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USAID/GAIN MICRONUTRIENT FORTIFICATION PROJECT IN CENTRAL ASIA, AFGHANISTAN AND PAKISTAN

ON THE NECESSITY OF LOW EXTRACTION WHEAT FLOUR FORTIFICATION WITH VITAMIN B12 IN CENTRAL ASIA REPUBLICS, AFGHANISTAN AND PAKISTAN

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1. Background

In October 2015, GAIN and USAID co-hosted a regional stakeholder meeting on food fortification that brought together representatives of governments, private sector, academia, civil society, and development partners from Kazakhstan, Afghanistan, Pakistan, Tajikistan, and Uzbekistan. Several joint priorities were identified, including facilitation of cross-border trade through harmonized standards of wheat flour. A Regional Expert Group on Wheat Flour Fortification Standards and Potential for Regional Harmonization was formed. It is chaired by the Kazakh Academy of Nutrition (KAN) and includes specialists representing national standards committees, government regulatory agencies, research institutions, wheat flour producers and respective business associations from six countries - Kazakhstan, Afghanistan, Pakistan, Tajikistan, Kyrgyzstan and Uzbekistan.

During November 2015-January 2016, in consultation with international experts, the KAN members of the group developed a technical justification for harmonizing fortification standards for low extraction (refined, white) flour used in all countries of the region, and high extraction (whole) flour mainly used in Afghanistan and Pakistan. These two analytical documents were shared with the expert group for comments and additional input. The documents highlight the need to ensure, at a minimum, adequate and comparable amounts of bioavailable micronutrients in all countries of the region as specified in the WHO recommendations.

Two options for the micronutrients in premix were proposed:

High extraction flour	Low extraction flour
Iron (NaFeEDTA)	Iron (NaFeEDTA and Ferrous sulfate, dried to increase iron content at a reasonable cost)
Zinc (Zinc oxide)	Zinc (Zinc oxide)
Vit B_9 (Folic acid)	Vit B_9 (Folic acid)
Vit B_{12} (Cyancobalamin)	Vit B_{12} (Cyancobalamin)
	Vit B_1 (Thiamine)
	Vit B_2 (Riboflavin)
	Vit B_3 (Niacin)

At the Technical Meeting of Regional expert group on harmonization of wheat flour fortification standards in Central Asian Republics, Afghanistan and Pakistan, in Almaty City, 14-15 March 2016, the experts arrived at a consensus and agreed on the following premix composition to harmonize standards on low and high extraction wheat flour fortification, which is fully consistent with the recommendations of the World Health Organization [1]:

High extraction flour		Low extraction flour	
Micronutrients	Levels, ppm	Micronutrients	Levels, ppm
Iron (NaFeEDTA)	15,0	Iron (NaFeEDTA)	15,0
Zinc (Zinc oxide)	30,0	Zinc (Zinc oxide)	30,0
Vit B_9 (Folic acid)	1,0	Vit B_9 (Folic acid)	1,0
Vit B_{12} (Cyancobalamin)	0,008	Vit B_{12} (Cyancobalamin)	0,008
		Vit B_1 (Thiamine)	2,0
		Vit B_2 (Riboflavin)	3,0
		Vit B_3 (Niacin)	10,0

At the same time, Regional Expert Group invited the Kazakh Academy of Nutrition (KAN) to develop a justification to support wheat flour fortification with vitamin B12 for target countries of the region before May 1, 2016.

2. Physiological functions of vitamin B12

Vitamin B12 (cobalamin) is a water-soluble vitamin. After the body uses these vitamins, leftover amounts leave the body through the urine. Vitamin B12, like the other B vitamins, is important for protein metabolism. It helps in the formation of red blood cells and in the maintenance of neurological function and the central nervous system. Vitamin B12 is also essential for DNA synthesis and for cellular energy production, the latter mainly from odd-chain fatty acids [2].

In mammalian cells there are only two vitamin B12-dependent enzymes [3]. Vitamin B12 functions as a cofactor for methioine synthase and L-methylmalonyl-CoA mutase [4].

Vitamin B12 (cobalamin) is a cofactor in the synthesis of an amino acid, methionine, and which is the main source of methyl groups for protein, nucleic acids, and neurotransmitter synthesis. Its metabolic role is closely linked to that of folate in that one of the vitamin B12-dependent enzymes, methionine synthase, is vital to the functioning of the methylation cycle in which 5-methyltetrahydrofolate acts as a source of methyl donor groups which are necessary for cell metabolism and survival. Deficiency of this vitamin can thus impair the utilization of folate and causes neurological deterioration, megaloblastic anaemia, elevated plasma homocysteine and possibly, impaired immune function. In infants and young children it can cause severe developmental delays [5].

Thus, the metabolism and the functions of vitamin B12 and folic acid are closely linked. Deficiency of vitamin B12, similar to that of folate, has been associated to the occurrence of neural tube defects [6-10].

3. Food sources

Food sources of vitamin B12 are the only animal products such as meat, eggs, and dairy and fish products [11, 12]. Plants do not contain this vitamin. Most microorganisms, including bacteria and algae, synthesize vitamin B12, and they constitute the only source of the vitamin, that is then transferred to tissues and organs of the animals where those microorganisms live [13]. The vitamin B12 synthesized in microorganisms enters the human food chain through incorporation into food of animal origin. In many animals, gastrointestinal fermentation supports the growth of these vitamin B12 synthesizing microorganisms, and subsequently the vitamin is absorbed and incorporated into the animal tissues. Because plants do not synthesize vitamin B12, individuals who consume diets completely free of animal products (vegan diets) are at risk of vitamin B12 deficiency.

4. Absorption

The absorption of vitamin B12 in humans is complex [14]. Vitamin B12 in food is bound to proteins and is only released by the action of a high concentration of hydrochloric acid present in the stomach. This process results in the free form of the vitamin, which is immediately bound to a mixture of glycoproteins secreted by the stomach and salivary glands. These glycoproteins, called R-binders (or haptocorrins), protect vitamin B12 from chemical denaturation in the stomach.

The stomach's parietal cells, which secrete hydrochloric acid, also secrete a glycoprotein called intrinsic factor. Intrinsic factor binds vitamin B12 and ultimately enables its active absorption. The vitamin B12–intrinsic factor complex then proceeds to the lower end of the small intestine, where it is absorbed by phagocytosis by specific ileal receptors [13].

Individuals consume about 2.4 μg vitamin B12 per day, of which **50-60% is absorbed** [15]. Body's ability to absorb vitamin B12 from dietary supplements is limited by the capacity of

intrinsic factor. Only about 10 mcg of a 500 mcg oral supplement is actually absorbed in healthy people [4]. Following absorption, vitamin B12 is stored in the liver. A small proportion (1–5%) of free vitamin B12 is absorbed from the intestine by passive diffusion in an intrinsic factor independent process.

5. Indicators of deficiency

Vitamin B12 status is usually assessed by measuring concentrations in plasma or serum. Although elevated urinary and plasma methylmalonic acid (MMA) levels are more specific, and often more sensitive, indicators of vitamin B12 deficiency. However, MMA concentrations are more difficult and expensive to measure than those of vitamin B12. Elevated homocysteine is a good predictor of vitamin B12 status (as also for folate status).

The following cut-offs are used to define of Vitamin B12 deficiency [16-18]:

<150 pmol/l (<203 μg/l) of Vitamin B12 in serum or plasma (reflects both recent intake and body stores) are indicative of deficiency; 150-221 pmol/l (203-300 μg/l) suggest depletion [19] and some authors have also suggested the values of 221-300 pmol/l (300-406 μg/l) for identifying marginal values [20]. If values above the cut-off do not necessarily indicate adequate status; if values are marginal, analysis of serum methylmalonic acid is indicated);

>271 nmol/l of MMA in serum or plasma (increased when supply of vitamin B12 is low; preferred indicator since increased levels are highly specific to vitamin B12 deficiency);

12–16 mmol/l (1.62–2.2 mg/l) of total homocysteine (free and bound) in plasma (is a good predictor of vitamin B12 status: it is also increased in cases of inadequate folate status, and therefore it is not specific. Homocysteine concentrations also vary by vitamin B2 and B6 status, and they are influenced by gender, race and renal insufficiency.

6. The causes of deficiency

The main risk factors for Vitamin B12 deficiency are: low intakes of animal products; malabsorption from food due to gastric atrophy induced by Helicobacter pylori, or bacterial overgrowth; genetic disorder of vitamin B12 metabolism. Deficiency linked to improper absorption rather than low consumption is the main reason of vitamin B12 in developed countries [21, 22]. However, in developing or in transition countries, However, in developing or in transition countries, the primary cause of vitamin B12 deficiency might be insufficient consumption of animal products. An inadequate intake, combined with increased requirement, and disturbed absorption (malabsorption) are exacerbating causes.

The presence of internal factors in the digestive tract is an essential condition for absorption of vitamin B12 from food. In this regard, the vitamin B12 deficiency in humans can be developed because of its absorption disorders in the gastrointestinal tract in atrophic gastritis, surgical resection and removing the stomach. This is due to the secretion disorders of "intrinsic factor" which is necessary for absorption of vitamin B12. Normally this factor is released by cells of the gastric mucosa. Anemia caused by a lack of or reduction in the secretion of intrinsic factor is called pernicious anemia [23]. Decreasing diet energy intake to reduce body weight, which leads to a decrease in vitamins and minerals consumption may contribute to the development of vitamin B12 deficiency.

Besides deficiency of intrinsic factor, the most common causes of vitamin B12 deficiency are the following: poor nutrition (especially veganism), old age (decreased absorption), increased demand (early childhood, pregnancy and lactation), alcoholism, long-term consumption of hypnotics, stomach injury (including surgical, autoimmune, and infectious and others), pancreas, and liver disease. Since vitamin B12 deposited in the liver, liver disease (especially viral) lead to a loss of vitamin in its depo.

Pernicious anemia.

Malabsorption of vitamin B12 can occur at several points during digestion [14]. By far the most important condition resulting in vitamin B12 malabsorption is the autoimmune disease called pernicious anaemia (PA). In most cases of PA, antibodies are produced against the parietal cells causing them to atrophy, and lose their ability to produce intrinsic factor and secrete hydrochloric acid. In some forms of PA, the parietal cells remain intact but autoantibodies are produced against the intrinsic factor itself and attach to it, thus preventing it from binding vitamin B12.

In another less common form of PA, the antibodies allow vitamin B12 to bind to the intrinsic factor but prevent the absorption of the intrinsic factor–vitamin B12 complex by the ileal receptors. The incidence of PA increases markedly with age. PA also results in an inability to reabsorb the vitamin B12, which is secreted in the bile. Biliary secretion of vitamin B12 is estimated to be between 0.3 and 0.5mcg/day. Interruption of this so-called enterohepatic circulation of vitamin B12 causes the body to go into a significant negative balance for the vitamin. Once PA has been established, the lack of absorption of new vitamin B12 is compounded by the loss of the vitamin because of negative balance. When the stores have been depleted, the final stages of deficiency are often quite rapid, resulting in death in a period of months if left untreated.

Atrophic gastritis.

Historically, PA was considered to be the major cause of vitamin B12 deficiency, but it was a fairly rare condition, perhaps affecting between one and a few per cent of elderly populations.

More recently, it has been suggested that a far more common problem is that of hypochlorhydria associated with atrophic gastritis, where there is a progressive reduction with age of the ability of the parietal cells to secrete hydrochloric acid [24]. It is claimed that perhaps up to one quarter of elderly subjects could have various degrees of hypochlorhydria as a result of atrophic gastritis. It has also been suggested that bacterial overgrowth in the stomach and intestine in individuals suffering from atrophic gastritis may also reduce vitamin B12 absorption.

The absence of acid in the stomach is postulated to prevent the release of protein-bound vitamin B12 contained in food but not to interfere with the absorption of the free vitamin B12 found in fortified foods or supplements. Atrophic gastritis does not prevent the reabsorption of biliary vitamin B12 and therefore does not result in the negative balance seen in individuals with PA. Nonetheless, it is agreed that with time, a reduction in the amount of vitamin B12 absorbed from the diet will eventually deplete vitamin B12 stores, resulting in overt deficiency.

When considering recommended nutrient intakes (RNIs) for vitamin B12 for the elderly, it is important to take into account the absorption of vitamin B12 from sources such as fortified foods or supplements as compared with dietary vitamin B12. Absorption of intakes of less than 1.5–2.0 mcg/day is complete - that is, for daily intakes of less than 1.5–2.0 mcg of free vitamin B12, the intrinsic factor-mediated system absorbs that entire amount. It is probable that this is also true of vitamin B12 in fortified foods, although this has not been specifically examined. However, absorption of food-bound vitamin B12 has been reported to vary from 9% to 60% depending on the study and the source of the vitamin, which is perhaps related to its incomplete release from food [16]. This has led many to **estimate absorption as being up to 50%** to correct for the bioavailability of vitamin B12 from food.

Vitamin B12 interaction with folate.

One of the vitamin B12-dependent enzymes, methionine synthase, functions in one of the two folate cycles, namely, the methylation cycle. This cycle is necessary to maintain availability of the methyl donor, S-adenosylmethionine. Interruption of the cycle reduces the level of S-adenosylmethionine. This occurs in PA and other causes of vitamin B12 deficiency, producing as a result demyelination of the peripheral nerves and the spinal column, giving rise to the clinical condition called subacute combined degeneration [13,15]. This neuropathy is one of the main presenting conditions in PA.

The other principal presenting condition in PA is a megaloblastic anaemia morphologically identical to that seen in folate deficiency. Disruption of the methylation cycle also causes a lack of DNA biosynthesis and anaemia.

The methyl trap hypothesis is based on the fact that once the cofactor 5,10-methylenetetrahydrofolate is reduced by its reductase to form 5-methyltetrahydrofolate, the reverse reaction cannot occur. This suggests that the only way for the 5-methyltetrahydrofolate to be recycled to tetrahydrofolate, and thus to participate in DNA biosynthesis and cell division, is through the vitamin B12-dependent enzyme methionine synthase. When the activity of this synthase is compromised, as it would be in PA, the cellular folate will become progressively trapped as 5-methyltetrahydrofolate. This will result in a cellular pseudo-folate deficiency where, despite adequate amounts of folate, anaemia will develop, which is identical to that seen in true folate deficiency. Clinical symptoms of PA, therefore, include neuropathy, anaemia, or both. Treatment with vitamin B12, if given intramuscularly, will reactivate methionine synthase, allowing myelination to restart.

7. Health consequences and manifestations of deficiency

The main health consequences and manifestations of vitamin B12 deficiency are the following: megaloblastic anaemia (the combination of severe folate deficiency and vitamin B12 deficiency can result in megaloblastic anaemia); severe deficiency can cause developmental delays, poor neurobehavioral performance and growth in infants and children, nerve demyelination and neurological dysfunction. vitamin B12 deficiency can serve as the risk factor for: neural tube defects; elevated plasma homocysteine; impaired cognitive function.

The following symptoms develops at the deficiency of vitamin B12:

✓ Megaloblastic anemia, fatigue, weakness, constipation, loss of appetite, and weight loss [12]. Vitamin B12 and folate are required for DNA synthesis. A deficiency of any of these vitamins can cause megaloblastic anemia, which is characterized by the production of large and immature red (erythrocytes) and white (leukocytes) blood cells. In addition, vitamin B12 deficiency can cause neuropsychiatric disorders irrespective of the presence of anemia.

✓ Neurological changes, such as numbness and tingling in the hands and feet [25]. The development of neuropathy in vitamin B12 deficiency is associated with a defect in the synthesis of myelin required for the formation of the shell of the nerve fibers. The appearance of neurological symptoms prior to the development of anemia may be associated with the introduction of folate without cobalamin. Treatment by folate of the patient with vitamin B12 deficiency can correct the formation and function of red blood cells, but does not prevent the neurological disorders. In this case, the bone marrow function is stimulated; thereby maintaining an adequate erythropoiesis, but neuropathy continues to develop.

✓ Difficulty maintaining balance, depression, confusion, dementia, poor memory and soreness of the mouth or tounge [26].

✓ By assessing bioavailable vitamin B-12 status in a large Canadian cohort accrued before and after FA fortification, it was found **a 3-fold increase in the risk of neural tube defects** (NTDs) in mothers who had vitamin B-12 status in the lower quartile, regardless of folic acid (FA) fortification [27].

✓ Infancy: failure to thrive, movement disorders, developmental delays and megaloblastic anemia [28].

✓ Elderly: vitamin B-12 deficiency is often associated with cognitive deficits. Cognition in the elderly may also be adversely affected at concentrations of vitamin B-12 above the traditional cutoffs for deficiency. By using markers such as holotranscobalamin and methylmalonic acid, it has been found that cognition is associated with vitamin B-12 status across the normal range. Possible mediators of this relation include brain atrophy and white matter damage, both of which are associated with low vitamin B-12 status. It is suggested that the elderly in particular should be encouraged **to maintain a good, rather than just an adequate, vitamin B-12 status** by dietary means [29].

8. Prevalence of deficiency

In considering the vitamin B-12 fortification of flour, it is important to know who is at risk of vitamin B-12 deficiency and whether those individuals would benefit from flour fortification. The recent review by WHO showed that the majority of data on the prevalence of folate and vitamin B12 deficiencies are derived from relatively small, local surveys, but these and national survey data from a few countries suggest that deficiencies of both of these vitamins may be a public health problem that could affect many millions of people throughout the world. Low blood concentrations of the vitamins occur across population groups and in countries in various stages of development [30].

In large surveys in the United States and the United Kingdom, it has been demonstrated that the prevalence of vitamin B12 deficiency varies by age range, affecting at least 3% of those aged 20–39 years old, 4% of those aged 40–59 years, and 6% of those 60 years or over. Marginal depletion, defined in this study as a serum cobalamin of 148–221 pmol/l, affects 15% of those aged 20–59 years old and more than 20% of those aged 60 years or over [31]. In developing countries, deficiency is much more common, starting in early life and persisting across the life span. Inadequate intake, due to low consumption of animal-source foods, is the main cause of low serum vitamin B-12 in younger adults and likely the main cause in poor populations worldwide. In older persons, food-bound cobalamin malabsorption becomes the predominant cause of deficiency, at least in part due to gastric atrophy, but it is likely that most elderly can absorb the vitamin from fortified food. **Fortification of flour with vitamin B-12 is likely to improve the status of most persons with low stores of this vitamin**.

In countries where vitamin B12 deficiency has been assessed at the national level, low serum vitamin B12 concentrations were prevalent, i.e. in Venezuela (11–12% in preschool and school-aged children), Germany (15% in women of reproductive age), the United Kingdom (31% of the elderly) and New Zealand (12% of the elderly). In smaller studies, a high proportion of low plasma vitamin B12 concentrations were found in Kenya (40% in schoolaged children), Zimbabwe (24% of the elderly), Israel (21% in adults), and India (46% in adults), while in other countries such as Botswana (preschool-aged children), Thailand (school-aged children) and Japan (adults), <1% of plasma vitamin B12 concentrations were low [19, 32-34].

So, there is a limitation connected with a lack of data on vitamin B12 deficiency simply because, to date, little investigation has taken place. This means that the prevalence of many deficiencies suspected of being relatively common (e.g. riboflavin (vitamin B2), vitamin B12, zinc and calcium) is not well known. In some cases, however, evidence of a deficiency in one micronutrient predicts the existence of deficiencies in others. For example, a high prevalence of anaemia and vitamin A deficiency is often accompanied by zinc, vitamin B12 and riboflavin (vitamin B2) deficiencies, because the underlying problem in all cases is an inadequate intake of animal source foods [5].

9. Prevention of deficiency

Prevention of vitamin B12 deficiency include three main approaches:

- ✓ consumption of animal products, which are the only sources of cobalamin;
- ✓ fortification of food with vitamin B12 (usually flour and intermediate cereal products are fortified);
- ✓ vitamin B12 supplementation by multivitamin or multivitamin-mineral preparations.

In poor countries, flour fortification would potentially improve vitamin B-12 status in a much larger proportion of the population because of low usual intake of the vitamin in animal-source foods. Here individuals could benefit across the life span, although it could be especially beneficial for pregnant and lactating women, children, and the elderly. Confirming the efficacy of different levels of addition of the vitamin to flour on vitamin B-12 status and functional outcomes in different populations, including the elderly in different stages of gastric atrophy, should be a

research priority. **In wealthier countries**, vitamin B-12 fortification of flour is most likely to lower the prevalence of inadequacy in the elderly, in those who consume low amounts of animal-source foods and fortified cereals, and in nonusers of supplements [35].

The work suggests that **vitamin B-12 fortification, analogous to the FA fortification program, may reduce NTDs more than FA fortification alone**. A multicenter randomized controlled trial comparing periconceptional vitamin B-12 in combination with FA against FA alone is warranted [27].

Deficiencies in at least some of "neglected" micronutrients (i.e. in zinc, vitamins B2 and B12, niacin, vitamin D and calcium) are likely to be common throughout much of the developing world and among the poorest populations in the industrialized nations. Fortification provides a means of lowering the prevalence of deficiencies in all of these micronutrients, and their inclusion in mass fortification programmes could produce significant public health benefits [5]. This approach is important for prevention of anemia as well, since the presence of vitamins A and B12, folate and riboflavin deficiencies also increases the risk of anaemia, and vitamin B12, as with folate deficiency, the prevalence of neural tube defects [36].

10. Requirements

The daily human requirements for vitamin B12 are minimal, and it is measured in micrograms, depending on age (Table 1).

Table 1 – Recommended nutrient intakes (RNIs*) for vitamin B12 in different groups of population [18]

Population groups	RNI (mcg/day)
Infants and children	
0-6 months	0,4
7-12 months	0,7
1-3 years	0,9
4-6 years	1,2
7-9 years	1,8
Adolescents	
10-18 years	2,4
Adults	
19-65 years	2,4
65+ years	2,4
Pregnant women	2,6
Lactating women	2,8

* RNI = Recommended nutrient intake is the daily intake which meets the nutrient requirements of almost all (97.5%) apparently healthy individuals in an age- and sex-specific population.

Upper limits. The absorption of vitamin B12 mediated by the glycoprotein, intrinsic factor, is limited to 1.5–2.0 mcg per meal because of the limited capacity of the receptors. In addition, between 1% and 3% of any particular oral administration of vitamin B12 is absorbed by passive diffusion. Thus, if 1000 mcg vitamin B12 (sometimes used to treat those with pernicious anemia - PA) is taken orally, the amount absorbed would be 2.0 mcg by active absorption plus up to about 30 mcg by passive diffusion. Intake of 1000 mcg vitamin B12 has never been reported to have any adverse side-effects [16]. Similar large amounts have been used in some preparations of nutritional supplements without apparent ill effects. However, there are no established benefits for such amounts. Such high intakes thus represent no benefit outside those with malabsorption and should probably be avoided for the population at large.

11. Is it needed to fortify flour with vitamin B12 in Central Asia Republics, Afghanistan and Pakistan?

Unfortunately, no data on the prevalence of vitamin B12 deficiency in Central Asia Republics (CAR), Afghanistan and Pakistan. There are only a nationally representative data on the levels of vitamin B12 intake in the various foodstuffs of animal origin in Kazakhstan in 2008 [37]. These findings will be discussed below and used to justify the need for fortification of flour with vitamin B12 in CAR, Afghanistan and Pakistan.

The main suppliers of vitamin B12 in the diet of people in Kazakhstan have been exclusively the following animal origin products (Table 2): meat – 2.5 mcg/day (87.3%), milk – 0.28 mcg/day (9.8%), fish – 0.05 mcg/day (1.6%) and eggs – 0.04 mcg/day (1.3%). Average daily consumption of cyanocobalamin by males was 3.3 mcg/day and by females - 2.6 mcg/day, average intake by males and females – 2.9 mcg/day at the requirement of adolescents and adults for this vitamin equal to 2.4 mcg/day.

Table 2 - Average daily consumption of animal origin products, and vitamin B12 intake (mcg/day) be males and females

Nutrients and food items	Measurement units	Males		Females		Total average
		Mean	Median	Mean	Median	
Vitamin B12, total	**mcg/day**	**3,3**	**2,2**	**2,6**	**1,7**	**2,9**
Meat and meat products	g/day	260	155	157	124	183
Vitamin B12 in the composition of meat	mcg/day	2,9	1,9	2,3	1,5	2,5
Milk and milk products	g/day	456	450	444	422	448
Vitamin B12 in the composition of milk	mcg/day	0,32	0,22	0,25	0,17	0,28
Fish and fish products	g/day	23	14	14	11	16
Vitamin B12 in the composition of fish	mcg/day	0,05	0,04	0,04	0,03	0,05
Eggs	g/day	16	9	9	7	11
Vitamin B12 in the composition of eggs	mcg/day	0,04	0,03	0,03	0,02	0,04

However, even at quite satisfactory average level of cobalamin intake, 33-56% of respondents had been exposed to the risk of vitamin B12 deficiency (Table 3).

Table 3 - Percentage of people aged 15-59 years with a high risk of vitamin B12 deficiency

Residence	The number of people surveyed	% of people with vitamin B12 intake level ≤2/3 of RNI *			
		15-18 лет		≥ 19 лет	
		males	females	males	females
Total	**3526**	**45**	**54**	**35**	**46**
Urban	1628	40	49	33	44
Rural	1898	48	56	36	47

* RNI = Recommended nutrient intake is the daily intake which meets the nutrient requirements of almost all (97.5%) apparently healthy individuals in an age- and sex-specific population

Such a high percentage of people at risk of vitamin B12 deficiency at a reasonable average level of cobalamin intake, may be due to inequalities of consumption of animal products, which are an expensive part of the diet. This is confirmed by statistically significant differences in the levels of vitamin B12 intake, depending on the income level of the population (Table 4).

Table 4 - The consumption of vitamin B12 (in mg / day) of men and women 15-59 years depending on the level of income (KZT/month[A])

Пол	**Percentile, income levels**			
	P10 ≤ 3 571,43 KZT/month	**P20** >3571,43 - ≤5000 KZT/month	**P80** ≥20 000-<30 000 KZT/month	**P90** ≥30 000 KZT/month
Males	1st group	5th group	6th group	2nd group
B12, mcg/day	2,66	2,91	3,54	3,56
Females[B]	3rd group	7th group	8th group	4th group
B12, mcg/day	1,89	2,09	3,35	3,49

[A] - Exchange rate of KZT to dollar averaged 120/1 at the end of July and in August 2008, when the fieldwork for data collection was conducted.

[B] - The difference was statistically significant at **t ≥2,64** ($p < 0.0042$) at using the multiple comparison analysis according to Holm-Bonferroni [38, 39], namely at comparison of Groups 3-4 (**t = -2,9**); Groups 7-8 (**t = -3,0**); Groups 7-4 (**t = 5,2**).

It can be expected that the non-uniformity of consumption of animal origin products takes place in other member-countries. This is confirmed by the World Bank data about the differences in gross domestic product per capita and income inequality in member-countries (Table 5). Moreover, the level of GDP in Kazakhstan many times higher than in other member-countries, and Gini indices on income inequality is lower than in Kyrgyzstan, Pakistan, Tajikistan, and Uzbekistan.

Table 5 - Gross domestic product (GDP) per capita and Gini indices on income inequality in the CARs and Afghanistan and Pakistan

Countries	GDP [40]		Gini indices on income inequality [41]		
	Year	Per capita/year, US$	Year	Gini index score[A]	Gini index rank[B]
Afghanistan	2014	633,6	2007	28	12
Kazakhstan	2014	12601,6	2010	29	13
Kyrgyzstan	2014	1268,9	2011	33	42
Pakistan	2014	1316,6	2010	30	18
Tajikistan	2014	1114,0	2009	31	25
Uzbekistan	2014	2036,7	2003	35	52

[A] 0 = Perfect equality, 100 = perfect inequality.
[B] The countries with a Gini index are ranked from most equal (#1) to most unequal (#145).

Moreover, a consumption of animal products, and thus vitamin B12, in other member-states are significantly lower than in Kazakhstan (Table 6). In particular, the intake of vitamin B12 from food in other member-countries are less of RNI for persons 10 years and older (2.4 mcg / day), ranging from 29.6% RNI (Afghanistan) to 91.2% RNI (Kyrgyzstan). Only in Kazakhstan vitamin B12 intake from food is above of RNI and it is equal to 122.3% of RNI. However, even in Kazakhstan, as described above, 33-56% of respondents were exposed to vitamin B12 deficiency due to uneven consumption of dietary sources of cobalamin, which may be caused by income inequality of population.

These data indicate the feasibility of wheat flour fortification with vitamin B12 in all participating countries, including Kazakhstan. In this case the level of flour fortification with vitamin B12, equal to 0,004 ppm, can be used as opposed to the recommended by the World Health Organization (WHO) level equal to 0,008 ppm, as there is currently no data on the prevalence of vitamin B12 deficiency in the participating countries. Later, after studying the prevalence of vitamin B12 deficiency in the member-countries, the level of flour fortification with vitamin B12 may be revised in the light of the data received.

Table 6 – Estimated intake of Vitamin B12 and its food sources in CAR and Afghanistan and Pakistan

Country	**Year**	**Per capita intake: all population[A)]**		**Per capita intake: adults, 15-59 years[B)]**		**Vit B12 intake, mcg [C)]**		**Vit B12 intake levels, % of RNI**
		kg/year	**g/day**	**kg/year**	**g/day**	**per 100 g of product**	**per capita/day**	
Afghanistan	2013							
Meat		12,33	33,78	14,796	40,536	1,41	0,572	
Milk		62,23	170,49	74,676	204,588	0,06	0,123	
Fish		0,07	0,19	0,084	0,228	0,3	0,001	
Eggs		1,30	3,56	1,56	4,272	0,35	0,015	
Total							**0,710**	**29,6**
Kazakhstan [D)]	2008							
Meat				66,80	183,00	1,41	2,580	
Milk				163,52	448,00	0,06	0,269	
Fish				5,84	16,00	0,30	0,048	

Country	Year	Per capita intake: all population[A)]		Per capita intake: adults, 15-59 years[B)]		Vit B12 intake, mcg [C)]		Vit B12 intake levels, % of RNI
		kg/year	g/day	kg/year	g/day	per 100 g of product	per capita/day	
Eggs				4,02	11,00	0,35	0,039	
Total							**2,936**	**122,3**
Kyrgyzstan	2011							
Meat		36,83	100,90	44,196	121,08	1,41	1,707	
Milk		208,22	570,47	249,864	684,564	0,06	0,411	
Fish		1,85	5,07	2,22	6,084	0,3	0,018	
Eggs		4,61	12,63	5,532	15,156	0,35	0,053	
Total							**2,189**	**91,2**
Pakistan	2013							
Meat		16,41	44,96	19,692	53,952	1,41	0,761	
Milk		183,13	501,73	219,756	602,076	0,06	0,361	
Fish		1,92	5,26	2,304	6,312	0,3	0,019	
Eggs		2,95	8,08	3,54	9,696	0,35	0,034	
Total							**1,175**	**49,0**
Tajikstan	2011							
Meat		13,95	38,22	16,74	45,864	1,41	0,647	
Milk		52,85	144,79	63,42	173,748	0,06	0,104	
Fish		0,13	0,36	0,156	0,432	0,3	0,001	
Eggs		1,79	4,90	2,148	5,88	0,35	0,021	
Total							**0,773**	**32,2**
Uzbekistan	2011							
Meat		33,89	92,85	40,668	111,42	1,41	1,571	
Milk		133,92	366,90	160,704	440,28	0,06	0,264	
Fish		0,46	1,26	0,552	1,512	0,3	0,005	
Eggs		4,81	13,18	5,772	15,816	0,35	0,055	
Total							**1,895**	**79,0**

Note:

A) – FAO stat data

B) - Estimated data obtained taking into account that the consumption of food by adults per capita 20% higher than by entire population. The exception is Kazakhstan, where the data of 2008 national studies are used.

C) - Estimated data in comparison with appropriate Kazakhstan's national studies data of 2008

D) - The results of National nutrition study in Kazakhstan, 2008

Table 7 summarizes estimated intake levels of vitamin B12 with animal origin food sources and with fortified wheat flour at the levels of fortification 0,004 ppm and 0,008 ppm in CARs and Afghanistan and Pakistan. These data confirm the feasibility of flour fortification with 0,004 ppm of vitamin B12 in CARs and Afghanistan and Pakistan, which could serve as a good means for preventing cobalamin deficiency based on actual consumption of its food sources.

Table 7 – Estimated intake levels of vitamin B12 by animal origin food sources and by fortified wheat flour* in CAR and Afghanistan and Pakistan

Countries	Vitamin B12 intake levels, % of RNI		
	By animal origin food sources, adults 15-59 years old	By fortified wheat flour, people 10-65 years old, at fortification levels:	
		0,004 ppm	0,008 ppm
Afghanistan	29,6	47,9-62,2	95,8-124,4
Kazakhstan	122,3	28,2-36,2	56,4-73,3
Kyrgyzstan	91,2	41,2-53,6	82,4-107
Pakistan	49,0	34,0-44,1	67,9-88,2
Tajikistan	32,2	38,2-49,7	76,4-99,3
Uzbekistan	79,0	51,0-66,2	101,9-132,4

* - Fortification level of wheat flour by vitamin B12 is 0,004 ppm

12. Conclusion

The above given analysis of the available data confirm the feasibility of flour fortification with 0,004 ppm of vitamin B12 in Central-Asian Republics and Afghanistan and Pakistan. It is proposed to use the following premix composition to harmonize standards on low extraction wheat flour fortification.

Low extraction flour	
Micronutrients	Levels, ppm
Iron (NaFeEDTA)	15,0
Zinc (Zinc oxide)	30,0
Vit B_9 (Folic acid)	1,0
Vit B_{12} (Cyancobalamin)	0,004
Vit B_1 (Thiamine)	2,0
Vit B_2 (Riboflavin)	3,0
Vit B_3 (Niacin)	10,0

Attachment: The updated version of the document "Analysis and justification the possibility of harmonizing standards for refined wheat flour fortification in Central Asia, Afghanistan and Pakistan". In this document, the level of flour fortification with vitamin B12 reduced from 0,008 ppm to 0,004 ppm, and all of appropriate data are recalculated

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