

Establishing safe and potentially efficacious fortification contents for folic acid and vitamin B₁₂

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Abstract

Determining the micronutrient contents in fortified foods depends not only on the health goal (additional intake to complement the diet), but also on ensuring that fortification does not raise micronutrient intakes beyond the Tolerable Upper Intake Level (UL), i.e., the safe limit. Technological incompatibility and cost may also restrict the fortification contents. For folic acid, the limiting factor is safety, while for vitamin B₁₂, it is cost. However, adequate fortification contents that are both safe and efficacious can be estimated for both nutrients. In order to obtain the maximum benefit from the fortification programs, three different formulas responding to three categories of consumption, as specified by the median and 95th percentile of consumption, are proposed.

The model presented is based on the estimation of a Feasible Fortification Level (FFL), which then is used to determine the average, minimum, and maximum contents of the nutrients during production, taking into consideration the acceptable variation of the fortification process. Finally, the regulatory parameters, which support standards and enforcement, are calculated by reducing the proportion of the nutrient that is degraded during the usual marketing process of the fortified food. It is expected that this model will establish a common standard for food fortification, and improve the reliability and enforcement procedures of these programs.

The model was applied to flours as vehicles for folic acid in the United States, Guatemala, and Chile. Analysis of the data revealed that, with the exception of Chile, where wheat flour consumption is very high and probably within a narrow range, supplementation with folic acid is still needed to cover individuals at the low end of

consumption. This is especially true when the difference in flour consumption is too wide, as in the case of Guatemala, where the proportional difference between consumption at the 95th percentile of the nonpoor group is as high as 100 times the consumption at the 5th percentile of the extremely poor group. Adoption of fortification content for staple foods near the safe limit brings together the need of restricting the voluntary addition of the specific nutrient to other foods and to dietary supplements.

Key words: Folic acid, fortification, micronutrients, vitamin B₁₂

Introduction

Methods to determine the micronutrient content in fortified foods have evolved over the years. First, there was a simple calculation of the quantity of the nutrient required over the average amount of the food consumed by the target population. In 1974, Guatemala established the amount of vitamin A content to be added to sugar as 15 µg/g, based on the daily requirement of vitamin A for preschool-aged children (at that time 300 µg/day), and the average sugar consumption of that group (20 g/day). A variation of 10% was considered acceptable around that value, i.e., 13.5 to 16.5 µg/g [1]. Other programs found that some nutrients are lost during handling, storage, and transportation. In the case of iodine in salt, it is estimated that 20 µg/g would be sufficient to provide the daily required amount of iodine for most individuals (assuming a daily salt consumption of 10 g/day), but it was advised that in order to compensate for losses, the content should be increased during production to 40 µg/g, and even more if the weather was warm and humid [2]. This additional amount is called the overage. Despite adjustments to guarantee the adequate content of iodine from losses during handling, storage, and transportation, acceptable ranges around the new value associated with variation in the fortification process were not considered.

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A manual for fortification of wheat flour with iron [3] described three procedures to estimate the fortification content. One was based on the calculated nutritional gap (the difference between the recommended intake and the actual intake over the average amount of wheat flour consumed). The second procedure was similar, but calculations were done more for reaching the recommended intake without taking into consideration the dietary intake. The third procedure reinforced that for some iron compounds, the mineral content should not change the sensory properties of the flour. In other words, the iron content was fixed by the technological compatibility between the nutrient compound and the food matrix rather than the estimated nutritional goal. This content was identified as the “known upper-level threshold.” In this case too, acceptable ranges around the estimated average values as a response to process variations were not included.

One of the first cases to recognize that the nutrient content in food fortification should not permit that the usual intake be beyond the Tolerable Upper Intake Levels (UL) was the sugar fortification program in Guatemala [4]. The UL is defined as the highest average daily intake level of a nutrient that is unlikely to pose a potential risk of adverse effects to all apparently healthy individuals in an age- and sex-specific group [5]. A manual on sugar fortification published in 1996 [4] recommended that the maximum content of vitamin A in sugar be 20 µg/g, based on the UL of 3,000 µg/day and the consumption of some individuals at around 150 g/day. The Guatemalan program also found that the narrow fortification range established in the regulation caused a conflict between the industry and the food control authorities when the latter tried to enforce the standards [6]. It was determined that the range was unreachable, for two reasons: the industrial variation of the fortification process was around 30% under optimal conditions, and vitamin A degrades by 50% during 1 year of storage. Thus, it would be normal to expect samples of sugar to contain between 9 and 24 µg/g during the fortification process and values as low as 4.5 µg/g after 1 year of storage. A larger variation would occur if the fortification process was not controlled.

The WHO *Guidelines on Food Fortification with Micronutrients* [7] systematizes the estimation of fortification formulas considering all the aspects mentioned above. This article applies the recommendations of that publication to calculations of the fortification content of folic acid and vitamin B₁₂ in wheat flour. The article also illustrates the use of the method in the United States, Guatemala, and Chile, countries with different patterns of flour consumption.

Analysis of regulations of wheat flour fortification

Current standards in food regulation are not supporting the nutritional goals

The United States [8] and countries in Central America [9] independently reached the conclusion that the enactment and enforcement of fortification content would be easier if only a lower minimum value was established. The industry would be held accountable to calculate suitable overages and maintain the fortification content during the marketing life of the product. Other countries, such as Chile, preferred to use an acceptable range [10].

Table 1 shows the fortification performance in wheat flour fortified with folic acid in Guatemala and Chile. In Guatemala, the results show that the industry is interpreting the “minimum” content as the average of addition. The problem originates from the formulation of the premix, because the millers are only following the instructions of addition that they receive from the manufacturers. As a result of using the minimum content as the average content, the program is only delivering half the amount of folic acid that it planned to provide [9].

In Chile, the variation of the fortification process (43%) was much larger than that calculated for preparing the standard (5%). As consequence, a large proportion of samples were outside of the regulatory range (2.0 to 2.4 mg/kg), and more than 50% of samples were either below or above the regulatory range. If the standard in Chile was enforced to the minimum content of 2.0 mg/kg, the estimated average would be 14.3 mg/kg, and the maximum content 26.6 mg/kg.

In the United States, the situation is different. The minimum content of 1.4 mg/kg for cereal flours was estimated to provide an average of 100 µg/day of folic acid. This content was selected as a compromise to benefit those in need without potentially risking those whose intakes may go beyond the UL [11–13]. The calculations were made as “averages,” but the standards were expressed as “minimums.” When the program was evaluated, it was found that the folic acid content in the

TABLE 1. Performance of wheat flour fortification with folic acid in Guatemala and Chile

Country	Legal levels (mg/kg)	True values (mg/kg)			Estimated values (mg/kg) ^a
		Mean	CV	Range	Range
Guatemala (2004) ^b	≥ 1.8	1.8	23%	0.9–2.5	1.0–2.7
Chile (2000) ^c	2.0–2.4	2.9	43%	1.0–5.9	0.4–5.4

a. Subtracting and adding twice the CV around the mean.

b. Data provided by the National Commission of Fortified Foods (CONAFOR), Guatemala.

c. Data provided by Eva Hertrampf, Institute of Nutrition and Food Technology (INTA), Chile.

foods [14, 15] and the additional intakes of folic acid in the population [16, 17] were nearly twice as much as those originally planned. Nevertheless, a recent paper reports that for women the average additional intakes from fortified cereal flours was as expected [18].

Content information is lacking for fortification with vitamin B₁₂, because this nutrient is just starting to be added to flours in a few countries. Nevertheless, there is no reason to think that the situation described for folic acid would be different in the case of vitamin B₁₂.

The WHO model to estimate fortification contents

The WHO *Guidelines on Food Fortification with Micronutrients* [7] introduced a model to calculate the fortification content and the associated parameters. This model is based on the steps described below.

Defining the program goals

The first consideration for a food-fortification intervention is safety. Most individuals should keep their total intake (from fortified foods, supplements, and in some instances diet) of certain nutrients below the levels that are recognized as safe (Tolerable Upper Intake Levels, ULs) [5, 7]. Folic acid is one of those nutrients, but only in this form and not as dietary folate, which is the natural form in foods. It means that food-fortification program managers must establish the maximum allowable supply of folic acid that would be provided by the program to most members of the population, including those who consume the food vehicles in large amounts. Adverse effects associated with excessive intakes of vitamin B₁₂ have not been found, and hence this nutrient does not have a UL. Nevertheless, it would be prudent to keep this vitamin at an adequate equilibrium with the intake of other nutrients.

The second consideration is related to the magnitude of the additional intake of a specific micronutrient based on the nutritional or health goals. This targeted intake is then used as a reference to assess the contribution of each intervention in a program. For most nutrients (except for iron because of the large individual variation in biological needs), it has been recommended that every individual of the population complement his or her diet to reach the Estimated Average Requirement (EAR) of a given micronutrient [7].

In the case of folic acid, the main motivation to incorporate it into foods is the reduction of neural tube defects (NTD). In the early 1990s two studies confirmed that the intake of folic acid by supplementation (large amounts in daily single doses) prior to conception was able to prevent NTD. Studies showed a daily dose of 4,000 µg/day reduced the recurrence of NTD in the United Kingdom, France, Hungary, the Soviet Union, Israel, Australia, and Canada [19], and a

dose of 800 µg/day prevented the first-time incidence of NTD in Hungary [20]. Almost immediately, the US Public Health Service [21], as well as the United Kingdom Expert Advisory Group [22], recommended that women of childbearing age consume 400 µg/day of folic acid to reduce the potential risk of having an infant with NTD. The proof that 400 µg/day of folic acid, given alone, in supplements was efficacious in reducing NTD came from a study carried out in China from 1993 to 1996, and published in 1999 [23]. Although the study showed 400 µg/day of folic acid is an effective dose, this does not necessarily mean that it is the minimum effective dose. Regrettably, the minimum effective dose for folic acid supplementation may never be known, because now it would be considered unethical to use a lower dose in human studies. However, it is important to point out that a study in Ireland [24] reported in 1992 that three daily doses of 120 µg each for a total daily supply of 360 µg was as effective as the 4,000 µg/day dose recommended in the intercountry study to prevent recurrence of NTD [19]. Although the Irish study may not have the desired statistical power, the fact that a dose 11 times lower than that used in the intercountry study was used deserves attention. Likewise, the minimum efficacious dose of folic acid through food fortification has not yet been determined. However, it is logical to expect that it may be lower than that for supplementation, because it has been found that small amounts of folic acid supplied during the day are better absorbed and converted into tetrahydrofolate (the active form of folate in living beings) than a single large dose. Kelly et al. [25] reported that unmetabolized folic acid is detectable in the blood after a 266-µg single dose or after 800 µg/day when received through small doses during the day. This finding suggested that the metabolic conversion of folic acid into tetrahydrofolate is saturable, and hence large intakes may not be more efficacious.

A temporary solution to the lack of a minimum effective dose of folic acid through food fortification to prevent NTD could be to provide sufficient folic acid for most individuals in the population to reach the EAR of folate. For adults, the EAR is 320 µg/day of Dietary Folate Equivalents (DFE) [26]. EAR is the dietary parameter to be used for population interventions, from which the Recommended Nutrient Intake (RNI) is derived by adding two standard deviations to the EAR in order to cover 97.5% of all individuals in the population. The accepted RNI of folate for adults is 400 µg/day of DFE [5, 27]. Folic acid is approximately 70% more bioavailable than food folate. Therefore, the additional intake of folic acid should be multiplied by 1.7 to calculate the equivalent folate intake in terms of DFE. Thus, 188 µg/day and 235 µg/day of folic acid supply the EAR and RNI values, respectively, of folate for an adult person.

Selecting the proper fortification vehicle

Fortification is a technology that should be supported by formal and centralized industries to ensure it is cost-effective and efficient. The challenge is to find an industrially produced processed food that is widely consumed by the target population in reasonable amounts and, if possible, within a narrow range of variation. Recently, there have been efforts to implement fortification at small-scale operations to reach wider segments of the population. Small-scale operations may prove to be efficacious (biological impact depends on the micronutrient quality and intake, but not too much on the delivery mechanism) with intense attention, training, and funding. However, in the long run, small-scale projects are difficult to extend, maintain, and supervise. In the case of folic acid and vitamin B₁₂, the most common food vehicles are flours (mainly wheat flour, and in some countries, flours from maize and other cereals). Regrettably, in most countries of the developing world, the production system or the consumption patterns of flours do not favor wide population coverage, and dependence on dietary supplementation still may remain.

Determining the feasible fortification level

The WHO *Guidelines on Food Fortification with Micronutrients* [7] define the Feasible Fortification Level (FFL) as the content that will provide the greatest number of potentially at-risk individuals with an adequate intake without exposing some individuals of the population to an unacceptable potential risk of excess intakes. At the same time, this content is technologically compatible with the food matrix, and the increment in the food price due to fortification is reasonably low.

The first constraint for the establishment of the FFL is safety, which is calculated by using the UL values and the largest level of consumption of the food in the population. It is the highest average fortification content that would be possible for avoiding the potential risk of approaching the UL, and it is calculated by equation 1.

$$\text{Safe limit (mg/kg)*} = \frac{\text{UL (mg) - amount of micronutrient from diet (mg) and supplements**}}{\text{95th percentile consumption (kg)}} \quad (1)$$

It is difficult to determine the 95th percentile of consumption of a specific food in a population. A practical way to estimate this value in the absence of true data is to multiply the per capita intake by three to four, depending on the estimated distribution of the consumption pattern. **Table 2** presents the case for folic acid with different consumption patterns of wheat flour.

Once the safe limit has been estimated, this value is assessed for its technological compatibility with the food matrix. In the case of folic acid, the contents present in **table 2** are unnoticeable in the flour, and hence the *technological constraint* is not an issue with folic acid.

The third constraint to evaluate is cost. In the case of the fortification of staple foods, experience has shown that a staple can tolerate up to a 2% increase in price. At the highest fortification content of folic acid presented in **table 2** (5.0 mg/kg), the increase in price would be around US\$0.45/MT (1 metric ton [MT] = 1,000 kg), is approximately 0.11% of the wheat flour price (assuming US\$0.40/kg). In summary, wheat flour fortification with folic acid does not have a *cost constraint*.

The FFL is determined by the lowest nutrient content among the safety, technological, and cost constraints. Therefore, in the case of folic acid in wheat flour, the limiting factor is safety, and the calculated safe limits become the FFL.

A similar analysis with vitamin B₁₂ demonstrates that this nutrient does not have a safety constraint because it does not have a known UL value. Furthermore, the contents that have biological significance (e.g., to supply 50% to 100% of EAR), as shown in **table 2**, are technologically compatible with flour. The limiting factor for vitamin B₁₂ is cost. Nevertheless, at the high-

* A more accurate calculation may consider losses during distribution and storage, as well as losses during food preparation. However, because it is very difficult to estimate these values for many different situations, and because the premix also contains overages for compensate in part for these losses, it is preferable to use this simplified approach.

** Folic acid supplements to prevent NTD are recommended only for women prior to conception, and therefore the amount supplied by this means may not be included in this calculation, because these supplements are intended neither for the general public nor on a permanent basis.

TABLE 2. Feasible fortification levels of folic acid and vitamin B₁₂ in wheat flour at different consumption patterns.

Amount of consumption (g/day)		Folic acid ^b (mg/kg)	Vitamin B ₁₂ ^c (mg/kg)
Median	95th percentile ^a		
200	600	1.7	0.005
100	350	2.9	0.010
50	200	5.0	0.020

a. From 3 to 4 times the median or the mean.

b. Using equation 1 and assuming that this is the only source of folic acid.

c. In order to supply 50% EAR for adults (2 µg/day) at the mean amount of consumption.

est content shown (0.020 mg/kg), it is about US\$0.84/MT, which is approximately 0.21% of the wheat flour price. This means vitamin B₁₂ fortification is still affordable in wheat flour.

Assessing the fortification content

Once the FFL has been established, it should be assessed for its contribution to reaching the biological goal. In the case of folic acid, the contents shown in **table 2** will supply to the average consumer 250, 290, and 340 µg/day, depending on the consumption values of wheat flour around 50, 100, and 200 g/day at each specified range of consumption. These intakes are equivalent to 425, 493, and 578 µg DFE/day, respectively, which in turn represent 133, 154, and 180% of the EAR of folate for adults, respectively.

Similarly, in the case of vitamin B₁₂, the selected contents (**table 2**) would provide 1.0 µg/day to the average consumer, which is equivalent to 50% of the EAR values of vitamin B₁₂ for adults (2 µg/day). As in the case with folic acid, absorption of the synthetic form may be higher than that of the forms available in foods. In summary, the fortification contents defined in **table 2** appear to be safe, feasible, and potentially efficacious.

If a country knows the nutritional gap of these nutrients in the diets of its population, it should be possible to design a program specifically for filling that gap, and therefore lower the contents and reduce the cost of the fortification intervention.

Establishing the production parameters

Once the fortification content has been decided, the rest of the work is a matter of simple calculations. The next step is to define the production parameters that would be used by the factories to base their quality control procedures. Those parameters are the **average**,* the **minimum**, and the **maximum** fortification contents.

The average content is calculated by adding to the selected fortification content the natural intrinsic content of each micronutrient in the unfortified food vehicle. Wheat flour contains folate, but not folic acid. Thus, if the analytical assay measures only folic acid, there is no need to add the intrinsic amount of folate. Otherwise, the natural amount of folate in the flour should be added. The intrinsic folate content in the unfortified food depends on the extrusion factor (degree of purity due to the removal of the bran and other structures of the wheat grain). For example, flour of 77% extraction (refined) has a folate content of 0.2 mg/kg, and flours with higher extrusion factors (less refined, or whole flour) have a folate content that can be around 0.4 mg/kg. Flours do not contain vitamin

B₁₂, and so the selected fortification content becomes the average fortification content.

The minimum fortification content is the amount calculated by reducing the average content by two coefficients of variation (CV) of the food-fortification process. **Equation 2** shows this calculation:

$$\text{Minimum (mg/kg)} = \text{Average} \times [1 - (2 \times \text{CV of the nutrient content during the fortification process } (\%)/100)] \quad (2)$$

The maximum fortification content is the amount calculated by increasing the average content by 2 CV of the food-fortification process. **Equation 3** shows this calculation.

$$\text{Maximum (mg/kg)} = \text{Average} \times [1 + (2 \times \text{CV of the nutrient content during the fortification process } (\%)/100)] \quad (3)$$

In the case of wheat flour fortification with folic acid, it has been found that a typical CV for a well-established and controlled mill is around 20% to 25%. Therefore, the minimum and the maximum contents are below and above 40% to 50% of the average content, respectively. **Table 3** shows those values using a CV of 25% for both folic acid and vitamin B₁₂. Larger values may be common in many countries, but if they are, then the allowed range of the micronutrient content would be too wide and it may lose its usefulness. Therefore, if the CV of a specific mill is found to be larger than 25%, efforts should be made to improve its performance.

Establishing the regulatory parameters

Regardless of whether the fortification intervention is voluntary or mandatory, suitable standards (technical specifications) should be enacted. Specific contents for the added micronutrients should be stipulated and government authorities should ensure industry compliance based on those values.

Two regulatory parameters are essential: the Legal minimum Level (LmL) for all nutrients and the Maximum Tolerable Level (MTL) for those nutrients whose consumption in excess is of concern. Vitamin B₁₂ does not have a UL value, and does not need to have an MTL, as illustrated in **table 4**.

The LmL is the minimum micronutrient content in the fortified food as defined by regulations and standards, and should appear on the label of the fortified food. This value is obtained by reducing the minimum fortification content by an amount equivalent to the average loss of the micronutrient during distribution and storage and within the marketing life of the product. **Equation 4** shows this calculation:

* This average content is identified as the **target** fortification level in the WHO Guidelines on Food Fortification with Micronutrients [7].

TABLE 3. Fortification parameters at production of flour fortification with folic acid and vitamin B₁₂^a

Amount of consumption (g/day)		Folic acid content (mg/kg)				Vitamin B ₁₂ content (mg/kg)			
Median	95th percentile ^b	Selected ^c	Minimum	Average ^d	Maximum	Selected	Minimum	Average	Maximum
200	600	1.5	0.9	1.7	2.6	0.005	0.003	0.005	0.008
100	350	3.0	1.6	3.2	4.8	0.010	0.005	0.010	0.015
50	200	5.0	2.6	5.2	7.8	0.020	0.010	0.020	0.030

a. Considering the intrinsic amount of the nutrient in the unfortified flour. In this case, 0.2 mg/kg of folate in low-extraction (refined) wheat flour was added to the selected fortification level to estimate the average content during production. The minimum and maximum in production are calculated by subtracting and adding 2 CV (25%) of the fortification process, respectively, to the average.

b. From 3 to 4 times the median (or the mean).

c. Assuming that this is the only source of folic acids; countries should select their levels according to the presence of other interventions.

d. Includes the intrinsic content of the nutrient in the unfortified food.

TABLE 4. Regulatory parameters of flour fortification with folic acid and vitamin B₁₂^a

Amount of consumption (g/day)		Folic acid contents (mg/kg)		Vitamin B ₁₂ content (mg/kg)	
Median	95th percentile ^b	Legal minimum	Tolerable maximum	Legal minimum	Tolerable maximum
200	600	0.6	2.6	0.002	N/A
100	350	1.1	4.8	0.004	N/A
50	200	1.8	7.8	0.008	N/A

a. The legal minimum is calculated by subtracting the expected loss during the marketing life of the product (30% for folic acid, and 10% for vitamin B₁₂). The tolerable maximum coincides with the maximum level of production, but it is not applicable (N/A) to vitamin B₁₂, because this nutrient does not have an established UL value.

b. From 3 to 4 times the median (or the mean).

$$\text{LmL (mg/kg)} = \frac{(\text{minimum fortification content mg/kg}) \times (\text{4})}{(1 - \text{proportion of losses})} \quad (4)$$

The Maximum Tolerable Level (MTL) is the maximum micronutrient content that a fortified food can present as established in food law; this minimizes the potential risk of excess intakes of micronutrients. **Table 4** shows the LmL and the MTL for folic acid and vitamin B₁₂ at different consumption patterns. It is assumed there will be a loss during storage and transportation of 30% for folic acid and 10% for vitamin B₁₂. These losses depend on the nature and quality of the fortificant, as well as the environmental conditions. High humidity has been identified as one of the most deleterious factors, and high temperatures accelerate the decay of the vitamins. The difference between the estimated values of LmL and the MTL is apparently large, but this is a normal situation, even for mills operating at high quality standards.

Defining the premix formulation

The last step in the preparation of a food-fortification intervention is the formulation of the micronutrient premix. The highest dilution rate, i.e., the lowest volume (or weight) of the premix added into the food to be fortified, is desirable for reducing costs of materials, storage, and transportation. The dilution rate is in

direct proportion to the size of the factory, so smaller industries may need to dilute the premix in order to reduce the dilution factor. This process may be done in-house. The largest dilution rate that is feasible is dictated by the weight, volume, purity, and proportions of the micronutrients in the fortificants. In general, both folic acid and vitamin B₁₂ are part of premixes with other micronutrients that are added into flour. That means the dilution factor depends on the whole mixture. In wheat flour fortification, the typical dilution factors are from 1:2,500 to 1:5,000. Any decision about the dilution factor must be made in coordination with the industry that produces premixes, because other ingredients such as fillers, stabilizers, and anticaking agents may be needed.

Usually, the composition of the premix has been left to the industry. However, experience has shown that it is important that governments also regulate the nutritional content of the premix, because the quality of the whole process depends on it. Furthermore, an important quality control component is based on the proportion of premix that is added into the food, which in many developing countries may be the most practical parameter of the food control system. If the quality of the premix is guaranteed, the probability of a well-fortified food increases.

The nutrient content of the premix can be easily calculated by multiplying the selected fortification

content by the dilution factor. In turn, the new value becomes the minimum nutrient content of the premix. The premix manufacturers may wish to add an overage to ensure that the minimum content for the premix remains valid until the expiration date. The premix overage should not be too large, because the packaging should be of the best quality to protect the micronutrient content against deleterious environmental factors. By using the procedure described in this article, there is no need to estimate overages for the fortified food itself.

Applying the method for folic acid fortification in three different settings

United States

The intake of folate and folic acid from the diet, fortified foods, and supplements by women of childbearing age has been recently reported [18]. This information corresponds to the years 2001–2002, as gathered by the National Health and Nutrition Examination Survey (NHANES). **Table 5** summarizes the estimated intakes through food fortification and supplementation by women of different ethnic groups.

Hispanic women showed the largest cereal flour

consumption value with a 95th percentile of about 300 g/day. Flours are also consumed by men, and in larger amounts. If one assumes that male consumption is 30% larger than that of females in the United States, then the consumption at the 95th percentile of males is approximately 400 g/day. Therefore the safe limit for folic acid fortification in the United States (using **equation 1**) is 2.5 mg/kg. If this content is selected as the safe limit of folic acid fortification in the United States, the corresponding LmL and the MTL would be 0.9 and 4.1 mg/kg, respectively. The calculated LmL is lower than the current regulatory minimum of 1.4 mg/kg, which means that the United States is using fortification of cereals above the maximum of its capacity.

Another interesting finding of the dietary analysis of consumption in 2001–2002 [18] is the increment differences in the total folate intake at the 95th percentile as compared with that at the 5th percentile. **Table 6** shows the folate intake, expressed as DFE, in the absence and in the presence of food fortification and supplementation with folic acid. Intake of folate increased 2.2 times for women at the 5th percentile and 5.4 times for women at the 95th percentile. Raising the fortification content will only magnify the differences between individuals at the 95th percentile over individuals at

TABLE 5. Additional intake of folic acid in fortified foods and estimated cereal flour consumption in the USA according to race or ethnicity

Race or ethnicity	Folic acid intake ($\mu\text{g}/\text{day}$) geometric means			Consumption of cereal flours (g/day) ^a		
	Cereal flours ^b	Breakfast cereals	Supplements ^c	5th percentile	50th percentile	95th percentile
Non-Hispanic black	98	11	81	17	70	245
Hispanic	117	11	79	21	84	294
Non-Hispanic white	104	29	56	18	74	259
All	105	23	63	18	75	262

a. Assuming a folic acid content in cereal flours of 1.4 mg/kg, and that 50th percentile = geometric mean, 5th percentile = 50th percentile/4, and 95th percentile = 50th percentile \times 3.5.

b. Information provided by R.J. Berry, Centers for Disease Control and Prevention (personal communication, May 2007).

c. Data from Yang et al. [18].

TABLE 6. Total folate intake in the USA in the absence and in the presence of food fortification and supplementation with folic acid^a

Race or ethnicity	Total dietary intake (DFE $\mu\text{g}/\text{day}$)			Total intake with interventions (DFE $\mu\text{g}/\text{day}$) ^b		
	5th percentile	50th percentile	95th percentile	5th percentile	50th percentile	95th percentile
Non-Hispanic black	39	128	366	75	378	1,709
Hispanic	57	147	395	100	448	1,796
Non-Hispanic white	52	158	393	125	614	2,192
All	49	152	392	108	527	2,126

a. Data from Yang et al. [18].

b. Intake of folic acid from fortified foods and supplements has been multiplied by 1.7 to express it in Dietary Folate Equivalents (DFE). The corresponding EAR and RNI values are 320 μg DFE/day and 400 μg DFE/day, respectively.

the 5th percentile, with small additional benefits for individuals at the 5th percentile, but large potential risks to individuals at the 95th percentile.

Table 6 also shows that the population of the United States is folate-deficient in the absence of folic acid interventions; the mean folate intake was lower than the EAR (320 µg/day) for all groups. In the presence of the interventions, most women of childbearing age have raised their folate intake above the EAR. However, those at the 5th percentile are still receiving only one-third of the EAR value of folate. If more folic acid is necessary, it should come through supplementation, because food fortification is already being used to its maximum potential.

In the United States, reduction of NTD has been reported from 10.86 to 7.44/10,000 pregnancies or 6.56 to 5.03/10,000 births [28], or 3.78 to 3.05/10,000 births [29]. Those are reductions of 31%, 23%, and 19%, respectively, which are attributable to the folic acid interventions.

The case of Canada can be compared with that of the United States. The Public Health Agency of Canada presents on its webpage a summary of the evaluation of food-fortification efforts with folic acid [30]. The prevalence of NTD decreased from 43.5/10,000 pregnancies in 1991–1993 to 9.6/10,000 pregnancies in 1998–2001, coinciding with the mandatory introduction of wheat flour fortification with folic acid. This is a reduction of 78%, which is associated with a large initial prevalence as compared with the United States. Similar information has appeared in other publications from Canada [31, 32]. The report by the Public Health Agency of Canada [30] estimated that prior to fortification, the mean folate intake was 260 µg/day, which, similar to the United States, is below the EAR of that nutrient, suggesting that the Canadian population was also folate-deficient. The mean and the maximum additional intakes of folic acid by Canadian women, through fortification, have been estimated at 70 and 235 µg/day, respectively [30], which are slightly lower than those calculated for the United States. The total

average intake of folate in Canada, expressed in DFE, is 379 µg/day, which is similar to the mean folate intake of non-Hispanic black women in the United States (**table 6**), the ethnic group in the United States that has showed the least improvement in folic acid intake. Here it is important to emphasize that, under this condition, the reduction of NTD in Canada was 78%, showing that the magnitude of the difference is associated with the initial prevalence values and not necessarily with the magnitude of the folic acid intake. These results confirm the deductions by Daly and colleagues [33] that folic acid intake raised by 100 µg/day would result in substantial reduction in NTD. Therefore, it is important to identify the causes of the new NTD cases in the United States and Canada. The results of these studies will clarify whether those cases are due to low folate intake or to other reasons.

Guatemala

The pattern of consumption of wheat flour by different socioeconomic groups in Guatemala was estimated using data of food purchases from the nationally representative 2000 Guatemalan Living Standards Measurement Survey [34]. **Table 7** presents the estimated consumption amounts for adult males and adult females, assuming that the latter group has a consumption that is 0.677 times that of the males based on the comparative energy intake of those two groups in the country. The consumption at the 95th percentile is estimated here by multiplying the median of consumption by 3.5, and the 5th percentile by dividing by 4. The results suggest that in Guatemala, wheat flour consumption is very different among the socioeconomic groups; the nonpoor women consume almost 15 times more of products made with wheat flour than the extremely poor group. The estimated consumption of the nonpoor group is similar to that of women in the United States. The difference between the the 95th percentile of the nonpoor group over the 5th percentile of the extremely poor and poor groups is 111 and 37 times higher, respectively, which shows that even if the

TABLE 7. Estimated consumption of wheat flour by adult men and women and folic acid intake by women in Guatemala^a

Socioeconomic group	Wheat flour consumption (g/day) ^b				Folic acid intake (µg/day) ^c		
	Men	Women			Women		
	50th percentile	5th percentile	50th percentile	95th percentile	5th percentile	50th percentile	95th percentile
Extremely poor (11%)	7	2	5	15	6	15	45
Poor (35%)	25	6	17	51	18	51	153
Nonpoor (54%)	110	12	74	222	36	222	666
All (100%)	50	11	34	102	33	102	306

a. Data from Imhoff-Kunsch et al. [34].

b. Median 50th percentile for women was estimated multiplying the male consumption by 0.677, which is the proportional energy intake of women as compared with that of men; 5th and 95th percentile levels of consumption were estimated by dividing the median by 3 and multiplying by 3, respectively.

c. Assuming a folic acid content of 3.0 mg/kg.

presence of folic acid fortification, many women of the extremely poor and poor groups would still receive very small amounts of folic acid. In conclusion, for Guatemala, the use of supplements is indispensable despite the presence of food fortification.

The consumption of wheat flour by males at the 95th percentile in Guatemala would be approximately 330 g/day (110 g/day \times 3.0). The safe limit of folic acid fortification would then be 3.0 mg/kg (1,000/330 = 3.0). If one accepts this value as the FFL of folic acid in wheat flour, then the calculated LmL and MTL, under the same conditions as specified above, are 1.1 and 4.8 mg/kg, respectively. The current content of folic acid in wheat flour in Guatemala (1.8 mg/kg) is lower than the calculated mean (3.0 mg/kg), but the legal minimum content of 1.8 mg/kg is higher than the expected LmL (1.1 mg/kg).

Chile

The consumption of refined wheat flour in Chile is much higher than in the United States and Guatemala, and the mean consumption by women has been estimated at 200 \pm 20 g/day [10]. If one assumes that the 95th percentile is twice the mean, because of the very large consumption pattern, and the 5th percentile is three times lower than the mean, then the range of wheat flour consumption is between 67 and 400 g/day. Males at the 95th percentile may be consuming around 30% more, i.e., around 500 g/day. Thus, in Chile the safe limit would be 2.0 mg/kg.

Table 8 shows the additional intakes for women of childbearing age at the estimated consumption profile using the contents of 2.0 mg/kg, as well as the current 2.9 mg/kg content found in the fortified wheat flour in the ongoing program. The following deductions can be obtained from this case: the two analyzed formulas provided relatively large amounts of folic acid (above 100 μ g/day) to most women, and hence supplementation may not be needed; the current fortification practice provides plenty of folic acid to everyone in the popu-

TABLE 8. Estimated intake of folic acid from consumption of fortified wheat flour in Chile

Folic acid fortification content (mg/kg)	Folic acid intake by women (μ g/day)		
	5th percentile (67 g/day) ^a	Mean/median (200 g/day)	95th percentile (400 g/day)
2.0 (estimated FFL ^b)	134	400	800
2.9 (current reported content)	194	580	1,160

a. 5th and 95th percentile levels of consumption were estimated by dividing the reported mean/median by 3 and multiplying by 2, respectively.

b. FFL, feasible fortification level.

lation, to the point that some men are having intakes as high as 1,508 μ g/day (i.e., 30% above the estimated 1,160 μ g/day for women at the 95th percentile) or even more; and in Chile, folic acid content around 2.0 mg/kg is useful for NTD prevention but requires monitoring for avoiding the potential risk of excessive intakes. If the selected fortification content were 2.0 mg/kg, then the LmL and the MTL would be 0.7 and 3.3 mg/kg, respectively. Thus, the current regulatory range of folic acid fortification (2.0 to 2.4 mg/kg) appears to be not only too large but also too narrow.

Final remarks

The model described here combines the public health interest with a systematic procedure to estimate the food control and regulatory parameters based on the inherent characteristics of the fortification process as well as the usual stability of nutrients during distribution and marketing. The application of the model might not only contribute to programs to reach their intended goals, but could also facilitate the food control actions for checking their performance using sensible standards.

The model is also useful for the planning, designing, and prediction of the health implications of food-fortification programs, and for demonstrating how to combine them with other interventions in order to reach the neediest individuals. It is clear that, with the exception of salt fortified with iodine, it is not possible to recommend a worldwide applicable fortification formula for other food vehicles and micronutrients, because the variation of consumption of the foods is too large among countries and even among different socioeconomic groups within countries. Thus, fortification formulas should be tailored to country conditions, although some categorizations can be made. For example, in the case of flours, the consumption ranges of 10 to 200 g/day, 25 to 350 g/day, and 50 to 600 g/day, with per capita values of 50, 100, and 200 g/day, may be useful. Here it is important to realize that the intake of individuals at the low end of consumption would be only a proportion of the intake of the individuals at the high end; for example, in this case, the proportions are 1/20, 1/14, and 1/12, respectively. The understanding of this basic relationship helps to explain why the inclusion of supplementation is still needed for some micronutrients, because the maximum fortification content is limited by the value of the UL and the consumption figure at the 95th percentile. This is the situation with folic acid. If the fortification formula is selected to avoid giving the individuals in the 95th percentile more than the current recommended UL (1,000 μ g/day), then the expected intakes for individuals in the specified 5th percentile values are 50, 72, and 85 μ g/day, respectively. If the health goal is to provide 400 μ g/

day additional folic acid, then the use of supplements is still essential. Furthermore, using the highest possible level of fortification implies that addition of this nutrient to other foods or dietary supplements should be restricted. Women planning to become pregnant might still consume supplements with folic acid, because in this case the treatment is temporary and targeted to a specific period of life.

The value of the safe limit for folic acid fortification, which also limits the additional supply of this substance to individuals in the 5th percentile consumption pattern, might change if the UL value were larger. However, the estimated benefit may also be different if the health goal would not require a large intake of folic acid. For example, if the efficacious dose were 100 µg/day using food fortification instead of the 400 µg/day as is recommended now for supplementation, then a smaller number of women would be targeted to receive supplements in addition to the food-fortification programs. This is why determining the minimum efficacious dose of folic acid using both food fortification and supplementation is important. The epidemiological results of the reduction of NTD in the United States [28, 29] and Canada [30–32] suggest that intakes of folic acid around 100 µg/day, through food fortification, are efficacious. However, the conditions may vary from country to country, and this is why the reduction of NTD should be associated with folate status instead of with the absolute additional intake of folic acid. In the absence of this information, it seems rational to establish as the temporary health goal for food fortification that the program reaches the EAR of folate.

Concerns have been raised about the possible adverse effects of high intakes of folic acid in nontarget subgroups of the population [35–37], especially for individuals who are vitamin B₁₂ deficient [38–40]. The incorporation of vitamin B₁₂ into flours is technically feasible and affordable, and therefore this nutrient should also be added as part of the fortification formula

of flours using the contents specified in **tables 2 to 4**. It is important to point out here that in developing countries, many other micronutrients are deficient in the diet, and some of them have important roles in the cycles where folate and vitamin B₁₂ intervene, such as vitamin B₂, vitamin B₆, and even niacin. Thus, the more sensible approach, not only for the prevention of NTD but also to improve the general well-being of the population, is the promotion of nutritionally balanced diets. Flours are excellent vehicles of fortification, and they are able to carry several micronutrients at the same time and at very affordable costs [41]. Thus, countries should not miss the opportunity to improve the overall diets of their populations by adopting more integrated fortification formulas. Finally, whatever the micronutrient added, and because flours are consumed by everyone in an almost permanent manner, the establishment of a monitoring and evaluation system should be a requirement of a food-fortification program [42]. Both the beneficial impacts and the potential adverse effects should be systematically and constantly monitored.

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