voluntary	15

Technical aspects of zinc fortification

Factors affecting zinc absorption from zinc-fortified foods

Although a number of dietary factors have been purported to influence the bioavailability of ingested zinc [16], systematic reviews of tracer studies conducted in human subjects have confirmed that just two of these dietary components exert a significant detectable impact on zinc absorption: the amount of zinc consumed in the current meal [1, 17], a recent meal [18–20], or as a recent dose of supplemental zinc [19]; and the quantity of myo-inositol phosphate (phytate) that is consumed with the meal [1, 21]. Another factor that is of practical importance is the age of the consumer, because term infants and children up to at least 4 years of age have approximately one-third of the maximal zinc absorptive capacity per day of that of adults [17, 22]. However, at the levels of intake necessary to meet physiologic requirements, the efficiency of absorption does not differ by such a wide margin [17].

Using the combined results of multiple studies of zinc absorption among adults, it is possible to develop equations to predict the amount of zinc that would be absorbed following consumption of particular amounts of dietary zinc and phytate. Two such equations have been published recently by IZiNCG [1] and by Miller et al. [21], both of which provide similar predictions of absorbed zinc. Examples of estimated zinc absorption under different dietary conditions are presented below, using updated parameter estimates for the Miller equation [21, 23, 24]. This model was selected for the

following simulations because it is based on current knowledge of zinc absorption physiology and was carefully evaluated with regard to model validity, goodness of fit, and a variety of statistical considerations [21]. The model predicts zinc absorption as follows:

$$TAZ = 0.5 * (A_{max} + TDZ + K_r(1 + TDP / K_p)) - \sqrt{(A_{max} + TDZ + K_r(1 + TDP / K_p))^2 - 4 * A_{max} * TDZ},$$

where

- TAZ = total absorbed zinc (mmol/day),
- A_{max} = the maximum possible amount of absorbed zinc = 0.11 mmol/day,
- TDZ = total dietary zinc (mmol/day),
- TDP = total dietary phytate (mmol/day),
- K_r = the equilibrium dissociation constant for zinc-receptor binding = 0.065, and
- K_p = the equilibrium dissociation constant for zinc-phytate binding = 0.77.

This model explains 86% of the variability in mean total daily absorbed zinc, as measured in 15 studies containing 32 sets of absorption data derived from 209 adult subjects.

As indicated above, the prediction equation demonstrates that the quantity of zinc absorbed each day continues to increase when greater amounts of zinc are ingested, and, as would be anticipated from our knowledge of zinc physiology, the amount of absorbed zinc is best described by saturation-response modeling [25]. In other words, the efficiency of zinc absorption (i.e., the fraction of any given amount of ingested zinc that is absorbed) decreases progressively with greater total daily zinc intake. Fortunately, the estimated physiologic requirements are equivalent to approximately half of the maximal total absorption capacity, both in adults and in young children [17], and the efficiency of zinc absorption (~ 30% to 40% of intake in the absence of dietary phytate) is relatively favorable when zinc intakes are less than this amount and phytate intake is negligible. It is apparent from these considerations that the effect of zinc fortification of wheat or maize flour on the quantity of zinc that is absorbed depends both on the usual dietary zinc intake and the level of fortification, as well as the amount of phytate in the diet.

Desirable levels of zinc fortification of cereal flours for mass fortification programs

Establishing the desirable level of zinc fortification for a mass fortification program requires consideration of the current shortfall in zinc intakes among the population subgroups at greatest risk for deficiency, namely, young children and pregnant and lactating women; the safe upper level of zinc intake among the population subgroup at greatest risk for excessive zinc intake, namely, adult men; potential adverse effects of high levels of fortification on organoleptic characteristics of the fortified foods; and the cost of the intervention in relation to the expected benefits. The first three issues will be reviewed in this section, and the cost of added zinc oxide was indicated briefly above. There is at present insufficient information to permit rigorous cost–effectiveness assessments.

For each of the following simulations of the total amount of zinc that would be absorbed following zinc fortification of wheat flour, we estimated the amounts of zinc and phytate consumed from the usual diet, excluding any zinc present in wheat flour; the amounts of zinc and phytate consumed from different hypothetical quantities of unfortified wheat flour, according to the assumed rates of extraction (either 80% or 95% extraction rates); and the amount of additional zinc that might be consumed from the flour following different levels of zinc fortification. We then estimated the total amounts of absorbed zinc (TAZ), using the Miller equation, under these different sets of dietary conditions, and we compared these estimated amounts of absorbed zinc with the age- and sex-specific physiologic requirements for absorbed zinc. When possible, we also compared the estimated TAZ with the level of zinc absorption that would be expected to occur following consumption of the theoretical safe upper level (UL) of zinc intake. It should be noted that in many cases, these calculations depended on extrapolations beyond the range of the experimental data upon which the prediction equation was derived, especially for the calculations involving ULs, so caution must be used in interpreting these results. Although the current examples refer to zinc fortification of wheat flour, the same approach can be used to simulate the effects of zinc fortification of maize flour, because zinc and phytate intakes appear to be the only two dietary factors that significantly affect TAZ.

Simulations of zinc absorption by adult men and women

To estimate the total amount of zinc that might be absorbed under different scenarios of zinc fortification of wheat flour, as described above, we first considered a hypothetical situation in which adults consume 5 mg zinc/day from dietary sources other than wheat flour and consume different assumed amounts of wheat flour, with either 80% or 95% rates of extraction during milling. The reason for using the stated extraction rates was the availability of information on both zinc and phytate contents for flour with these degrees of milling. Although some countries may use slightly lower extraction rates than 80%, this would not be likely to cause any further effect on the final zinc and phytate contents of the flour compared with 80% extraction flour, because the endosperm constitutes about 75% of the wheat kernel, so with 80% extraction almost all of

the flour content is derived just from the endosperm, as would also be the case with 70% or 75% extraction. Although it is not practical to provide recommendations for all possible levels of extraction in the space available in this article, there is little change in flour composition when 80% extraction flour and lower-extraction flours are compared, so the general recommendations for 80% extraction flour also should be reasonable for lower-extraction flours as well.

We assumed that 100 g of 80% extraction wheat flour contains 1.4 mg zinc and 350 mg phytate, and that 100 g of 95% extraction wheat flour contains 2.4 mg zinc and 900 mg phytate, as was found in recent studies in Mexico [26]. We then estimated the TAZ that would result from different daily amounts of flour consumption and different levels of zinc fortification, for each of the respective degrees of flour extraction. As an example, in **table 2** we present the results for predicted TAZ by adult men or women who consume 50 to 500 g wheat flour per day, fortified at a level of 50 mg zinc/kg flour.

As shown in **table 2**, these simulations indicate that with 5 mg zinc intake and no phytate intake from other dietary sources, consumption of 200 g of 95% extraction wheat flour per day fortified with 50 mg zinc/kg flour would be adequate to provide the current estimate of mean physiologic requirements for women (i.e., 3.3 mg absorbed zinc/day [27]). At the same level of fortification, women could meet their mean physiologic requirements with less than 75 g/day of 80% extraction flour. By contrast, men would fall short of their mean physiologic requirements (i.e., 3.84 mg absorbed zinc/day), even if they consumed 500 g of 95% extraction wheat flour fortified with 50 mg zinc/kg flour. However, 200 g of 80% extraction flour fortified at this level would be adequate.

By using a series of such simulations and the same underlying assumptions about the preexisting diet, we estimated the minimum level of zinc fortification of wheat flour that would be necessary to permit men and women to meet their estimated mean physiologic needs for absorbed zinc. **Table 3** shows the estimated range of zinc fortification levels that are needed to ensure adequate zinc absorption under these different conditions. For 80% extraction wheat flour, the level of zinc fortification that would be needed to ensure adequate zinc intakes ranges from approximately 13 to 94 mg zinc/kg flour, depending on the level of flour consumption and whether the program is targeting men or women. For 95% extraction flour, the respective levels of fortification range from approximately 42 to 136 mg zinc/kg flour. The foregoing estimates

TABLE 2. Predicted amount of total absorbed zinc (TAZ), according to amount of wheat flour intake and rate of wheat extraction during milling, assuming wheat flour is fortified with 50 mg zinc/kg flour[a]

Level of wheat extract (%)	Flour intake (g/day)	Zinc intake from diet and unfortified flour (mg/day)	Total zinc intake (mg/day)	Phytate intake (mg/day)	Predicted TAZ (mg/day)[b]
80	50	5.7	8.2	175	3.0
	75	6.0	9.8	262	3.5
	100	6.4	11.4	350	3.7
	200	7.8	17.8	700	4.1
	300	9.2	24.2	1,050	4.4
	400	10.6	30.6	1,400	4.5
	500	12.0	37.0	1,750	4.6
95	50	6.2	8.7	450	3.0
	75	6.8	10.6	675	3.1
	100	7.4	12.4	900	3.2
	200	9.8	19.8	1,800	3.3
	300	12.2	27.2	2,700	3.4
	400	14.6	34.6	3,600	3.4
	500	17.0	42.0	4,500	3.5

a. Assumes 5 mg zinc intake from all dietary sources other than wheat flour and all dietary phytate provided only by wheat flour. The estimates assume no fermentation of wheat flour, and the results could vary depending on the degree of fermentation (and hence phytate content) of flour.

b. TAZ is predicted from the model of Miller et al. [21], which is based on data from adults. The estimated average requirement for TAZis 3.8 mg/day for adult men and 3.3 mg/day for nonpregnant, nonlactating adult women [27].

TABLE 3. Estimated minimum levels of zinc fortification of wheat flour that are needed to ensure adequate zinc absorption by adult men and women, according to level of flour intake and rate of wheat extraction during milling[a, b]

Flour intake (g/day)	Minimum level of zinc fortification (mg zinc/kg flour)			
	80% extraction wheat flour		95% extraction wheat flour	
	Men	Women	Men	Women
50	94	48	136	78
75	69	37	112	68
100	57	30	99	59
200	38	21	81	50
300	32	18	75	46
400	29	16	72	45
500	27	15	70	44
600	26	14	68	43
700	25	14	67	43
800	24	13	67	42

a. The analyses assume that there is 5 mg zinc intake from all dietary sources other than wheat flour and that all dietary phytate is provided only by wheat flour. The assumed zinc and phytate contents of the flour are 1.4 mg zinc/100 g and 350 mg phytate/100 g for 80% extraction wheat flour and 2.4 mg zinc/100 g and 900 mg phytate/100 g for 95% extraction wheat flour. The mean requirement for TAZ is 3.84 mg zinc/day for men and 3.30 mg zinc/day for women [27]. The estimates assume that the wheat flour is not fermented. Fermentation could reduce the phytate content of the flour and thereby reduce the necessary level of zinc fortification.

b. TAZ is predicted from the revised model of Miller et al. [21], which is based on data from adults.

are further simplified in **table 4**, which displays the recommended levels of zinc fortification for different ranges of flour intake, using the same assumptions (and caveats) about the usual diet. In this summary table, we have capped the recommended maximum level of zinc fortification at 100 mg zinc/kg wheat flour (100 ppm) until more information is available, because some studies suggest that greater levels of fortification may adversely affect the sensory properties of food items prepared with such flour, as discussed below in greater detail. It is also important to note that the foregoing calculations do not adjust for any possible fermentation of flour, which would be expected to lower its phytate content and thereby reduce the level of zinc fortification that would be required to meet the theoretical requirements for absorbed zinc.

TABLE 4. Summary of recommended levels of zinc fortification of wheat flour (mg zinc/kg flour) that are needed to ensure adequate zinc absorption by adult men and women, by level of flour intake and rate of wheat extraction during milling[a]

Per capita wheat flour intake (g/day)	Low-extraction wheat flour (~ 80% extraction)	High-extraction wheat flour (~ 95% extraction)
< 75	95 mg zinc/kg flour	100 mg zinc/kg flour
75–149	55 mg zinc/kg flour	100 mg zinc/kg flour
150–300	40 mg zinc/kg flour	80 mg zinc/kg flour
> 300	30 mg zinc/kg flour	70 mg zinc/kg flour

a. The estimates are based on the assumptions that there is 5 mg zinc intake from all dietary sources other than wheat flour, all dietary phytate is provided only by wheat flour, and the wheat flour is not fermented. Fermentation could reduce the phytate content of the flour and thereby reduce the necessary level of zinc fortification. The recommended levels of zinc fortification are capped at 100 ppm to avoid possible adverse effects on sensory properties of finished food products.

As explained in the preceding paragraphs, the amounts of zinc and phytate present in the diet from sources other than wheat flour also affect the desirable level of zinc fortification. **Tables 5A** and **5B** show how differences in dietary intakes of zinc and phytate from other sources would affect the recommended level of zinc fortification of 80% and 95% extraction wheat flour, respectively. The tables are based on the amounts of zinc fortification that would be necessary under the different dietary conditions to permit absorption of 3.84 mg zinc per day, which is the amount of absorbed zinc required by an adult man. The tables can be used to determine the desirable level of zinc fortification for a particular population, by locating the appropriate cell in the table for the population of interest, according to the population's usual level of wheat flour intake, the degree of milling that is commonly practiced, and the amounts of zinc and phytate that are provided by all other dietary sources. As shown in this table, there are

TABLE 5A. Amount of zinc fortification of wheat flour (mg zinc/kg flour) that is necessary to ensure 3.84 mg absorbed zinc/day, considering different amounts of usual zinc and phytate intakes from sources other than wheat flour and the stated amounts of flour consumption (80% extraction flour)[a]

Flour intake (g/day)	Dietary zinc from sources other than wheat = 3 mg/day			Dietary zinc from sources other than wheat = 5 mg/day			Dietary zinc from sources other than wheat = 7 mg/day		
	Dietary phytate (mg/day)			Dietary phytate (mg/day)			Dietary phytate (mg/day)		
	0	500	100	0	500	1,000	0	500	1,000
50	134	229	325	94	189	285	54	149	245
75	96	160	223	69	133	197	42	106	170
100	77	124	172	57	104	152	37	84	132
200	48	72	96	38	62	86	28	52	76
300	38	54	70	32	48	64	25	41	57
400	34	46	58	29	41	53	24	36	48
500	31	40	50	27	36	46	23	32	42
600	29	37	45	26	34	42	22	30	38
700	28	34	41	25	32	38	22	29	36
800	27	33	39	24	30	36	22	28	34

a. Shaded values indicate fortification levels > 100 mg zinc/kg flour, which need to be examined for sensory acceptability. The estimates assume that the wheat flour is not fermented. Fermentation could reduce the phytate content of the flour and thereby reduce the necessary level of zinc fortification.

TABLE 5B. Amount of zinc fortification of wheat flour (mg zinc/kg flour) that is necessary to ensure 3.84 mg absorbed zinc/day, considering different amounts of usual zinc and phytate intakes from sources other than wheat flour and the stated amounts of flour consumption (95% extraction flour)[a]

Flour intake (g/day)	Dietary zinc from sources other than wheat = 3 mg/day			Dietary zinc from sources other than wheat = 5 mg/day			Dietary zinc from sources other than wheat = 7 mg/day		
	Dietary phytate (mg/day)			Dietary phytate (mg/day)			Dietary phytate (mg/day)		
	0	500	100	0	500	1,000	0	500	1,000
50	176	272	368	136	232	328	96	192	288
75	138	202	266	112	176	239	85	149	213
100	119	167	215	99	147	195	79	127	175
200	91	115	139	81	105	129	71	95	119
300	81	97	113	74	90	106	68	84	100
400	76	88	100	72	83	95	66	78	90
500	74	83	93	70	79	89	66	75	85
600	72	80	88	68	76	84	65	73	81
700	70	77	84	68	74	81	65	72	78
800	69	75	81	67	73	79	64	70	76

a. Shaded values indicate fortification levels > 100 mg zinc/kg flour, which need to be examined for sensory acceptability. The estimates assume that the wheat flour is not fermented. Fermentation could reduce the phytate content of the flour and thereby reduce the necessary level of zinc fortification

sizeable differences in the recommended level of zinc fortification, depending on the amount of zinc and phytate consumed from other sources, thus emphasizing the importance of collecting population dietary data to be able to adjust fortification levels optimally. Likewise, as stated above, it is important to consider the possible effects of fermentation of flour on its phytate content when completing these simulations.

Because it is possible that zinc fortification levels greater than 100 mg zinc/kg wheat flour may have adverse effects on its sensory properties, as discussed in more detail below, cells in **tables 5A** and **5B** have been shaded when the estimated levels of fortification exceed this amount. In these cases, fortification should not exceed 100 mg zinc/kg flour without first conducting sensory trials to ascertain acceptability. In general, such high levels of fortification are estimated as being necessary only when the usual levels of flour consumption are fairly low, in which situations it might be more appropriate to seek other vehicles for fortification in

addition to wheat flour, so the level of zinc fortification of wheat could be reduced accordingly.

Simulations of zinc absorption by children

Similar attempts to estimate the effects of different levels of zinc fortification on TAZ among young children are complicated by the fact that specific absorption prediction equations are not yet available for children. The adult model [21] provides a good fit for data from children aged 2 years consuming a low-phytate diet [22] if adjusted for the difference in their small intestinal length compared with adults [17], but there are no data available to model for the effect of higher amounts of phytate in younger children, and the "gut length adjustment" is very crude. Thus, it is still not known whether it is appropriate to apply the same prediction equation derived from absorption studies conducted among adults to estimate zinc absorption by children. For this reason, we are not able to present estimates of the effects of zinc fortification of cereal flours on TAZ among young children.

Safe upper level of zinc intake

The maximal acceptable limit for zinc fortification is determined by the safe upper level (UL) of zinc intake, that is, the highest level of zinc intake at which no adverse effects occur among the vast majority (> 97%) of the population. The Food and Nutrition Board of the Institute of Medicine estimated the UL for zinc to be 40 mg zinc/day for adults [27], but this estimate did not make any adjustment for the phytate content of the diet. Because any adverse effects of zinc are presumably related to bioavailable zinc and not to zinc that is chelated with phytate (and which therefore remains bound within the intestinal lumen), this UL estimated by the Institute of Medicine presumably can be adjusted upward, in relation to the phytate content of the diet [24].

With the use of Miller's revised model to predict total zinc absorption from the diet in relation to zinc and phytate intakes [21, 28], approximately 6.4 mg zinc/day is estimated to be absorbed by adults who have a dietary intake of 40 mg zinc/day (the UL for adults) and no phytate in the diet. Thus, 6.4 mg of absorbed zinc can be considered to be the highest level of absorbed zinc that is consistent with no adverse effects of zinc intake. This so-called absorption UL (or TAZ UL) can then be used to assess whether a particular level of flour intake and level of fortification would result in a greater amount of absorbed zinc than the TAZ UL.

On the basis of the information provided above and in **table 3**, the levels of zinc fortification that would ensure adequate TAZ for both men and women if average wheat flour intakes were just 100 g/day are 57 mg zinc/kg for 80% extraction flour and 99 mg zinc/kg for 95% extraction flour. Assuming that 800 g flour/day (providing ~ 2,900 kcal/day from flour) is the greatest amount that is likely to be consumed by an adult man on a regular basis, and assuming a total intake of 5 mg zinc/day and no additional phytate intake from other dietary sources, the estimated TAZ would be approximately 4.5 mg zinc/day with 800 g consumption of 80% extraction wheat flour and approximately 4 mg zinc/day with 800 g/day consumption of 95% extraction wheat flour, with both types of flour fortified at the respective levels. In both cases, this is considerably less than the TAZ UL of 6.4 mg zinc/day, so even with these improbably high levels of usual flour intake, the upper level for absorbed zinc would not be exceeded. Even if wheat were fortified at the level proposed for populations that consume just 50 g of wheat flour per day, the TAZ would be less than the TAZ UL with 80% extraction flour, and the TAZ would just approximate the TAZ UL with 95% extraction flour. Thus, there appears to be a very broad range of safety for zinc fortification, and the major factors that will determine the acceptable upper range of zinc fortification are sensory factors and cost considerations.

Recommended form of fortificant

Several zinc compounds are generally regarded as safe (GRAS) for human consumption, and these compounds are therefore available for use in food fortification. Zinc oxide is the cheapest chemical form of GRAS zinc compounds, although concerns have been raised about its bioavailability because it is insoluble at neutral pH. However, three separate tracer studies found no difference in zinc absorption when zinc oxide was compared with zinc sulfate, a more soluble form of zinc, regardless of the level of phytate that was present in the test meals [18, 29, 30]. Likewise, there was no difference between zinc absorption from meals fortified with zinc oxide and from meals fortified with zinc methionine, even though the methionine-containing fortificant also contained other putative enhancers of zinc absorption [31]. Thus, it seems appropriate to use zinc oxide preferentially in mass fortification programs because of the cost considerations.

There is current interest in the possible role of sodium ethylenediaminetetraacetate (NaEDTA) as an enhancer of zinc absorption, primarily because NaEDTA is known to enhance iron absorption [32]. Moreover, the equilibrium dissociation constant for ZnEDTA is lower than that for zinc-phytate and higher than that for binding of zinc to intestinal mucosal receptors (Boyd O'Dell, personal communication), findings that are consistent with a possible beneficial effect of EDTA in enhancing zinc absorption from phytate-containing meals. However, the results of currently available studies of ZnEDTA are inconsistent, possibly because of differences in the NaEDTA:Zn

molar ratios in the products that were examined [30, 31, 33]. Thus, additional research is needed, both to clarify this issue and to define its practical importance relative to the additional cost of the product.

Stability of zinc in fortified foods

Zinc losses during storage and distribution of fortified flour are expected to be minimal. The physical form of zinc oxide that is used for fortification is a fine-particle, "light grade" compound that mixes well with flour, without clumping. On the basis of the authors' personal experience, there do not seem to be any problems with settling out, separation, or nonhomogeneity of zinc oxide in flour, although this has not been studied systematically under different industrial conditions.

Effect of proposed levels of zinc fortification on absorption of other minerals

Possible adverse effects of zinc fortification on the absorption of other minerals have been examined through short-term tracer studies of iron absorption and longer-term studies of the impact of zinc fortification on biochemical indicators of iron and/or copper status. Four studies are available in which iron absorption from six zinc-fortified products was compared with iron absorption from similar, non-zinc-fortified products [29, 31, 33] (D. López de Romaña, unpublished). Except for one study in which iron absorption was reduced by approximately one-fourth when wheat dumplings were fortified with zinc sulfate [29], none of the other comparisons, including two other studies of zinc sulfate, found any adverse effects of zinc fortification on iron absorption.

Six studies are available in which the impact of zinc fortification on indicators of iron status was measured, including four studies of zinc-fortified cereal products [33–36] and two studies of zinc-fortified milks [37, 38]. No adverse effects of zinc fortification on iron status indicators were identified in any of these studies. In addition, three studies of zinc-fortified cereal products [34, 36, 39] and four studies of zinc-fortified milks [37, 40–42] provided information on final serum copper concentrations, and none of these studies found a significant impact on serum copper concentration. In summary, the current weight of evidence suggests that there are no clinically important adverse effects of zinc fortification on iron or copper status. Information is still lacking with regard to any possible effects of zinc fortification on other minerals.

Effect of proposed levels of zinc fortification on organoleptic characteristics of final products

Several studies of zinc fortification of wheat flour used for baking bread found that zinc fortification levels as high as 100 mg zinc/kg flour, provided either as zinc oxide or zinc sulfate, were undetectable by consumers [43, 44]. Although it was possible to detect a slight difference in the flavor and texture of pasta prepared from flour fortified with 60 mg zinc/kg as zinc oxide, compared with pasta prepared from non-zinc-fortified flour, the zinc-fortified products were still very acceptable, and the differences were completely masked when the pasta was served with tomato sauce. Another study compared intakes of children who received 30-g daily portions of porridges that contained either 150 mg zinc, as zinc sulfate, per kilogram of porridge (dry weight) or no zinc fortification for a period of 6 months [36]. The children in both groups consumed the respective porridges on more than 80% of days, although the children who received the zinc-fortified porridge consumed approximately 10% less of the porridge.

These combined sets of results suggest that low-extraction wheat flour can be fortified with at least 100 mg zinc/kg flour without producing important adverse effects on the sensory properties or acceptability of products prepared from zinc-fortified flour. Information is still lacking on the effects of zinc fortification on the sensory properties of products made with high-extraction flour. Therefore, sensory evaluations should be completed before scaling up mass fortification programs based on high-extraction wheat flour.

Evidence regarding the efficacy and/or effectiveness of zinc fortification

The nutritional effect of zinc fortification can be assessed in several ways, namely, by measuring its impact on dietary zinc intake, TAZ, and biochemical and functional indicators of zinc status, among either individuals or populations who are exposed to zinc-fortified foods. The results of small-scale efficacy trials clearly show that zinc fortification can increase total daily zinc consumption and the amount of absorbed zinc in both adults and young children [45]. Despite the fact that fractional zinc absorption (i.e., the percentage of dietary zinc that is initially absorbed by the intestinal tract) declines when more zinc is consumed, studies indicate that the TAZ increases in relation to total zinc consumption, and fortification has a positive overall impact on TAZ. Although several studies indicate that the phytate present in cereal flours inhibits zinc absorption from zinc-fortified foods [45], the total amount of zinc that is absorbed from phytate-containing foods is greater when the foods are fortified with zinc than when they are not fortified, so the presence of phytate in cereal flour should not be considered a contraindication for zinc fortification programs.

There is relatively little published information regarding the impact of zinc-fortified cereal products on biochemical or functional indicators of zinc status,

and many of the studies that are available focused on specialized foods used for targeted fortification rather than cereal flours that are appropriate for mass fortification programs. The available results suggest that zinc fortification of cereal-based products can produce a positive impact on serum zinc concentration among school-aged children [46], although similar studies have not yet confirmed these findings for younger preschool children [46]. There is still insufficient information to determine whether zinc fortification of cereal products could enhance growth or reduce morbidity among children at risk for zinc deficiency, both because of the small number of available studies and because of the fact that these studies sometimes enrolled children who were not growth restricted initially, so the interventions would not be expected to induce a growth response to zinc. In some cases, the studies also were too short to be able to detect such changes. We were unable to locate the results of any efficacy trials of the biochemical or functional impact of zinc fortification among adolescents or adults.

Mass fortification of cereal flour has been evaluated in Mexico and China. The results of the Mexico evaluation are not yet available, but preliminary results of the China evaluation have been kindly provided to this working group (Junsheng Huo, China Center for Disease Control, personal communication). The zinc-fortified wheat flour studied in China contained 25 mg zinc/kg flour, as zinc oxide, and 20 mg iron/kg flour, as either FeEDTA or electrolytic iron. At 24 and 36 months following the introduction of the zinc-fortified flour, there was a small, but statistically significant, increase in serum zinc concentration among the women of childbearing age who were exposed to the zinc-fortified flour compared with those who received the unfortified flour, regardless of the type of iron fortification (**table 6**). Thus, there is evidence from this program that zinc fortification of wheat flour can boost population zinc status.

Population subgroups that might benefit from zinc fortification

From a theoretical perspective, the population subgroups that are most likely to benefit from zinc fortification are those that currently have the greatest shortfall in zinc intakes relative to their physiologic requirements and those that consume a sufficiently large amount of the zinc-fortified food to produce a meaningful impact on zinc intake and TAZ. The population subgroups that are believed to have the greatest risk of dietary zinc insufficiency are infants and young children beyond the age when exclusive breastfeeding provides sufficient zinc, and possibly pregnant and lactating women, adolescents, and the elderly [1].

Of the foregoing population subgroups, most information on dietary inadequacy is available for young children, which is the subgroup that would seem, upon first consideration, to be least likely to benefit from mass fortification of cereal flours, both because of their relatively low level of consumption of these food products and because of the relatively small amount of zinc that is currently added to zinc-fortified flours. As shown in **table 1**, the level of zinc fortification in existing flour fortification programs ranges from 14 to 33 mg zinc/kg flour. If we assume that breastfed children 6 to 8, 9 to 11, and 12 to 23 months of age obtain half of their nonbreastmilk energy from cereal products, as was the case in studies conducted in Nigeria [47] and Bangladesh [48], they would consume approximately 25 g of cereal per day (~ 100 kcal/day) from 6 to 8 months, approximately 40 g of cereal per day (~ 150 kcal/day) from 9 to 11 months, and approximately 70 g of cereal per day (~ 275 kcal/day) from 12 to 23 months.

Table 7 shows the amounts of additional zinc that would be consumed by children in these respective age groups if the cereal in their diets were consumed as flour fortified with 15, 30, or 50 mg zinc/kg of flour. As shown in the table, these children would receive an

TABLE 6. Impact of zinc fortification of wheat flour on mean serum zinc concentration (μg/dL), by month of intervention and type of iron fortification, among women of reproductive age in China[a]

Month of intervention	EDTA arm		Elemental iron arm	
	Control group	EDTA iron + zinc group	Control group	Elemental iron + zinc group
0	0.73 ± 0.25	0.75 ± 0.27	0.73 ± 0.16	0.72 ± 0.17
12	0.72 ± 0.24	0.75 ± 0.28	0.72 ± 0.14	0.74 ± 0.18
24	0.72 ± 0.19	0.78 ± 0.16*	0.74 ± 0.13	0.76 ± 0.12*
36	0.71 ± 0.19	0.79 ± 0.16*	0.75 ± 0.13	0.78 ± 0.11*

EDTA, ethylenediaminetetraacetate

* Zinc-fortification group significantly different from control group ($p < .05$).

Source: Unpublished information provided by Dr. Junsheng Huo, China Center for Disease Control.

a. Wheat was fortified with 25 mg zinc, as zinc oxide, and 20 mg iron/kg flour.

additional 0.4 to 1.2 mg zinc/day from 6 to 8 months, 0.6 to 1.9 mg zinc/day from 9 to 11 months, and 1.0 to 3.4 mg zinc/day from 12 to 23 months. Although these amounts are, for the most part, considerably less than the estimated dietary requirements for zinc, especially among the younger children, the additional zinc provided by mass fortification of cereal flours could certainly make an important contribution to reducing the existing gap between these children's dietary zinc intakes and their physiologic requirements, as discussed below.

If the same reasoning is applied to adult women with assumed dietary energy requirements of 1,800 kcal/day, they would consume approximately 225 g flour per day (~ 900 kcal/day) if they were to receive half of their energy needs as cereal flour. This amount of flour fortified at levels of 15, 30, or 50 mg zinc/kg flour would provide an additional 3.4, 6.8, or 11.2 mg zinc/day, respectively, compared with estimated dietary zinc requirements of approximately 9 mg/day (unadjusted for dietary phytate content). Thus, zinc fortification could augment zinc intake considerably if women were to consume this amount of zinc-fortified flour.

Evaluation of zinc fortification programs

A detailed review of the major issues concerning monitoring and evaluation of food fortification programs is available in a recently published World Health Organization (WHO) document [49]. As indicated in that publication, it is appropriate to consider evaluating the nutritional impact of a food fortification program only after monitoring activities have confirmed that the program has been properly implemented and is reaching the targeted beneficiaries. Of particular concern with regard to zinc fortification programs are the specific indicators of zinc status to be used and the segment of the population that should be examined to detect changes in zinc status. There is now general consensus that the best indicators of population zinc status and risk of zinc deficiency are serum zinc concentrations, rates of nutritional stunting, and dietary zinc intakes [50, 51]. In the case of zinc fortification programs, dietary intake assessment is useful to assess the adequacy of zinc intake and the appropriate level of fortification, but dietary studies are not very useful for assessing program impact. Because mass fortification programs generally do not target children under 2 years of age, which is the period of maximum growth restriction, rates of nutritional stunting also would probably not be affected by zinc fortification of cereal flours, except perhaps through changes in maternal zinc status during pregnancy.

For these reasons, the most appropriate indicator for assessing the nutritional impact of zinc fortification programs is the change in serum zinc concentration in a representative sample of the population before and after introduction of the fortification program. Detailed information on appropriate methods for collecting and processing samples for serum zinc analyses have been published previously [1]. For the aforementioned reasons, this evaluation could focus on either older children or adults, which are the population subgroups most likely to be affected by the intervention. As suggested by the results of the previously cited evaluation conducted in China, it may be necessary for more than 1 year of regular consumption of fortified flour to elapse before changes in population zinc status are detectable. Of course, it is not possible to draw causal inferences from the usual pre-post evaluation design because of the inability to preserve a nonintervention control group in the context of a mass fortification program. Nevertheless, the proposed evaluation plan can be useful to determine whether any changes in zinc status have occurred following successful introduction of the fortification program.

Summary of recommendations

In summary, zinc fortification is an appropriate strategy for increasing zinc intake and zinc absorption. Additional information is needed to confirm the efficacy of zinc fortification of cereal flours for improving the

TABLE 7. Estimated amount of additional zinc intake from zinc-fortified cereal flour among young breastfed children, by age group and selected levels of zinc fortification

Age (mo)	Assumed energy intake from cereal flour (kcal/day)[a]	Flour intake (g/day)	Estimated amount of additional zinc intake (mg/day), by level of fortification (mg zinc/kg flour)			Zinc RDA (mg/day)[b]
			15	30	50	
6–8	101	25	0.4	0.8	1.2	3
9–11	154	38	0.6	1.1	1.9	3
12–23	274	68	1.0	2.0	3.4	3

a. Assumes 50% of non-breastmilk energy provided by cereal flour

b. Recommended Dietary Allowance (RDA) of the Institute of Medicine [27], without adjustment for estimated zinc absorption from different diets

zinc status of high-risk subgroups of the population, such as young children, and information is needed on the overall effectiveness of zinc fortification programs. The necessary levels of zinc fortification that would be required to mitigate a shortfall in dietary zinc intake in a particular population depend on the population subgroup considered, their usual amount of flour intake, the degrees of milling and fermentation that are practiced, and their usual levels of zinc and phytate intake from other food sources. Taking all these issues into account, recommendations for levels of zinc fortification are specified in **tables 5A** and **5B** in relation to the average level of flour consumption and the degree of flour extraction. The range of suggested levels of zinc fortification is based on the amounts needed to ensure adequate TAZ by adult men, using the stated assumptions regarding usual dietary zinc and phytate intakes. These estimates do not take into consideration the effects of any further processing of flour, such as yeast fermentation, on zinc absorption. It is also important to recognize that if the usual dietary zinc intake from other food sources is less than the estimated amounts, a higher level of fortification will be required to meet the requirements for absorbed zinc. On the other hand, when the usual dietary zinc intake from other foods is greater than the present estimates, a lower level of fortification would be appropriate.

The proposed levels of zinc fortification should also benefit young children who consume wheat flour in the assumed amounts, but a shortfall in zinc intake relative to theoretical requirements will probably still exist for young children. Thus, mass cereal flour fortification programs should not be considered, by themselves, as sufficient to meet the zinc needs of young children in most settings, unless higher levels of fortification are used. It is very unlikely that the currently proposed levels of zinc fortification will have adverse effects on the absorption of other minerals from zinc-fortified foods or their sensory properties.

The levels of zinc fortification of cereal flour that are currently being applied in national fortification programs are generally less than the fortification levels recommended in the present document. Thus, these existing programs—and those that are currently under development—should be reexamined with regard to the desired level of zinc fortification. The impact of these programs on population zinc status should be monitored periodically.

Research needs

Based on the information reviewed for the present document, several knowledge gaps were identified. The following research questions were deemed to be of the greatest importance for the design and successful implementation of zinc fortification programs:

- Development of a valid prediction equation for estimating zinc absorption among young children, and expansion of the range of data used for the existing prediction equation for adults (especially with data from high-zinc diets and high-phytate diets);
- Reassessment of the safe upper level of dietary zinc intake (as opposed to intake of zinc supplements consumed between meals), including additional studies of zinc absorption, with appropriate considerations for the degree of flour extraction and dietary phytate content;
- Efficacy and effectiveness of zinc fortification programs in high-risk population subgroups, such as young children, adolescents, pregnant and lactating women, and the elderly;
- Possible effect of absorption enhancers, such as EDTA and exogenous phytase, on zinc absorption;
- Evaluation of the responsiveness of serum zinc concentration and functional indicators of zinc status to zinc fortification;
- Development of simple new indicators of individual and population zinc status.

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Miller's best/enhanced practices for flour fortification at the flour mill

Quentin W. Johnson and Annie S. Wesley

Abstract

***Background**: Cereal flour fortification has been identified as an effective mass fortification intervention as part of a national public health strategy to overcome micronutrient deficiencies and improve the health status of populations, especially women and children.*

***Objective**: The effectiveness of cereal flour fortification programs requires the use of micronutrient premixes that provide the desired health benefits.*

***Method**: The Miller's Best/Enhanced Practices for Flour Fortification at the flour mill has been developed to provide specific guidance to millers and government officials to ensure that flour fortification practices are carried out in a way that results in the anticipated public health impact.*

***Results**: The paper provides information specific to the use of micronutrient premixes, feeders, the fortification process, and quality control systems to ensure that both minimum and enhanced practices can be followed by the millers.*

***Conclusions**: Guidelines for basic and best/enhanced practices to be followed for each stage of the flour fortification process at the flour mill are presented. The paper is designed to be a companion to the Recommended Practices for the Production and Procurement of Premix used in Cereal Fortification Programs and supplementary to existing food quality manuals and systems, such as Good Manufacturing Practices, Food Quality Systems, and Hazard Analysis and Critical Control Points. Other stakeholders involved in flour fortification programs have the responsibility to follow best practices of their own to ensure optimum effectiveness.*

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Introduction

The United Nations in 1992 identified micronutrient deficiencies as a significant public health problem in many developing countries, and anemia still affects 1.6 billion people globally [1]. One of the nutritional strategies to address these vitamin and mineral deficiencies is through fortification of staple foods with micronutrients [2], particularly products containing wheat and maize flour that are commonly used by large segments of population [3].

Although developed countries have fortified flour for decades, in recent years a growing number of developing countries have designed and implemented flour fortification programs, drawing upon the expertise and financial resources of United Nations agencies, national and international nongovernmental organizations, and other donor agencies. Data compiled by the Flour Fortification Initiative indicate that up to 2009, at least 57 countries worldwide were fortifying wheat flour [4]. These efforts have also involved close collaboration with the milling industries of each country where fortification has occurred.

Such work is often guided by technical information provided to millers and regulatory agencies overseeing flour fortification programs [5]. However, to date, there has been no overarching effort to standardize best practices for flour fortification. This document sets forth a series of best or enhanced practices for flour millers as support for the industry to meet basic norms and requirements of fortification. It should also help establish consistency in fortification practices across various countries where such programs exist.

Adherence to fortification best practices requires investment of time, money, and resources by government and industry partners. Where the milling industry is already following internationally accepted practices for the production of flour, the best practices

for the fortification of the flour can be easily integrated. Although this document is intended to guide parties involved in fortification, regulatory agencies that choose to make best practices mandatory should consider additional cost burdens put on the milling industry, which may or may not be recovered through pricing mechanisms.

Government agencies also need to prepare for circumstances involving the mixing of domestic and imported flours, particularly if the importing and exporting countries have different milling standards. To minimize such problems, all decisions involving mandatory best practices should be made with the full involvement of both the milling industry and the agencies responsible for food control.

This document covers the elements of flour fortification at the flour mill level that millers need to follow to meet the acceptable basic fortification practices and/or the best fortification practices. The topics that are covered include quality systems, premix and preblending guidelines, premix measurement, feeder installation and guidelines for feeder operation and the fortification process, quality control, monitoring premix usage and inventory control, and parameters for basic and best fortification practices at the flour mill.

Quality systems and monitoring

Quality systems

Systems to help manufacturers meet quality standards are common throughout the global food industry. One important feature of any quality system is documentation. Without documentation of correct procedures and processes, industry standards cannot be maintained or adjusted to meet changing conditions. The quality systems described below are used by millers worldwide.

Good Manufacturing Practices

Good Manufacturing Practices (GMPs) can be established by government regulations or developed internally by individual firms or industry groups. In many cases, GMPs are published in print or electronic manuals for employees and/or managers. GMPs are sometimes closely associated with Standard Operating Procedures (SOPs), which are used to ensure quality and consistency for repetitive tasks or for performing a specific action. GMPs include Quality Assurance and Quality Control procedures and testing. The minimum requirement for flour mills is a set of documented GMPs.

ISO 9000 Series

The International Organization for Standardization developed the ISO 9000 Series [5] to be an internationally recognized set of standards for quality assurance and quality control. Companies wishing to meet ISO 9000 standards must be certified and their production processes inspected. Certification also requires sound record keeping, proper maintenance of equipment, worker training, and proven customer relations. The ISO 9000–2000 Series is more rigorous than the previous ISO Series.

Hazard Analysis and Critical Control Points

Hazard Analysis and Critical Control Points (HACCP) are "closed-loop" systems that help identify potentially unsafe links in the food processing chain, including risks of exposure of food to biologic, chemical, and physical hazards. Such systems also help prevent the manufacture and distribution of unsafe foods by identifying key control points. A distinguishing characteristic of HACCP systems is that they can be tailored to specific industry sectors. In some countries, HACCP systems are mandatory for firms that process meats, dairy products, or seafood, as well as for low-acid food processing. The ISO has developed an international standard for HACCP, ISO 22000 [6].

Premix and preblending guidelines [7–11]

Premix specifications

Every flour mill should keep a set of premix specifications on site. The premix specifications must include the correct amounts of micronutrients to meet fortification regulations, and the addition rate should be measured in grams per metric ton of flour [8–11]. With regard to premix and blending guidelines, millers should also refer to a companion document, *Recommended Best Practices for the Production and Procurement of Premix used in Cereal Fortification Programs* [7].

Premix ordering

Each mill should have a list of approved premix suppliers. Premix orders should be based on a mill's flour production rates and the estimated delivery time(s) from supplier(s). Reliance on a single supplier raises the risk of interruption of supplies and noncompetitive pricing. Premixes also have a limited shelf-life, usually between 1 and 2 years (depending upon the composition and number of micronutrients). Extra supply should be managed to ensure that the premix is used prior to reaching its "best before" date [8].

Packaging, storage, and handling of premixes

Vitamin and mineral (fortificant) premixes should be packaged in airtight and watertight containers and

stored in cool, dry areas with minimum light exposure. Typical premix packaging is a polyethylene bag placed inside a heavy cardboard box. Premix storage bags should be resealed after a portion of the product has been removed, then placed in a closed box. Unopened premixes should be kept in their original containers and stored in a cool, dry place. Once opened, light and air exposure should be minimized to prevent product degradation.

Handling of premixes at the mill

When handling premixes, the following precautions should be taken:

1. Wear a dust mask to prevent inadvertent inhalation of the active ingredients.
2. Wash any skin surface exposed to the premix material during the filling of the feeder hoppers.
3. Ensure the fortificant premix is clearly labeled to prevent accidental replacement with another flour additive or premix. Consider using a color-coded system for identifying different additive feeders and additive boxes.
4. Tightly seal and store open containers of premix in a cool, dry place with minimum light exposure.
5. Dispose of any spilled material according to the supplier's instructions. Spilled premix should not be mixed with flour.
6. Do not eat premix.
7. Wear a long-sleeved shirt and gloves to avoid risk of allergic skin reactions to vitamins and minerals in the fortificant. (A common occurrence is skin reddening caused by the vasodilatation effect of niacin. This condition is usually transitory and not dangerous, but it can be annoying.)
8. Premix should be stored in a secure location away from direct sources of light and heat.
9. It is advisable to protect the manual intake of a premix with a sieve.

Recording premix deliveries and inventory

With any new premix shipment, the production lot number(s) should be recorded and retained. A first-in, first-out (FIFO) stock rotation system should be used, since the vitamins in the fortificant premix have a limited shelf-life in terms of their biologic effectiveness and stability. This is particularly true of vitamin A [7].

Unopened packages of premix containing vitamin A may have a supplier-guaranteed shelf-life of 6 months in warm climates. In cooler climates, the shelf-life may be guaranteed for as long as 12 months. The shelf-life for unopened premixes containing minerals and only B vitamins is up to 2 years. Once a premix box is opened, it should be used within a few weeks.

Mill preblends

Mills may need to dilute premixes with flour or flour improvers before use. Premix dilution may be required when a flour mill has low production capacity or when premixes are concentrated. For example, a mill with a capacity of less than 60 MT per day would need to dilute by two- to threefold premix that is packaged for mills with a 150-MT capacity. This is usually done in a small mixer, making only enough preblend for 1 or 2 days of production. Mixing extra preblend is discouraged, since the flour–nutrient blend will have a reduced shelf-life.

Example of preblend formulation

Premix dosage rate, according to supplier's specification: 200 g/MT.

Dilution formula: **table 1** shows a dilution formula for a mill preblend.

Mill preblend dosage rate: 600 g/MT of flour.

Mill preblends with other ingredients

Flour improvers may be included in the preblend. These include enzymes, azodicarbonamide, and ascorbic acid. **WARNING**: Additives that should **never** be included in preblend are concentrated forms of potassium bromate and benzoyl peroxide (flour bleach), as they are strong oxidizers that can adversely react with fortificants.

Premix measurement [7, 9]

Two general principles apply to the measurement of premix or fortificant to be added to flour:

Volumetric addition. Volumetric addition adheres to the principle that the **volume** of the material being added has a set weight when handled in a uniform manner. Volumetric addition is similar to using a cup or spoon to measure out ingredients in home baking. The same procedure can be used to fortify flour in small-batch mixing processes. The most common method for fortifying flour at a mill is with volumetric feeders that dispense a set volume of a premix at a constant rate. The weight of the premix dispensed depends on its bulk density. The minimum error of measurement for volumetric addition is ± 3%. The precise measurement of the dosage is not possible with the use of the volumetric addition system

TABLE 1. Dilution formula

Component	Weight (kg)	%
Premix as supplied	3.333	33
White flour (low-extraction)	6.667	66
Total	10	100

Loss-in-weight addition (gravimetric addition). Loss-in-weight addition relies on **continuous weight readings** of the premix and feeding equipment. This is achieved by mounting the feeder on electronic load cells that send out an electronic signal proportional to the total weight of the feeder and the premix in the hopper. The rate at which the weight drops over time determines the true addition rate. This system is more complex and expensive than is required in most cereal milling operations. Loss-in-weight feeders are recommended for new mills, as the incremental cost is small compared with the overall construction cost. With a loss-in-weight system, the dosage is more precise and the actual dosage can be measured and recorded.

Feeders (dosifiers)

Types of powder feeders and designs

Many types of powder feeders are available to fortify flour, and they range from simple to complex. Feeders also vary in terms of their cleaning, repair, and maintenance needs. Three types of powder feeders are used in most flour mills: screw type, revolving disk, and drum type. The feeders use different mechanisms to deliver powder at a constant rate. Most feeders manufactured today are of the screw type.

Screw feeder

The screw-type feeder is powered by a variable-speed electric motor (using either alternating current or direct current) so that feed rates can be adjusted to meet production demands. The feed screw's shape, number (single or twin screw), and diameter determine the feed rate capacity. Large-capacity feeders may use a gearbox to increase and adjust the feed rate capacity. Continuous flow of premix is maintained by a large conditioning screw or flexible pulsating plates attached to the bottom of the hopper. Flow rates for large hoppers may be aided by the intermittent running of a vibrator to prevent bridging. A low-level detector can be installed on the bottom of the hopper to indicate when the premix is close to running low. The on/off switch, speed control, and low-level indicator light can be located near the feeder or at a remote location.

Screw feeders offer several advantages over other models, including their ability to sustain a constant addition rate, their wider range of delivery rates and hopper capacity, and the fact that they have fewer mechanical parts and are less expensive to build. Screw feeders also can be more sanitary and easier to maintain than the other types. The most common feeder for fortifying flour at a mill is a volumetric screw feeder that dispenses a set volume of a premix at a constant rate.

Revolving disk feeder

The revolving disk feeder is an older technology that can run on an AC or DC motor. It relies on a revolving disk equipped with a slide mechanism to control the rate of powder discharge. Effective operation requires that the disk spin at a constant speed. The hopper size on revolving disk feeders is usually smaller than that found on other types of feeders, requiring more frequent powder refills. This can be a disadvantage for large mills, since refilling takes up valuable time. This type of feeder also has more mechanical parts than the screw feeder.

Drum- or roll-type feeders

Considered a workhorse in flour treatment, with thousands of units still operating, drum- or roll-type feeders allow powder to pass between two revolving cylinders. The feeder can be powered by either an AC or a DC motor, and its rotation speed is controlled by a gearbox and pulley system. Adjustments in the feed rate capacity are made by using pulleys and wheels of various diameters. Drum feeders also come equipped with an adjustable gate that can be positioned to make fine adjustments in the feed rate. Although popular among millers, drum-type feeders require more parts and have greater maintenance requirements than other feeders. For example, shear pins in the drive mechanism cause the feeder to stop working if foreign objects such as bolts or plastic get stuck between the rolls.

Feeder calibration and maintenance

Calibration

Most mills fortify flour continuously using an ingredient feeder or dosifier to meter the premix into the flour as it flows through the mill. Millers also can use a batch mixing system whereby a set quantity of premix is blended into a set quantity of flour. In continuous systems, the fortification feeder must be adjusted to ensure that the correct amount of premix is added based on both the flour flow rate and the premix addition rate. Mills typically determine the flour flow rate in terms of metric tons per hour, kilograms per minute, or another unit of measurement.

The premix addition rate should be checked regularly, such as on every shift or production day, to ensure adequate quality control. Millers should routinely perform feed rate checks and change the premix addition rate to meet any change in the flour flow rate. A feed rate check involves placing a plate or cup under the feeder discharge spout for 60 seconds and weighing the amount of premix collected to an accuracy of 0.1 g. A standard lab balance or electronic scale can be employed for this purpose. This allows verification of actual addition rates as compared with the target addition rate.

Maintenance

All feeders require maintenance to ensure consistent performance. This includes regular lubrication and replacement of broken or worn parts and materials. Also, a feeder may require recalibration after it has been powered down for maintenance. Feeder suppliers should be able to provide a recommended maintenance schedule.

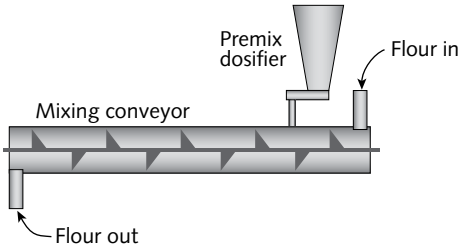

FIG. 1. Gravity feed method of premix delivery

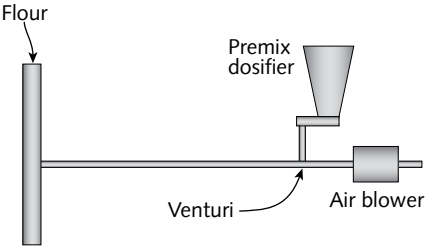

FIG. 2. Pneumatic method of premix delivery

The fortification process [9, 11, 12]

Addition and mixing design delivery systems

Once the feeder has delivered its required quantity of premix, there are two ways to introduce the material into the flour stream: gravity feed and pneumatic conveying.

Gravity feed is by far the most common system. In a gravity feed system, the feeder is placed above a conveyor and the premix is dropped directly into the flour as it flows through the conveyor (**fig. 1**). The flour collection conveyor is designed to collect and blend the individual flour streams coming from the sifting equipment so that the final mixed flour is uniform. It is critical that the fortificant be introduced to the flour conveyor at the proper location. This should occur at the front half of the collection conveyor above the blades of the mixing screw. If the fortificant is introduced too close to the discharge end of the conveyor, it may not sufficiently blend with the flour.

This distance requirement may not apply in some mills where the flour is pneumatically blown from the collection conveyor to either a packing bin or a flour storage bin, or where the flour collection conveyor discharges into another conveyor where additional mixing can occur.

Pneumatic conveying involves dropping premix into a venturi tube, which in turn injects the premix into the air stream (**fig. 2**). The material is blown by positive pressure or sucked by a vacuum through a pipe into the flour collection conveyor. If positive or vacuum pressure is not available, the mixing can occur at a downstream location as long as some flour mixing occurs.

Pneumatic conveying has one key advantage in that it allows the feeder to be located anywhere in the mill. However, a pneumatic conveying system requires additional equipment, such as a blower, valve, and piping. The pipes used to convey the material should have as few as bends as possible to prevent clumping and the blocking of the pipes by the flour fortificant. The venturi tube should be routinely checked for premix residues and cleaned if such buildup occurs. Pneumatic conveying is less effective at mixing premix into flour, so it is recommended that the flour collection conveyor lead to a mixing conveyor or sieve rather than directly into a flour holding bin.

Electrical interlock system

Electrical interlock systems are strongly recommended for feeder systems. An interlock causes the feeder to stop if the flour collection conveyor or plansifter stops and will prevent the inadvertent overtreatment of the flour in the event of a mechanical breakdown. Such systems should be installed between the feeder motor and the motor driving either the flour collection conveyor or the first break plansifter. An interlock system can be installed directly into the mill control panel.

In pneumatic delivery systems, an interlock should be made between the feeder and the blower to ensure that the two components always operate simultaneously. This will prevent buildup of premix in the pneumatic lines in the event of an overtreatment of flour. An alternative is to equip the feeder with an automatic shutoff switch that is triggered by a flour flow indicator or pressure indicator in a pneumatic system.

Continuous monitoring system

Most mills are designed for constant flour flow, but blockages, also known as "chokes," may occasionally interrupt flow. Overtreatment can occur if the flour conveyor stops or slows while feeders continue operating at a normal rate. Such problems can be eliminated by installing an electronic controller to slow the speed of the feeder to that of the flour flow. Such controllers

generate an electronic signal that is proportional to the flour flow rate; the signal is then used to regulate the feeder's motor speed.

Additional mixing conveyor

For mills without automated control capabilities, operators must adjust the feeders manually. Such manual adjustments can make continuous fortification more difficult, particularly in smaller and/or older mills that lack the technology to ensure a constant flow of flour. Millers can overcome such problems by installing a mixing conveyor between the flour holding bin and the packing bin. The mill's feeder would be configured to drop or blow premix into the mixing conveyor.

Batch blending system

Some small mills may choose to use a batch blending system with or without a feeder. These systems use a large-capacity mixer, usually ranging in size from 1 to 5 MT, and either feed or add the premix based on proper mixing ratios. **Table 2** shows a typical formulation for a premix with a specified dosage of 200 g/MT. Blending duration for batch systems will be determined by an experimental design that relies on mixing times, sampling of at least six different locations in the mixer (top, middle, and bottom), and quantitative analysis using iron as the marker (if iron is in the premix).

Low-level indicator

A low-level indicator should be installed in the feeder hopper that will trigger an alarm when premix needs to be added to the hopper.

Instructions for fortification operations [9, 10]

The following step-by-step procedures should be used to set up and calibrate feeders.

Feeder setup

1. Locate and install the feeder based on optimal mill equipment configuration. Note position requirement on flour collection conveyors for gravity feeder setup. Ensure there is adequate mixing of flour after the point of premix addition.
2. Install voltage regulator if there is a large variation in electrical voltage (more than ± 20%).
3. Install the electrical interlock system either directly to the flour collection conveyor motor or to the mill control panel.
4. Install the low-level indicator.
5. Ensure that the light indicating low premix level in the hopper is operating.

TABLE 2. Premix dosage

Component	Weight (kg)	50-kg bags
Flour	1,000	20
Premix	0.2	

Feeder calibration

1. Fill hopper about half full with premix.
2. Set feeder to maximum discharge.
3. Run feeder for 2 minutes.
4. Weigh premix.
5. Calculate maximum discharge per minute.
6. (Optional) Repeat steps 1 to 5 with different speed or percent settings.
7. Using graph paper or spreadsheet software, prepare a chart (see theoretical chart in **fig. 3**) showing feed discharge rates for different feeder speed settings of between 0% and 100% of maximum discharge.

Determining the flour production rate

Flour production rates should be determined at the point of fortification, usually by recording measurements from the wheat flour production scale. In some cases, operators pack the flour as it is being milled. In such circumstances, flour production can be determined by counting the number of bags packed per hour.

Determining the premix feed rate

The following procedures should be used to determine the premix feed rate to meet fortification standards. Feed rates are usually expressed in grams per minute.

1. Determine the recommended addition rate of premix from supplier specifications.
2. Calculate premix feed rate per minute using the following formulas:
   a. Premix weight in grams per ton divided by 1,000

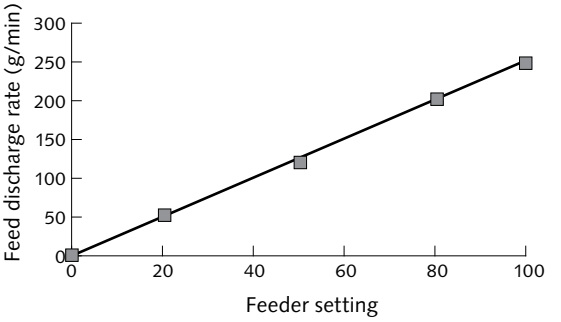

FIG. 3. Example of feeder calibration (theoretical) curve

= g/kg flour.
   b. Premix weight per kilogram multiplied by production rate per minute in kilograms = premix weight in grams required per minute.
3. Adjust feeder controls to deliver the proper amount of premix per minute. Note: If using diluted preblend, millers must set the feed rate in accordance with the dilution rate of the preblend.

Fortification operation

The following steps should be taken to achieve the optimum results in a fortification operation.
1. Start mill and allow equipment to warm up to expected production rate.
2. Start feeding premix at required setting.
3. Ensure feeder hopper contains sufficient premix.
4. Perform "check weighs" at the start, middle, and end of mill production shift to verify premix addition is correct. Adjust feeder if addition rate is above or below target. Recheck addition rate using "check weigh" procedure.
5. Visually check feeder during production run to ensure that there is sufficient premix in the hopper and that the feeder is operating properly.
6. At end of production run, turn off feeder before shutting down mill.
7. Maintain production records showing:
   a. Lot number of premix used
   b. Check weights
   c. Time(s) of check weighing
   d. Start and finish times of production run

Quality control

Most large mills adhere to the following quality control standards to ensure that flour is properly fortified:
1. Use a quality feeder whose premix delivery rate can be calibrated to flour flow and whose controls allow the feeder to stop operating when flour flow stops.
2. Check feed rates at the start, middle, and end of each production shift.
3. Perform regular iron spot tests on flour at the start, middle, and end of each production shift or when otherwise required under a quality control sampling schedule (See iron spot testing guidelines below). Iron spot tests should also be performed on both packing and shipment samples.
4. Note: For premixes containing sodium iron EDTA (NaFeEDTA), the colorimetric test still under development by the Chinese Center for Disease Control and AkzoNobel should be used (personal communication, C. A. De Wolf and C. T. J. Wreesmann, AkzoNobel Chemicals).
5. Check premix usage against production of fortified flour. Such checks should be done weekly or monthly.
6. (Optional) Perform quantitative tests of iron on a weekly or monthly basis. Such testing should be done by an outside laboratory.
7. (Optional) Perform quantitative testing of all fortification components in a composite sample on a monthly or quarterly basis. Again, testing on all added micronutrients should be done by a contract analytical laboratory.
8. Note: Some premix companies offer independent testing at no charge.

Record keeping

Proper record keeping is key to quality assurance and quality control in flour fortification. Each mill should have a written plan for what records are to be kept, how the data are to be collected and entered, who should manage the data, and where the records are to be stored. All records should be kept at the mill and made available to government agencies or flour customers when requested for inspections or audits.

Sampling procedures [9, 10]

Careful obtaining and handling of flour samples is important to achieving sound analysis, particularly when the samples are to be submitted for quantitative testing. Proper sampling techniques should be explained and documented in the mill's quality assurance plan. Sampling should occur immediately before flour is packed, since this represents the final mill product. Composite samples are generally preferred for quantitative testing, but spot samples are acceptable for the iron spot test.

A good composite sample will include seven spot samples taken over an 8-hour period. A minimum composite sample consists of three spot samples taken over an 8-hour period. One technique for acquiring a good composite sample is to place 500-g spot samples into a laboratory blender with a capacity of least 5 kg of flour. When all samples are collected, the composite is mixed by blending using a paddle attachment. A single sample of 500 g is then taken for testing. The miller should retain at least another 500 g sample of the composite in case the original sample is contaminated or damaged.

Fortified flour or meal samples must be **kept away** from direct sunlight or strong indoor light to prevent degradation of light-sensitive vitamins such as vitamin A and riboflavin (vitamin B_2). Samples should be labeled with the name of the mill, the date of collection, the type of flour, and whether the sample is fortified (unfortified samples are often collected to establish baseline natural levels). Regularly collected samples should be numbered consecutively, and their data should be entered into a mill sample record book.

Undocumented samples **should not be tested.**

Mills fortifying flour for the first time may want to keep spot samples of fortified product on site to be tested against unfortified samples. Such testing will allow mill operators to get a better sense of their fortification capacity, including expected variations in their final product. During early production runs, millers may wish to take a spot sample every 6 hours over a 3-day period, then measure iron in the samples as an indicator nutrient. Millers may also choose to take several composite samples and have them tested for all added micronutrients.

Once full-scale fortification has commenced, it is not practical to run multiple samples on a regular basis. Medium- and small-capacity mills may wish to perform a composite sample two or three times annually, whereas larger mills may run a composite sample monthly or quarterly. The largest-volume mills should consider running composite samples more regularly to ensure their product meets high fortification standards. Whereas composite sampling generally tests all added micronutrients, tests that sample only one or two indicator nutrients may be sufficient for many mills.

When a government agency or outside inspector requests samples for testing, millers should collect three samples: one for the outside test, one to be retained at the mill, and a third to be used in the event of conflicting or contradictory testing results.

Analytical testing [9, 10, 13]

Flour samples should be tested to verify that the finished product has been properly fortified. Three types of testing are possible: qualitative, semiquantitative, and quantitative assay tests.

Qualitative tests

Qualitative tests are the easiest to apply and usually involve measures that simply indicate the presence or absence of a micronutrient. One example of this type of test is the UV light test for riboflavin. Another example is the iron spot test (American Association of Cereal Chemists method 40-40, Iron-Qualitative Method), which is used worldwide by millers to ensure that flour meets fortification standards [14]. This simple procedure should be used on a regular basis, typically every 2 to 4 hours, during a large mill's production run. At a minimum, iron spot testing should be done once during every 8-hour production shift. Sampling should be done during the production process, but it also can be used to sample packaged flour to verify it has been properly labeled as fortified.

The qualitative iron spot test can detect ferrous and ferric iron. The red spot test for NaFeEDTA is adapted from the semiquantitative method [13]. Although iron spot testing measures only iron content, the test is generally considered a suitable surrogate for other micronutrients, since they are added as a single premix.

Semiquantitative tests

These tests include the semiquantitative iron spot test and the color test recently developed for vitamin A. These tests are more precise than qualitative tests, because they can show if fortification levels are low, normal, or high. The semiquantitative iron spot test is an indicator of whether a mill's flour is underfortified or overfortified [10]. If either condition occurs to a sufficient degree, the mill may need to take corrective action. The density of the spots provides an estimate of how much iron was added, which is best done by comparison to standard flours having known levels of added iron [14]. The semiquantitative iron spot test can detect ferrous and ferric iron.

Quantitative assay tests

Quantitative tests using spectrophotometric and other methods provide the highest level of testing certainty, because they measure an actual value for the level of a micronutrient in a sample. Unlike qualitative and semiquantitative tests, which respond only to added micronutrients, quantitative assay tests generally measure total content—or both the natural and the added levels—of a micronutrient. Some quantitative tests can show only the added micronutrient levels.

Although millers can accomplish many of their quality control requirements by good record keeping, regular feed rate checks, and iron spot tests, there are times when more detailed quantitative analysis is necessary. Quantitative analytical procedures are available for all micronutrients added to flour. The tests differ, however, in cost, complexity, analytical error (CV), type of equipment required, and the skill needed to run the tests.

Because of these complexities, it is strongly recommended that quantitative tests be contracted to an outside laboratory. The benefits of independent testing are as follows:

- The costs of equipment and trained personnel to run many of these tests are beyond the resources of most milling companies,
- Quantitative tests need to be run regularly and consistently to obtain accurate results. Millers performing such tests only occasionally would not be able to develop the proficiency necessary to ensure accurate results,
- Mill owners will generally achieve cost savings by contracting quantitative tests to an outside lab, and some premix suppliers provide such testing free of charge,
- Mills with decades of experience in flour fortification have proven that internal quantitative testing is not necessary for maintaining quality assurance and quality control,

» Test results from independent laboratories are generally viewed as having higher credibility by government agencies and customers.

Monitoring premix use and inventory control [7, 9, 10]

A key tool for successful fortification is a premix usage and inventory control system. Such systems are designed to verify that premix is being added at the correct levels and is meeting the target addition rate within a specified range.

Figure 4 is intended to help millers measure their inventory of premix and to determine how close the actual premix addition rate is to the target rate. The target rate is defined by the recommended addition rate of premix in grams per metric ton of flour. This recommended rate is provided by the premix supplier.

This inventory control system can be used daily, weekly, or monthly. At the start of a flour fortification program, it may be helpful to use such a system daily until the fortification process becomes consistent and quality control/quality assurance is assured.

Basic and best/enhanced fortification practices

Table 3 is designed to help fortification program managers identify basic practices as well as best/enhanced practices at the mill level. The table also includes indicators for millers wanting conduct self-assessments and for auditing purposes.

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FIG. 4. Fortification premix control records

FORTIFICATION PREMIX

A. STARTING INVENTORY	_____*KG*
B. AMOUNT PURCHASED	_____*KG*
C. ENDING INVENTORY	_____*KG*
D. AMOUNT USED (A+B–C)	_____*KG*
E. PRODUCTION OF FORTIFIED FLOUR	_____*MT*
F. ADDITION RATE (D*1000/E)	_____*G/MT*
G. TARGET ADDITION RATE	_____*G/MT*
H. PERCENT OF TARGET (100*F/G)	_____%

TABLE 3. Basic and best/enhanced fortification practices

Fortification component	Basic practice	Indicator	Best/enhanced practice	Indicator
Quality system	GMPs	GMP manual	HACCP and/or ISO 9001 or 9002	Manuals Third party audits certificates
Premix				
Premix specifications	On file in GMPs	Documents	Quality system manuals	Documents
Premix ordering	SOPs	Documents	Quality system manuals	Documents
Packaging, storage and handling	SOPs, dry, out of sunlight, boxes closed, premix lot numbers recorded	Inspection and documents	SOPs, dry, out of sunlight, boxes closed, premix lot numbers recorded	Inspection documents
Handling practices	SOPs, feeder hoppers covered, employee protection masks and gloves	Inspection	SOPs, feeder hoppers covered, employee protection masks and gloves	Inspection
Mill preblends	SOPs, production records	Inspection and documents	SOPs, production records	Inspection documents
Feeders				
Type	Volumetric, screw, disk, or drum	Inspection	Gravimetric, loss in weight	Inspection
Feeder calibration	SOPs	Documents	SOPs	Documents
Feeder maintenance	SOPs	Documents	SOPs	Documents

continued

TABLE 3. Basic and best/enhanced fortification practices *(continued)*

Fortification component	Basic practice	Indicator	Best/enhanced practice	Indicator
Fortification process				
Feeder location or addition point	At least 3 m from discharge of flour collection conveyor OR additional blending system, pneumatic or conveyor	Inspection	At least 3 m from discharge of flour collection conveyor OR additional blending system, pneumatic or conveyor	Inspection
Feeder control	Electrical interlock system	Inspection	Electrical interlock system	Inspection
Continuous monitoring			Feeder controls tied in with flour scale and computer or microprocessor controlled	Inspection
Production rate determination	SOPs Calculations	Inspection and documents	SOPs Calculations	Inspection and documents
Premix feed rate determination	SOPs Calculations	Inspection and documents	SOPs Calculations	Inspection and documents
Routine check weighing	SOPs, feeder discharge check weighing every 8 h or once per shift by miller	documents	SOPs, feeder discharge check weighing every 4 h or once per shift by miller	Documents
Mill QC				
Sampling schedule	SOPs, iron spot test every 4 h	QC records and documents	SOPs, iron spot test every 2 h	QC records and documents
Analytical testing qualitative	SOPs, QC methods iron spot test	QC records and documents	SOPs, QC methods Iron spot test compared with standard sample	QC records and documents
Analytical testing quantitative	Composite samples, monthly basis using external lab—iron only	QC records and documents	Composite samples, monthly basis using external lab—all added micronutrients	QC records and documents
Usage and inventory control	SOPs, calculations on monthly basis	QC records and documents	SOPs, calculations on weekly basis	QC records and documents

GMP, Good Manufacturing Practices; HACCP, Hazard Analysis and Critical Control Points; SOPs, Standard Operating Procedures QC, quality control

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Maximizing the impact of flour fortification to improve vitamin and mineral nutrition in populations

Food fortification should be guided by a fundamental public health principle to effectively and safely prevent vitamin and mineral deficiencies and assure a healthful dietary intake of essential nutrients. Fortification of wheat and maize flours, widely consumed and centrally processed food staples, could have an enormous impact on micronutrient malnutrition. Compared with other interventions, fortification programs have the advantage of reaching broad population groups through existing food delivery systems, but without requiring changes in existing consumption patterns [1]. Furthermore, because flour can be fortified with several micronutrients, fortification can lower the risk of multiple deficiencies where they exist. Compared with other interventions, food fortification may be more cost-effective and, if fortified foods are regularly consumed, has the advantage of maintaining body stores.

Successful flour fortification programs require commitment of many sectors, including the commercial sector, particularly the milling industry and premix manufacturers, the public health sector, and the medical private sector. Recognition of the importance of industry is particularly key to a successful program. Years of deliberation and extensive consultation with stakeholders may be required before mandatory flour fortification is adopted. Three recent efforts could prove useful for countries moving toward such standards—those of Food Standards Australia and New Zealand (FSANZ), the Food Safety Authority of Ireland, and the Food Standards Agency UK on mandatory fortification of flour with folic acid [2–4]. The documents prepared as part of those deliberations are excellent sources of information for other countries considering their own programs.

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Specific observations and Workshop conclusions

The Workshop deliberations affirmed that wheat and maize flour fortification:

- Improves iron status among consumers if a sufficient level of bioavailable forms of iron is added;
- Increases folic acid intake by women and reduces neural tube defects (NTDs) (folic acid may also have additional public health benefits);
- Could be a feasible approach to improve vitamin B_{12} status of populations;
- Can increase vitamin A intake and improve status;
- Improves zinc status among consumers if a sufficient level of zinc is added.

Considerations in determining levels of vitamins and minerals to add to flour

To help determine the specific level of vitamins and minerals to add to fortified wheat or maize flour, program managers should:

- Estimate the per capita consumption of nationally produced and imported flour milled by industrial roller mills with a minimum production capacity of > 20 MT/day. Such industrially produced flour is called "fortifiable" flour.
- Avoid risk of human exposure to excess levels of vitamins and minerals caused by very high consumption of fortified flour products.
- Consider potential sensory and physical effects of added nutrients on flour and flour products. Sensorial incompatibility is more common in high-extraction flours, especially those coming from corn. Prior to selecting a fortification level, countries should conduct basic sensorial tests to confirm that the proposed amounts of fortificants (mainly iron and zinc) are technologically compatible not only with the flour but also with the products manufactured with it.
- Understand that there is much less experience in

fortifying maize flours than wheat flours. A detailed evaluation of maize flour fortification was not attempted in the Workshop. Compared with wheat flour, fortification of maize flour with iron and zinc is more likely to result in sensorial incompatibility. For iron and zinc, it is not possible to combine the fortification guidelines for wheat flour and maize flour. However, it is possible to extrapolate guidelines for wheat flour to folic acid, vitamin B_{12}, and vitamin A, because sensorial incompatibility is uncommon with these fortificants.

» Consider the cost implications of the fortificant premix formulation.

These recommendations are based on four levels of flour consumption. **Table 1** shows the distribution of per capita flour consumption across a number of countries using Food Balance Sheet data from the Food and Agriculture Organization (FAO) and World Bank supported Household Income and Expenditure Survey (HIES) data [5, 6]. The recommended levels of iron, folic acid, vitamin B_{12}, vitamin A, and zinc to be added to flour are based on these ranges of flour consumption.

In planning a national flour fortification program, the per capita consumption of "fortifiable" flour should be estimated for each country. Approaches for estimating such consumption in a country are as follows:

» When population-based flour consumption data are available from individual dietary intake assessments (e.g., 24-hour dietary recall data), use that information to estimate the distribution of individual flour intake (in grams per day). Such data may be available from population-based surveys, but they are difficult and costly to collect and few countries have up-to-date dietary data. Furthermore, dietary recall data do not usually distinguish between "nonfortifiable," "fortifiable," and fortified flour products. Thus, additional knowledge of the flour and flour products industries in the country should be used to interpret the national dietary data with regard to estimating individual consumption of "fortifiable" or fortified flour.

» When flour consumption data are available only at the household level, use that information to estimate the distribution of per capita flour intake per adult equivalent (in grams per day). Such household-level data may be available in many countries through recent World Bank-supported HIES data on household purchases, including flour and flour products. An important limitation of HIES or other household-level food expenditure data is that individual food intake is not measured; thus, adult equivalent per capita consumption is roughly estimated. Furthermore, it is assumed that commercially purchased flour and flour products are potentially "fortifiable"; the calculated per capita intake of such flour may be an overestimate, depending on the profile of the flour industry in a particular country.

» When estimates of per capita flour intake are based only on national level data, such as from the FAO food balance sheets [5], use that source to estimate the average per capita "flour consumption."

» When no published data are available, estimates of "fortifiable" flour may be obtained using industry information as follows:
  – Number of mills with > 20 MT/day capacity
  – Running times of the mills, and the percentage of capacity at which they operate (usually confidential industry information)
  – Flour extraction rates
  – Estimated flour imports and exports.

Such milling industry information can also augment any population-based flour consumption data to maximize the public health impact of flour fortification.

It is essential not to rely on national-level estimates of flour consumption when not all population groups of a country are regular consumers of flour. Furthermore, in countries with large differences in geographic distribution of flour consumption, monitoring the potential impact of a "national" flour fortification program should distinguish between national and geographically specific data and information. The paucity of data giving rise to this tiered approach to rapid estimation of intake distributions underscores the need for countries to consider conducting nationally or regionally representative wheat flour intake surveys prior to scaling up national flour fortification initiatives.

TABLE 1. Estimated percentile distributions of per capita wheat flour intake (g/day) from the Household Income Expenditure Surveys for countries stratified by ranges of per capita wheat flour intake

Percentile of wheat flour intake	Wheat flour intake (g/day)			
	< 75	75–149	150–300	> 300[a]
5th	7.5	15	30	60
50th	50	100	200	400
95th	150	300	600	800

a. Few countries have per capita consumption of > 300 g/day.

Iron fortification

The iron fortification Working Group focused on developing updated guidance based on a thorough review of both published efficacy and effectiveness trials of iron-fortified foods and of current wheat and maize flour fortification regulations [7]. The Working Group concluded that the Cuernavaca recommendations were valid, but they had not been implemented by the majority of national flour fortification programs around the world.

The working group members also agreed that the fortification of flour with appropriate levels of the

most bioavailable forms of iron will improve the iron status of populations with very little risk of adverse effects. Efficacy studies indicate that daily consumption of 7.1 mg iron as ferrous sulfate (equivalent to 7.1 mg iron as ferrous fumarate, 4.6 mg iron as sodium iron ethylenediaminetetraacetate [NaFeEDTA], or 10 mg electrolytic iron) through fortified flour products will improve iron status in women of childbearing age. Such public health benefits could also be achieved in high-extraction flours—or low-extraction flours without a yeast fermentation process—by fortifying with the same level of NaFeEDTA. Encapsulation of NaFeEDTA, ferrous sulfate, and ferrous fumarate would be especially helpful in making bioavailable forms of iron compounds "fortification friendly" by eliminating the adverse sensory and physical effects of adding such iron compounds to flour. Although an encapsulated form of ferrous sulfate, which would not be separated by sieves in industrial mills, has been developed and documented to be efficacious [8], such products are not yet commercially available.

A review of current national flour fortification programs suggests that many programs are not effective in reducing the burden of iron deficiency because most use reduced elemental iron powders (e.g., atomized reduced and hydrogen-reduced iron) that have low bioavailability. Furthermore, in many countries with fortification programs, less than 80% of flour is fortified. Consequently, the iron status of only those who have regular access to fortified flour products—rather than the population as a whole—would be expected to improve. Better approaches are therefore needed to ensure that the maximum number of women have regular access to fortified flour.

Iron recommendations

- **Table 2** presents the recommended levels and types of iron fortificants based on ranges of per capita flour consumption and extraction of wheat flour.
- The preferred order of iron fortificants for wheat flour is NaFeEDTA, ferrous sulfate, and ferrous fumarate. If these fortificants cannot be used, then electrolytic iron powder is the only alternative iron compound recommended, provided flour consumption is high enough.
- Atomized, reduced, and hydrogen-reduced elemental iron powders should **not** be used in flour or food fortification programs.
- If other forms of iron fortificants are demonstrated to be adequately bioavailable in human efficacy studies, they could be considered for use in flour fortification.
- Maize flour is processed into several different products, including degermed flour (the most similar to refined wheat flour), whole flour, and lime-treated (nixtamalized) flour. The Pan American Health Organization (PAHO) has issued fortification guidelines for these types of maize flour [9].
- Countries currently regulating fortification of flour with iron should reexamine their standards to account for the above recommendations on the types

TABLE 2. Average levels of nutrients to consider adding to fortified wheat flour based on extraction, fortificant compound, and estimated per capita wheat flour intake

Nutrient	Flour extraction rate	Compound	Level of nutrient to be added (ppm) according to estimated per capita wheat flour intake (g/day)[a]			
			< 75 g/day[b]	75–149 g/day	150–300 g/day	> 300 g/day[c]
Iron	≤ 80%	NaFeEDTA	40	40	20	15
Iron	≤ 80%	Sulfate/fumarate	60	60	30	20
Iron	≤ 80%	Electrolytic powder	NR[d]	NR[d]	60	40
Iron	> 80%	NaFeEDTA	40	40	20	15
Folic acid	All	Folic acid	5.0	2.6	1.3	1.0
Vitamin B_{12}	All	Cyanocobalamin	0.04	0.02	0.01	0.008
Vitamin A	All	Vitamin A palmitate	5.9	3.0	1.5	1.0
Zinc[e]	≤ 80%	Zinc oxide	95	55	40	30
Zinc[e]	95%	Zinc oxide	100	100	80	70

NaFeEDTA, sodium iron ethylenediaminetetraacetate

a. These levels consider only wheat flour as the main fortification vehicle in a public health program. For maize flour programs, levels can be extrapolated for folic acid, vitamin B_{12}, and vitamin A, but not iron and zinc. If other mass fortification programs with other food vehicles are implemented effectively, these suggested fortification levels may need to be adjusted accordingly. These recommendations have been adopted by WHO as a Meeting Report Interim Consensus Statement [33].

b. Per capita intake of < 75 g/day does not allow for addition of a sufficient level of fortificant to cover micronutrients for women of childbearing age. Fortification of additional food vehicles should also be considered.

c. Few countries have per capita consumption > 300 g/day.

d. NR, not recommended because the very high levels of electrolytic iron needed would negatively affect the sensory properties of fortified flour.

e. These are the recommended amounts of zinc fortification assuming 5 mg zinc intake and no additional phytate intake from other dietary sources.

and levels of iron fortificants and should consider per capita consumption of "fortifiable" flour when revising guidelines.
» Development of small particle size (< 150 μm) encapsulated NaFeEDTA, ferrous sulfate, and ferrous fumarate should be encouraged to help eliminate sensory and physical effects of adding iron compounds to flour.
» Further evaluation, including human efficacy studies, of potentially less expensive forms of highly bioavailable iron fortificants, including mixtures of NaFeEDTA and other iron compounds, should be encouraged.
» Better monitoring and documentation of the biologic impact of existing national flour fortification programs on iron status of relevant population groups using World Health Organization/Centers for Disease Control and Prevention (WHO/CDC) guidelines [10] should be encouraged.
» National studies assessing consumption patterns of dietary iron, flour and flour products, and vitamin and mineral supplements should be supported.

Folic acid fortification

It is widely recognized that adequate consumption of folic acid before pregnancy and during the early weeks of gestation protects most, but not all, fetuses from prenatal death or birth with debilitating NTDs. Evidence also suggests that adequate folic acid consumption is associated with reduction in the risks of other types of birth defects (e.g., cardiac defects) [11, 12].

Recommending that women take a periconceptional supplement containing 400 μg folic acid has been the mainstay of public health measures for the primary prevention of NTDs in many countries. Although education campaigns can increase the use of supplements, their effectiveness is limited, because the maximum level of use is usually much less than 50%, and not all segments of the population are reached equally; women at highest risk for having NTD-affected infants are those least likely to be taking supplements. Even in the United States, where substantial promotion efforts have been implemented, only 30% to 35% of women of childbearing age report adhering to the daily supplementation guideline of 400 μg folic acid [13, 14]. In addition, because women move through their childbearing phase within a relatively short time (just a few years), educational activities to promote the use of supplements must be designed to reach at-risk women in perpetuity.

To protect newborns from NTDs, both the United States and Canada mandated fortification of cereal grain products at a level of 1.4 ppm in 1998. Soon after, Chile mandated fortification of wheat flour used for making bread with 2.2 ppm folic acid. Currently, more than 50 countries mandate folic acid fortification of flour [15, 16]. Still, folic acid in fortified foods provides only about one-quarter of the daily recommended amount.

Clinical folate deficiency is defined as serum folate < 3 ng/mL (~ 7 nmol/L) or red blood cell folate < 140 ng/mL (~300 nmol/L). In the only prospective study of the subject to date [17], the lowest prevalence of NTDs was associated with a higher serum folate concentration of > 7 ng/mL (~ 16 nmol/L) and a red blood cell folate concentration of > 400 ng/mL (~ 900 nmol/L). Thus, the definition of folate deficiency in women of childbearing age should not be based solely on avoiding signs of clinically defined folate anemia, as seen with blood folate levels below the clinical laboratory cutoff (as is the case with other vitamins). Rather, "sufficient" blood folate levels need to be established by experts. Currently, limited evidence suggests that a serum folate concentration of 7 ng/mL or higher would be sufficient to protect all women of reproductive age from having an NTD-affected pregnancy. Raising blood folate to the higher concentrations associated with maximum NTD risk reduction requires that women consume folic acid from supplements and/or fortified foods in addition to a healthy diet containing natural folate. Mandatory folic acid fortification of cereals and flours has been proven to be a low-cost and highly effective public health strategy in various countries [18].

Although concerns about potential negative health consequences associated with folic acid fortification have arisen since 2004, the general view from this Workshop is that most of the questions raised in recent literature on folic acid probably result from conditions where study participants received high doses of folic acid in supplements not at levels typically found in fortified foods [19, 20]. Furthermore, it was agreed that when flour is fortified with appropriate levels of folic acid, based on appropriate estimates of per capita consumption of "fortifiable" flour, the intervention does not appear to pose a public health risk.

Folic acid recommendations

The Workshop reaffirmed the Cuernavaca recommendations supporting national efforts for mandatory fortification of wheat and maize flour with folic acid [7]. The recommended levels of folic acid to add to flour, based on per capita consumption of flour, are presented in **table 2**.

Vitamin B_{12} fortification

The only dietary sources of vitamin B_{12} are animal-based food products, including meat, dairy products, and fish. Thus, populations with poor access to these

animal-source foods are at high risk for vitamin B_{12} deficiency. Even among well-nourished US populations, people who reported consuming the lowest amounts of dairy and meat products and who also did not take nutrient supplements had low or deficient plasma vitamin B_{12} concentrations [21]. Furthermore, individuals of all ages, both male and female, are at risk for vitamin B_{12} deficiency when they have limited intake of animal-source foods.

Elderly people are at special risk for vitamin B_{12} deficiency because with aging, the gastrointestinal tract may gradually lose its ability to absorb the vitamin. The primary cause of this "food-bound cobalamin malabsorption" is loss of gastric acid resulting from gastric atrophy (often caused by chronic *Helicobacter pylori* infection) and subsequent inability to release vitamin B_{12} from proteins in animal-source foods, although there may be multiple causes that are poorly understood [22]. In addition, people affected by HIV/AIDS suffer from reduced vitamin B_{12} absorption and thus require adequate daily intake of this nutrient.

The consequences of vitamin B_{12} deficiency include megaloblastic anemia (although this condition tends to be most prevalent among infants exclusively breastfed by mothers who are strict vegetarians and patients with severe vitamin B_{12} deficiency due to lack of intrinsic factor); neurologic disorders, including subacute degeneration of the spinal cord; cognitive impairment, including depression; and possibly increased risk of NTDs [23].

Individuals with vitamin B_{12} deficiency should take high-dose oral supplements to counteract the condition. Fortification of flour with vitamin B_{12} is intended to protect populations against becoming deficient, but there are no documented studies of the effectiveness of mass flour fortification. A pilot study in Israel showed that vitamin B_{12} added to flour was stable during baking, did not affect the quality of the bread, and increased plasma B_{12} concentrations slightly within 6 months (personal communication, S. Gabriel-Levy). Addition of vitamin B_{12} (9.6 μg/day) to dough before baking increased serum vitamin B_{12} by 45% in 12 weeks in healthy adults aged 50 to 75 years in The Netherlands [24]. However this is a larger dose than is likely to be provided through mass fortification. A study by the American Institute of Baking reported that the addition of vitamin B_{12} in quantities up to 1,000 μg/100 g flour did not impact dough handling or fermentation rates of white pan breads, nor did it produce any noticeable sensory and physical changes in the flour [25].

Vitamin B_{12} recommendations

- Vitamin B_{12} (as cyanocobalamin) should be included in the mix of nutrients used in flour fortification. The addition levels are presented in **table 2**.
- Both low- and high-extraction flour should be fortified.
- Efficacy trials to determine effective fortification levels in several locations and among subjects with different ranges of vitamin B_{12} status should be supported.
- Research on the bioavailability of vitamin B_{12} from fortified products, especially in persons with food-bound cobalamin malabsorption, should be supported.
- The impact of flour fortification on the vitamin B_{12} status of target populations should be monitored.

Vitamin A fortification

Vitamin A deficiency is estimated to affect approximately 190 million children under 5 years of age, possibly as many school-aged children and adolescents, and nearly 20 million women during pregnancy and early lactation in the developing world [26]. Fortification of foods with vitamin A is intended to help at-risk groups whose daily requirements are not met because of inadequate intake, absorption, and/or utilization of vitamin A. Fortification of margarine and milk with vitamin A has been practiced in some European countries and North America for many years, while fortification of sugar has also been shown to be effective and sustainable in a number of Latin American countries. Typically, vitamin A fortification programs have sought to deliver 30% to 60% of the Recommended Dietary Allowances (RDAs) for specific target populations [27]. (Quantitatively, the RDAs and Recommended Nutrient Intakes [RNIs] are indistinguishable.)

As with other nutrients, the biologic effectiveness of vitamin A fortification depends on whether target populations consume enough of the proposed food vehicle(s). In addition, the cost of fortification must usually be absorbed by the marketplace for it to be sustainable. Thus, in countries where per capita daily consumption of wheat flour is 75 g or less, adding relatively high amounts of vitamin A (e.g., 5.9 μg retinol activity equivalents [RAE]/g) will only provide approximately 24%, 21%, and 22% of the RDA for adult women, school-aged children, and preschool-aged children, respectively, while the cost of fortification will amount to US$8.62/MT of flour. Alternatively, where wheat flour consumption is high (e.g., > 300 g/day per adult equivalent), a substantially lower level of vitamin A fortification (0.75 μg RAE/g) will increase vitamin A intake by comparable fractions of the RDA, but at a much lower cost of about US$1.10/MT of flour.

The most common vitamin A fortificant for cereals is vitamin A palmitate, a dry form that is stable in flour. With addition levels of up to approximately 500 μg retinol equivalents (RE)/kg, there is little evidence of sensory and physical changes either in the flour or in wheat-based flour products. However, baking of flour

products typically results in approximately 30% loss of the vitamin.

Vitamin A recommendations

Vitamin A fortification of wheat and maize flour in commodity foods should be considered when fortification of more cost-effective food vehicles is not feasible and when the target population consumes enough flour to deliver sufficient amounts of vitamin A. **Table 2** presents the fortification levels necessary to meet roughly 25% of the RDA for women of childbearing age.

When possible, multiple foods should be fortified with fractions of RDA for vitamin A. Options for edible oil fortification should be specifically considered.

Zinc fortification

Although the importance of zinc nutrition is increasingly understood and the International Zinc Nutrition Consultative Group (IZiNCG) has developed guidelines on intervention programs [28], this Second Technical Workshop on Wheat Flour Fortification was the first forum for multisector experts and specialists to directly address zinc fortification of wheat and maize flours.

Infants and young children are believed to be at greatest risk for dietary zinc insufficiency, as are pregnant and lactating women, adolescents, and the elderly. Small-scale efficacy trials indicate that zinc fortification can increase total daily zinc consumption and the amount of absorbed zinc in both children and adults [29].

The quantity of zinc absorbed from fortified flour depends on the amount of regularly consumed fortified flour and the levels of total dietary zinc and phytate. Recent information suggests that flour can be fortified with at least 100 mg zinc/kg flour without adverse effects on the sensory properties or acceptability of zinc-fortified flour products. Although there is no evidence of clinically important adverse effects of zinc fortification on iron or copper status, questions remain about the potential effects of zinc fortification on other minerals [30].

The Workshop concluded that fortification of flour with zinc should be encouraged as a strategy to increase zinc intake in countries with an elevated risk of zinc deficiency, although more information is needed to confirm the effectiveness of zinc flour fortification. In the United States, a number of zinc fortificants meet Generally Recognized as Safe (GRAS) standards, although current evidence suggests that zinc oxide is the most suitable fortificant because of its low cost and negligible effect on sensory characteristics of fortified flour and flour products.

To determine what level of zinc to add to fortified flour as a commodity food staple, the Workshop relied upon simulations of estimated zinc absorption under different dietary conditions based on updated parameter estimates of the Miller equation [31, 32]. In the simulations (based on fortification of 80% to 95% extraction flour), adults were assumed to consume about 3, 5, or 7 mg zinc and 0, 500, or 1,000 mg phytate per day from dietary sources other than flour.

Zinc recommendations

» **Table 2** presents the recommended levels of zinc (as zinc oxide) to add to fortified wheat flour based on ranges of per capita flour consumption and extraction of flour, assuming 5 mg zinc intake and no additional phytate intake from other dietary sources.
» Countries currently regulating addition of zinc to fortified flour should reexamine their requirements based on the above recommendations regarding levels of zinc fortification based on per capita consumption of "fortifiable" flour.

General observations and conclusions of the Workshop

The Workshop participants agreed that flour fortification should be considered as a public health intervention when industrially produced flour is regularly consumed by large population groups in a country. The recommended levels of micronutrients to add to fortified wheat flour based on ranges of per capita flour consumption (**table 2**) have been adopted by WHO as a Meeting Report Interim Consensus Statement [33].

Decisions about which nutrients to add and the appropriate amounts to add to fortified flour should be based on a series of factors. These include the nutritional needs and deficiencies of the population; population coverage and per capita consumption of "fortifiable" flour (i.e., the estimated amount of flour milled by industrial roller mills with a minimum production capacity of > 20 MT/day, produced domestically or imported); sensory and physical effects of the fortificant on flour and flour products; fortification of other food vehicles; dietary intake and consumption of vitamin and mineral supplements; cost; and feasibility. Depending on the target population, fortification of food vehicles other than flour could be considered. The selection of the type and quantity of vitamins and minerals to add to flour, either as a voluntary standard or a mandatory requirement, lies with national decision makers in each country, and therefore the choice of compounds as well as quantities should be viewed in the context of each country's situation.

The Workshop participants also acknowledged that:

» Fortified flour is more nutritious than nonfortified flour.
» Flour fortification is only **one** food-based intervention and is not the only solution to preventing micronutrient malnutrition (fortification of other appropriate food vehicles with the same and/or other nutrients should also be considered if feasible).
» Flour fortification is not a "curative" intervention, but rather a preventive approach to equitably improve the micronutrient status of populations over time.
» Nutrients other than iron, zinc, folic acid, vitamin B_{12}, and vitamin A also are important. During the wheat milling process, many essential vitamins and minerals are removed. Restoration would include fortification with additional B vitamins as well as other vitamins and minerals.
» Although all age and sex subgroups can benefit from flour fortification to some extent, women of childbearing age are the primary target group for most flour fortification programs, because such women are often at risk for vitamin and mineral deficiencies and because they consume sufficient quantities of flour to benefit nutritionally. Flour fortification will be less likely to improve the micronutrient status of infants and toddlers because of low consumption of flour in these age groups.
» The public health impact of flour fortification programs is maximized through the adoption of legislation mandating fortification and enforcement of the program at the national level. Compared with voluntary fortification, mandatory fortification is more likely to result in sustained delivery and availability of fortified flour [1]. Even so, the success of mandatory programs requires a well-organized and supportive milling industry and competent premix manufacturers. Strong public sector collaboration is needed to ensure the safety and efficacy of fortification through regulatory enforcement programs and to build public acceptance through consumer education and social marketing programs.
» Population-based dietary assessments should be encouraged in order to estimate intake distributions of potentially fortifiable food vehicles in countries that are considering fortifying staple commodities.
» Questions remain about fortification standards and practices, and there is no single best approach to flour fortification. Programs should move forward guided by the best available knowledge and practices. National decisions are often based on compromising between fully meeting nutritional needs and other factors, such as not affecting sensory characteristics of flour, consumer acceptance of fortified flour and flour products, and cost. Fortification recommendations need to balance public health benefit against risk, as is done with any other public health intervention program.
» Fortification programs should include a monitoring and evaluation component to determine whether the program is being implemented as planned. The risk of excessive intake and safety of the flour fortification program should be monitored. Flour fortification programs should include appropriate quality assurance procedures in the mills and quality control inspections by food control and regulatory agencies, as well as public health monitoring of the nutrient content of fortified foods and assessment of the population coverage.
» When possible, the efficacy and effectiveness of fortification programs should be evaluated. Better monitoring and documentation of the biologic impact of existing national flour fortification programs on vitamin and mineral status of relevant population groups using WHO/CDC guidelines [1, 10] should be encouraged. Impact measures include the effect on human health and function, including birth defect rates, as well as biochemical and hematologic changes in the population.

Disclaimer

The selection of the type and quantity of vitamins and minerals to add to flour, either as a voluntary standard or a mandatory requirement, lies with national decision makers in each country. This meeting fully recognized this, and any guidance or recommendations should be viewed in the context of each country's situation. In addition, the official normative-setting international organizations that guide countries on food standards are the World Health Organization and the United Nations Food and Agriculture Organization, the CODEX Alimentarius Commission, and the Joint FAO/WHO Expert Committee on Food Additives (JECFA).

The findings and conclusions in this report do not necessarily represent the official position of the organizations of individuals participating in the Workshop, including the Centers for Disease Control and Prevention.

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Acknowledgments

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