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Report from the Working Group on Vitamins and Minerals on food fortification by vitamins and minerals: meeting the nutritional and safety needs of the consumer

ANNEXES

List of annexes

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Annex 2. Glossary

Commonly eaten foods and relevant populations
The working group refers to commonly eaten foods as all foods available to the consumer, in all markets and for all members of the population regardless of:

- age (including children of 4 years and above, adults and older people)
- physiological state (i.e. pregnancy or intense physical activity)
- nutritional typology, including regional

Clinical deficiency
Clinical manifestation of nutrient insufficiency.

Biological store depletion
Biological deficiency as manifested by depleted stores or functional effects due to a lack of the nutrient in question; although not clinically manifested, nutrient store depletion could affect disease states or otherwise alter the state of health of an individual.

Inadequate intake
When the average nutrient intake is lower than the recommended nutrient intake (RNI) (as per criteria defined in Annex 3) but there are no neither biological markers of store depletion (or the validity of these are questionable), nor clinical manifestations of deficiency, or the nutrient composition tables are unreliable, or the epidemiological evidence is poor. Inadequate intake does not necessarily imply deficiency or store depletion as defined above.

Proportion of enriched foods consumed
For a given individual, the amount of fortified foods consumed, as a proportion of all potentially fortifiable foods consumed by that person.

Maximum level
The amount of a given micronutrient that can be added to a food without being a safety risk to the highest consumers (95^{th} percentile).

Optimum level
The amount of a given micronutrient which can be added to a food without being a safety risk to the highest consumers (95^{th} percentile) **and** which provides a potential nutritional value as it allows the lowest consumers (10^{th} percentile) to reach or approximate the RNI.

Annex 3[1]. Vitamins and minerals: nutritional status in France assessed from 3 main studies

Nine vitamins (excluding vitamin D) and 5 minerals are addressed. There are no biological values for PP, B_{12}, calcium and magnesium.
Recommended Nutrient Intakes (RNI) from 2001 (4)

AGE GROUP	SEX	RISK OF DEFICIENCY= median<0.9ANC and biological ≥ 10%*	MARGIN OF UNCERTAINTY = median< 0.9 RNI and biology< 10%** or median > 0.9 RNI and biology≥ 10%*	SATISFACTORY STATUS = median > 1 RNI and / or biology < 10%**	HIGH INTAKES= median > 1.5 RNI 95th perc. > 5 RNI maximum> 10 RNI or > tolerable upper intake levels
FREE LIVING OLDER PEOPLE	**M**	**B1, C**	**B2, B6 (βcarotene), E Mg, (Zn), (Cu)**	**PP, B9, B12 A Ca, Fe**	**B12 A, retinol, (βcarotene) (Zn)°°**
(> 62/65 yrs)	**F**	**B1, B6, C Ca**	**B2 E Mg, (Zn), (Cu)**	**PP, B9, B12 A, (βcarotene) Fe**	**B12 A, (βcarotene) (Zn)°°**
ACTIVE ADULTS	**M**	**Iode**	**B6, C (βcarotene), E Mg, (Zn), (Cu)**	**B1, B2, PP, B9, B12 A Ca, Fe**	**B12, A, retinol, (βcarotene) Ca°, (Zn)°°, iodine°°°**
18-65 yrs	**F**	**Fe (18-30 yrs) Ca, iodine**	**B6, B9 (18-30 yrs), C E Mg, (Zn), (Cu)**	**B1, B2, PP, B9, B12 A, (βcarotene) Fe**	**B12 A, retinol,(β carotene) Ca, (Zn)°°, iodine°°°**
ADOLESCENTS	**B**	**E**	**B2, B6 Mg**	**B1, PP, B9, B12, C A, (βcarotene) Ca, Fe**	**B12 A, retinol (Zn)°°**
14-18 yrs* **	**G**	**E Ca, Fe**	**B2, B6, B9 Mg, (Cu)**	**B1, PP, B12, C A, (βcarotene)**	**B12 retinol (Zn)°°**
CHILDREN	**B**		**B2**	**B1, PP, B6, B9, B12, C A, (βcarotene), E Ca, Fe, Mg, (Cu)**	**B12 A, retinol Ca°, (Zn)°°**
6-10 yrs* **	**G**		**B2**	**B1, PP, B6, B9, B12, C, A, (βcarotene), E Ca, Fe, Mg**	**B12 retinol Ca°, (Zn)°°**

The 10-14 yrs age group is not dealt with for lack of sufficient biological values.
* over 10% of the population below the biological limit for high risk of deficiency
** less than 10% of the population below the biological limit for high risk of deficiency
*** : the margin of uncertainty for these two age groups is based on biological values from a single study (Hercberg *et al.*, 1994) and must be taken with caution: the nutritional data are satisfactory but the biological upper limits for B2 and B6 are still under discussion.
°: intakes > 2 RNI of the 95th percentile and/or > 4 RNI of the highest value
°°: intakes above tolerable upper intake levels as defined by the CSHPF
°°°: above the biological upper limits defining risk of excess (> 35-50 µg/dl)
(): in brackets, nutrients whose level in foods is still uncertain or under-assessed (Zn et Cu), the needs ill defined (Cu), or RNI is only estimated (βcarotene=50% RNI of total vitamin A)

[1] ESVITAF, 1986; Hercberg et al., 1994; Costa de Carvalho et al., 1996

Annex 4. Comments and table on tolerable upper intake levels of minerals

Annex 4a. French tolerable upper intake levels of minerals, as per 2001 RNI (Cnerna-Afssa)

Léon Guéguen – 12 March 2001

Re-examination, by individual mineral, of the section entitled "Au-delà des ANC, toxicité" (Beyond RNI, toxicity) of chapter 7 of the publication "Apports Nutritionnels Conseillés pour la population française (ANC)" (Recommended Nutritional Intake (RNI) for the French population) (*CNERNA-AFSSA, 2001)*.
Justification provided when French reference values differ from those adopted by the ILSI project, (not the case for magnesium and iodine).

Calcium

The new American "adequate intakes" aimed at maximum calcium retention already exceed the RNI as defined here. They are however below the 1500 mg per day recommended after 50 years of age by some (literature, and consensus meeting on osteoporosis) to slow down bone resorption by limiting secondary hyperparathyroidism in older people. Substantial calcium supplements (500-1500 mg/day) help reduce cortical bone loss (without affecting trabecular bone) and the incidence of fractures (Chapuy *et al.,* 1995; Recker *et al.,* 1996; Cumming *et al.,*, 1997; Dawson-Hughes *et al.,*, 1997; Meunier, 1999).

Intakes greater than the RNI can be legitimately given on an individual basis for therapeutic ends, not for common nutritional needs. Contrary to the US where fortified foods and calcium supplements are easily obtained, such high intakes cannot be ensured in France through the normal diet. Recommendations of such high intakes would therefore be unreasonable at population level, as only a small proportion could benefit. Such supplements could be given to subjects at risk of bone fracture (Riis *et al.,* 1987; Prince *et al.,* 1995; Reid *et al.,* 1995; Kanis, 1999), once they have been diagnosed by modern methods such as bone densitometry and biochemical markers of bone resorption. Menopausal women may benefit from such high calcium supplements (Cumming 1990) associated with hormone replacement therapy (Nilves *et al.,* 1998).

Emphasis should also be put on other well-known methods of osteoporosis such as vitamin D intake, hormone replacement therapy in menopause, physical activity, and so forth.

Recent results on the effectiveness of high calcium intakes to reduce arterial pressure (McCarron *et al.,* 1991; Allender *et al.,* 1996; Osborne *et al.,* 1996) and for the prevention of colon and prostate cancer still do not justify higher calcium RNI, but support a recommendation for an adequate dietary intake of calcium.

High calcium intakes (up to 2 g/day) do not seem to have deleterious effects on healthy subjects (Whiting & Wood 1997). However, long-term intake can lead to hypercalciuria, and in turn urolithiasis and nephrocalcinosis, especially in vulnerable subjects. This is aggravated with hypervitaminosis D. Oxalic lithiasis (the most common) is not an issue of concern here since excess intake of calcium interferes with oxalic acid absorption in the intestine and is therefore a protective factor (Liebman & Chai 1997; Curham *et al.,* 1997). Hypercalcaemia and renal failure from a very high calcium intake (syndrome of milk drinkers) is exceptional (Whiting & Wood 1997). Finally, excessive dietary calcium can also inhibit intestinal absorption of other elements such as magnesium, zinc and in particular iron (Hall berg *et al.,* 1992).

The tolerable upper intake level of 2g/d calcium is therefore maintained, and not the American recommendation of 2.5g/d (Institute of Medicine 1999).

Phosphorus

Phosphorus intake from in commonly eaten foods (see section 5.8) always exceeds RNI, the Ca:P ratio being 0.5-0.6. The issue with phosphorus is therefore one of risk of absolute or relative excess intake.

Phosphoraemia increases with increased phosphorus intake, leading to a risk of hyperphosphoraemia, the main consequences of which are problems in the hormonal regulation of calcium metabolism (particularly parathormone), ectopic calcifications (i.e. renal) and interference with intestinal absorption of certain oligo-elements. There is virtually no risk of acute or chronic toxicity from intake of phosphorus by the normal diet (Ellinger 1977). In 1963, a joint FAO/WHO Expert Committee set an "unconditional" tolerable intake level of 30 mg/kg, regardless of calcium intake, and a "conditional" intake level of 30-70 mg/kg for diets with normal to high levels of calcium, i.e. 5 g/d phosphorus under the best circumstances.

However there are potential side effects of a slightly excessive P intake on Ca metabolism and bone mineralization. If P intake helps bone retention of Ca by diminishing Ca urinary excretion (even though endogenous faecal loss also increased), it can also at longer-term (especially when associated with inadequate Ca intake) have deleterious effect on bone remodelling due to secondary hyperparathyroidism provoked by a slight fall in plasma Ca ion. The urinary excretion of excess P could also active hyperparathyroidism. Hypocalcaemia of newborn babies fed with cow's milk which has a high P:Ca ratio (not so with human milk) is well known (Specker *et al.,* 1991b).

The negative effect on bone of a P:Ca imbalance (due to excess P or insufficient Ca) is well established in animal (reviews: Draper & Bell, 1979, Guégen, 1982, Pointillart & Guégen, 1985; Calvo, 1994). There is no question that excess P associated with a Ca:P ratio < 1 provokes bone loss.

The Food and Nutrition Board in the US (Institute of Medicine, 1999) finds that there is little evidence pointing to deleterious side effects of excess P on bone (due to difficulties in carrying out human studies) arguing that first, this effect has not been demonstrated in humans and one cannot extrapolate from animal studies as animals have diet much higher in P and Ca; and second, although hyperparathyroidism is a known side effect, there is no evidence that it negatively affects bone.

In response to the first argument, the conclusions herein are based on human data obtained using an imprecise method: 2 standard deviations of ingested and excreted quantities lead to a high overestimation of retained quantities. A difference of 20-30 mg/d of calcium is not statistically significant in a study with too few adult subjects, but does have significant consequences on the bone (see section 4.4). Two studies show unfavourable effects of P on bone (Heaney & Recker 1987; Bizik *et al.,*, 1996), a moderate excess (1.6-2.2 g/d) being associated with adequate intake of Ca and no increased chance of phosphoraemia. There is strong evidence that excess P affects parathyroid activity and bone mineralisation (Goldsmith *et al.,* 1976; Bell *et al.,* 1977; Zemel & Linkswiler 1981; Silverberg *et al.,* 1986; Calvo & Health 1988; Calvo & Park, 1996); it results from increased bone resorption, specifically from inactive bone formation in its initial phases (Kärkäinen & Lamberg-Allardt 1996).

In terms of the second argument, it is true that hyperparathyroidism can have an anabolic effect on bone. This has been shown on the iliac bone of patients with primary hyperparathyroidism (Charhon *et al.,* 1982) and supported in a review carried out by Dempster *et al.,* (1993). However, this anabolic effect might limit itself to the trabecular bone and might occur only with intermittent injections of PTH and not for regular administrations (Hock & Gera 1992; Hodsman *et al.,* 1993).

Although the effect of excess P on PTH secretion is independent of Ca or calcitriol (Hernandez *et al.,* 1996), this must be taken into consideration particularly with low Ca intake, especially as excess P decreases renal synthesis of calcitriol (Portale *et al.,* 1989; Calvo & Park 1996).

The regulatory mechanisms of calcium homeostasis and of low intake adaptation might thus be disturbed in more vulnerable subjects such as post-menopausal women and older people (Calvo & Park 1996).

The tolerable upper intake levels adopted in the US (Institute of Medicine 1999) are 4 g/d P for adults and adolescents and 3 g/d for children and older people. Such high levels are unacceptable, first, as they disregard the potential side effects of excess P on bone, and second, as they are methodologically incoherent: an acceptable dietary intake of 10 g/d in adults corresponds in theory to the tolerable upper limit of "normal" phosphoraemia in children (whereas this limit in adults corresponds to an intake of 3-3.5 g/d!) and an arbitrary factor of uncertainty of 2.5.

Such a high dietary intake would be artificial, put at risk those with impaired renal function, and increase the risk of osteoporosis. A tolerable upper intake level of 2.5 g P per day is therefore maintained, not actually provided by normal diets, unless through fortification or uncontrolled intake of polyphosphate-rich foods. Excess P is better tolerated with adequate intakes of Ca, for example in the consumption of dairy products (Andersen 1991; Bizik *et al.,* 1996).

Iron

Although the maintenance of iron stores is needed, there is no evidence that maintaining maximum iron stores is health beneficial. Certain genetic predispositions, such as hemochromatosis (Brissot & Deugnier 1993), lead to increased iron stores, where excess iron is linked to a digestive hyperabsorption and (Lynch *et al.,* 1989) and can even develop in normal diets. Iron accumulates in parenchymatous cells and leads to organ damage (i.e. liver, heart, pancreas…(Salonen *et al.,* 1992)).

Moreover, excess iron especially with vitamin C can increase oxidative stress due to the reactivity of iron in the production and propagation of free radicals (Rehema *et al.,* 1998). Recently Lund *et al.,* (1999) showed that the administration of ferrous sulphate in humans increases the level of free radicals in faeces; these free radicals may be involved in the aetiology and development of many chronic or acute diseases such as cardiovascular diseases and cancers.

The accidental intake of over 3 g ferrous composites can be lethal, especially in children.

The tolerable upper intake level set by CEDAP is 28 mg/d (2 RDA) (CEDAP 1998).

Zinc

Zinc should only be administered in confirmed cases of zinc deficiency. In pregnant women interventions studies showed that 15-90 mg/d could diminish the risks of birth, as well as prematurity and increase birth weight (Bouglé *et al.,* 1995). Also, doses of 10-15 mg/d are clinically effective on immune function and growth when zincaemia is low, although questionable if zincaemia is normal.

The benefit of long-term poly-supplementation to prevent cardiovascular and cancers is still unclear.

Doses of zinc over 50 mg/d are associated to low plasma ferritin, copper, copper-zinc dependent superoxide dismutase, HDL cholesterol and an increase in lipoperoxides leading to an increased risk of oxidative diseases. Pharmacological doses have a negative effect on the immune system (Sandström 1995). It is proposed that intakes from 15 mg/d (Martin 1996) to 40 mg/d (Hathcock 1996) be the tolerable upper intake level. A long-term mono-supplementation (over 30 days) of over 20 mg/d warrants medical supervision.

Copper

The joint FAO/WHO Expert Committee (1971) concluded that deleterious effects of copper in humans occurs from 0.5 mg Cu/kg body weight/day (>35 mg/d in adults). Excess copper intake can lead to

hepatitis or severe haemolytic icterus, depending on the individual, the meal composition and protective or hepatotoxic factors. Toxic effects of copper such as lipidic peroxidation or DNA damage are directly linked to the production of oxygen free radicals (Bremner 1998) although superoxide dismutase helps destroy these. **A recent study demonstrated that the ingestion of 3-6 mg Cu/d over a 6-week period does not have positive effects in humans (FOODCUE 1998)**. Copper intoxication for the diet is rare and is usually related to drinking water or cooking in copper pots (Indian cirrhosis). Uncontrolled supplementation of oligo-elements can also lead to copper intoxication.

Selenium

Other than a nutritional effect (i.e. restoration of depleted stores), selenium has therapeutic properties on cancer and possibly on rheumatological and cardiovascular diseases (Nève 1993). At least 200 μg per day is thus recommended (Nève 1995).

It is not clear whether dietary intakes can lead to selenium toxicity. It appears that one can take up to 1000 μg per day without clinical signs of toxicity. Oral DL_{50} in humans is estimated between 0.5-1 g mineral selenium (Nève 1995).

It has been established that a daily dose of 5 μg/kg during a lifetime does not have deleterious effects on health (Levander & Whanger 1996). A tolerable upper intake level of 150 μg /d has however been proposed in France using a tolerance factor of 10 (Martin 1996); this level is low compared to the daily selenium intake of certain Europeans (Finland), between 100-200 μg/d.

Chromium

The effectiveness of high-dose chromium picolinate (1000 μg/d) has been demonstrated in non-insulin dependent diabetes (Anderson *et al.,* 1997c), but the benefits of supplementation (25-50 μg/d) in a healthy individual are only evident when chromium status is low, such as in older people, malnourished children and those with syndrome X. False allegations of beneficial supplementation must stop as . Uncontrolled supplementation is not desirable, but it must be recognised that chromium is essential for the above at-risk groups.

Chromium III toxicity is traditionally considered nonexistent. High doses of picolinate or chromium chloride in rats over a 6-month period show no blood or histological anomalies. However chromium VI is very toxic, leading to dermatitis, nephritis and hepatitis with chronic intoxication.

Cited references can be consulted in the general reference list at the end of the document entitled "Apports nutritionnels conseillés pour la population française" Cnerna-Afssa, 2001, ed Tec & Doc, Lavoisier, Paris

Annexe 4b: Tables on tolerable upper intake levels of minerals

- Tolerable upper intake levels

	Nutritional value	**tolerable upper intake levels stemming from RNI**	**tolerable upper intake levels proposed by the SCF**	**tolerable upper intake levels proposed by ILSI**	**Interactions**
Ca (mg)	RNI 800 RNI 900 66% RNI 594 Individual <u>therapeutic</u> benefits At-risk groups: adolescents and post-menopausal women	➢ 2g /d *(Whiting &Wood, 1997)* In particularly sensitive individuals, prolonged intakes= risk of hypercalciuria (urolithiasis and nephrocalcinosis)	- Expected	➢ 2.5 g/d *US Food and Nutrition Board tolerable upper intake levels* Supplemental intake recommended (18% RNI/100kcal and 25% market share)	Counteracts intestinal absorption of Mg, Zn and Fe. *(Hallberg et al., 1992)* Increased risk of hypercalciuria when there is hypervitaminosis D.
P (mg)	RNI 800 RNI 750 66% RNI 495 average consumption in France is excessive (1500 - 1600 mg/d)	➢ 2.5 g/d Low risk of severe toxicity in food: hyperphosphoremia, atopic calcifications, interaction with intestinal absorption of certain oligo-elements. Risk in case of slight excess: side effect on calcium metabolism and bone mineralization.	- expected	➢ 4 g/d *US FNB Tolerable Upper Intake Levels* Can be added with risk of excess (18% RNI/100kcal and 25% market share) ▲ possible side effects of excess phosphorus on bone are overlooked in the FNB analysis ▲ questionable method of calculation (see **RNI** 2001) (4)	Helps maintain bone density in case of P:Ca imbalance due to inadequate intakes in calcium.

	Nutritional value	**tolerable upper intake levels stemming from RNI**	**tolerable upper intake levels proposed by the SCF**	**tolerable upper intake levels proposed by ILSI**	**Interactions**
Mg (mg)	RNI 300 RNI 6 mg/kg/d i.e. 180 mg/d per 60 kg 66% RNI 118.8 18% of men and 23% of women are below 2/3 RNI	➢ 650mg/d Substantial pharmacological intakes can be insignificant due to renal homeostasis	- expected	➢ 700 *US FNB Tolerable upper intake levels* Can be added without risk of excess (18% RNI/100kcal and 25% market share)	
Fe (mg)	RNI 14 RNI ♀ 16 ♂ 9 66% RNI ♀ 10.5 ♂ 5.9 There is no evidence that high iron doses benefit health	➢ 28 mg/d *CEDAP, 1998* *Increased iron stores in vulnerable individuals (Brissot &Deugnier, 1993)* This risk exists if diet is normal. Risk of increasing oxidative stress (particularly in the presence of vitamin C) *(Rehema et al., 1998)* Accidental intake of high iron doses (over 3g ferrous elements) can induce fatal intoxication (particularly in children)	- expected		Increased oxidative risk with vitamin C

	Nutritional value	**tolerable upper intake levels stemming from RNI**	**tolerable upper intake levels proposed by the SCF**	**tolerable upper intake levels proposed by ILSI**	**Interactions**
Zn (mg)	RNI 15 RNI[2] ROA ♀ 7 ♂ 9 POA ♀ 12 ♂ 14 66% RNI ROA ♀ 4.6 ♂ 5.9 POA ♀ 7.9 ♂ 9.2 benefits demonstrated in the administration of zinc <u>only when insufficiency is noted</u>: e.g. 1 : pregnant women (Bougle *et al.*, 1995) e.g. 2 : clinical efficiency on immune function and growth (10-50 mg/d)	➢ 15 *(Martin, 1996)* ➢ 40 *(Hatchcock, 1996)* above 50 mg/d : decrease in plasma ferritin, copper, dependent copper-iron superoxide dismutase, HDL-cholesterol and increase in lipoperoxides → increased risk of oxidative pathologies Pharmacological doses have a negative effect on immunity *(Sandström, 1995)* A long-term mono-supplementation (> 30 d) over 20 mg/d warrants medical supervision.	- expected	Can be added without risk of excess (18% RNI/100kcal and 25% market share)	Copper

[2] Intestinal absorption of zinc varies according to the animal and vegetable composition of the meal: ROA : meal rich in products of animal origin, POA : meal poor in products of animal origin

	Nutritional value	**tolerable upper intake levels stemming from RNI**	**tolerable upper intake levels proposed by the SCF**	**tolerable upper intake levels proposed by ILSI**	**Interactions**
Cu (mg)	RNI - RNI ♀ 1.5 ♂ 2 66% RNI ♀ 0.9 ♂ 1.3	No fixed tolerable upper intake level due to insufficient elements Risk of toxicity linked to oxidative elements Deleterious effects in humans once intake exceeds 0.5 mg/d/kg (*FAO/WHO, 1971)* Risk of hepatitis and severe haemolytic icterus. Toxic effects (lipidic peroxidation, DNA damage) are directly linked to its role in the production of oxygen free radicals (*Bremmer, 1998)* The intake of 3 or 6 mg of copper per day over a 6 week period is not recommended *(FOODCUE, 1998)*	-		Zinc

	Nutritional value	**tolerable upper intake levels stemming from RNI**	**tolerable upper intake levels proposed by the SCF**	**tolerable upper intake levels proposed by ILSI**	**Interactions**
F (mg)	RNI - RNI ♀ 2 ♂ 2.5 66% RNI ♀ 1.3 ♂ 1.6 Since 1985 fortification of salt with fluoride (specifically KF) has been authorised (250 mg/kg fluoride ion). Water in drinks can be fortified with up to 0.5 mg/l (problem in certain mineral waters)	➢ 4.0 Tenir compte de la fluoration de l'eau du robinet et du sel. Tap water and salt fluoridation must be taken into consideration. Fluorosis (discoloured teeth) in children who drink water containing over 1.6 mg/l of fluoride. Bone fluorosis when doses > 10 mg/d over more than 10 years *(Institute of medicine, 1999)* Severe intoxication when doses >500 mg/d.	- expected	*US FNB Tolerable Upper Intake Levels*	

	Nutritional value	**tolerable upper intake levels stemming from RNI**	**tolerable upper intake levels proposed by the SCF**	**tolerable upper intake levels proposed by ILSI**	**Interactions**
I (µg)	RNI - RNI 150 66% RNI 99 Salt iodization (by NaI): currently 10- 15 mg/kg salt (an increase from 15 to 20mg/kg is expected; this issue will be discussed by the CES Nutrition over the next months.	➢ 500 µg/d *LiM (1987)* *Gardner DF (1988)* *Paul T (1988)* *Chow CC (1991)* *See other references at end of document* Thyrotoxicosis in individuals with pre-existing functional anomalies. In developing regions, poorly implemented salt iodisation leads to a reappearance of hyperthyroidism (Basedow's disease) *(Gomo et al., 1999)* Carcinogenic potential of iodates?	- expected	1000 µg/d	children

	Nutritional value	**tolerable upper intake levels stemming from RNI**	**tolerable upper intake levels proposed by the SCF**	**tolerable upper intake levels proposed by ILSI**	**Interactions**
Se (µg)	RNI - RNI ♀ 60 ♂ 70 66% RNI ♀ 39.6 ♂ 46.2 To deal with clinical deficiencies Therapeutic properties at doses of 200 µg/d Daily intakes of Finns are 100-200 µg/d	➢ 150 µg/d *(Martin, 1996)* Lifetime dose at which there are no deleterious effects: 5 µg/kg/d *(Levander et Whanger, 1996)* Possibility of neuropathies at doses of 50 mg/d *(Guilland, 1996)*	➢ 300 µg/d 1-3 years 60 µg/d 4-6 years 90 µg/d 7-10 years 130 µg/d 11-14 years 200 µg/d 15-17 years 250 µg/d (Variable by weight) *(SCF, 2000)* 18% RNI/100kcal and 25% market share	➢ 300 µg/d *(SCF, 2000)*	
Cr (_g)	RNI - RNI 50 à 70 66% RNI 33 à 46.2 Of nutritional value only if chromium status is low Supplements of 25-50 µg/d) to older people, malnourished children, and those suffering from Syndrome X.	*No tolerable upper intake level* Cr III is considered non-toxic		*Not addressed*	

	Nutritional value	**tolerable upper intake levels stemming from RNI**	**tolerable upper intake levels proposed by the SCF**	**tolerable upper intake levels proposed by ILSI**	**Interactions**
Na	Excessive consumption relative to needs Reduce sodium and chloride intakes (6-8g/d)	-	-	-	
K		-	-	-	
Cl		-	-	-	
Mn (mg/d)	RNI - RNI 1-2.5 66% RNI 0.6-1.6 Needs met by commonly eaten foods (2-9) Manganese-rich waters (high bioavailability of manganese, over 2 mg/l) can have deleterious effects on the CNS.	➢ 4.2 -10 mg/d potentially deleterious effects on the central nervous system *(Gregger, 1998)* Needs met by commonly eaten foods and excess intake is risky without any potential health benefit. No quantified upper intake level.	No tolerable upper intake level. Data too scarce. Neurotoxicological with oral intake *(SCF, October 2000)*	Not addressed	
Mo (_g)	RNI - RNI 30-50 66% RNI 19.8-33 Largely covered by consumption of commonly eaten food	➢ 350 µg/d	➢ 600 µg/ d *SCF 2000*	Not addressed	

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Annex 5. Comments and table on tolerable upper intake levels of vitamins

Annexe 5a. Data used to define tolerable upper intake levels: water-soluble vitamins.

M Garabédian.

Signs of excess vitamin D have been detected after prolonged intake of 250-1250 µg /day vitamin D in adults (1) and 112-750 µg/day in infants and young children (2). However, a daily intake of 250 µg/d over a 7-week period in adults has induced neither hypercalcaemia nor hypercalciuria (3), and daily doses of 25-50 µg/days seem to be tolerable to infants (4, 5).

Based on these data, tolerable intake limits of 25 µg/day for adults were proposed in France (NOAEL/10; NOAEL=LOAEL=250 µg/day) and of 50 µg/day for infants (6).

Since then, several studies have demonstrated that vitamin D-fortified milk can be toxic to adults (7, 8). These studies and others have also shown the important discrepancy between labelled and actual vitamin D levels in fortified milk (7, 9-11).

Moreover, a recent publication shows a linear correlation between vitamin D intakes over 8 weeks and available vitamin D stores in healthy adults (11). It is estimated, based on these results, that an intake of 25 µg /day of vitamin D increases 25-hydroxyvitamin D stores by 12 ng/ml. This intake alone is enough to increase the stores of a vitamin D deficient person (i.e. one who has no sun exposure and does not consume vitamin D-rich foods) to above the upper limit for deficiency (i.e. 10-13 ng/ml).

These data emphasize the need to proceed cautiously when identifying tolerable upper intake levels for vitamin D. It seems unreasonable to fortify commonly eaten foods to provide doses of vitamin D exceeding the recognised doses needed to prevent and correct vitamin D deficiency in children, pregnant women and older people i.e. 10-25 µg /day.

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Annexe 5b. Data used to define upper intake levels: water-soluble vitamins.
(J-C Guilland – UFR de Médecine – Dijon)

1. Thiamine

The oral ingestion of high quantities of thiamine does not have negative side effects in people. The absence of toxicity is undoubtedly linked to the fact that intestinal absorption of thiamine occurs via a saturable active transport mechanism. Maximum absorption via this mechanism is 5 mg thiamine per day. Thiamine can be absorbed by a process of passive diffusion when the active transport mechanism is saturated. However, this mechanism is inefficient.

It is however well established that high doses of intravenous or parenteral thiamine can induce certain side effects. In humans, side effects to thiamine are now rarely observed due to reduced posologies. Accidents from the 1940s and 1950s due to parenteral administration had to do with the cardiovascular and neuromuscular systems: headaches, weakness, convulsions, paralysis, vasodilation, tachycardia, and cardiac arrhythmia. The most frequent side effect was a hypersensitive reaction with anaphylactic shock.

Overall, the toxicity of orally administered thiamine is limited because of limited absorption when doses are high and the fact that body has limited capacities to store thiamine. As there have not been any reported cases to data of thiamine toxicity when orally administered to date, no upper intake level has been established for this vitamin.

2. Riboflavin

It appears that riboflavin is not toxic to humans. High doses have been administered as part of Schoenen *et al.* (1994, 1998) and Zempleni *et al.* (1996) studies. Zempleni *et al.* found no side effects in subjects taking in up to 60 mg orally and 11.6 mg intravenously. Schoenen *et al.* (1994) also reported no side effects in patients given 400 mg Riboflavin/day over three months as migraine treatment. In a second study, Schoenen *et al.* (1998) 55 patients suffering from migraines were given a placebo during three months. Of those receiving the riboflavin, two experienced diarrhoea and polyuria; there was one case of abdominal cramps in the placebo group. It is difficult to account for these symptoms, as they are non-specific. As for thiamine, riboflavin is not easily absorbed by the intestine nor stored in the body, and risk of toxicity is therefore not high. It is however important to note that no studies have yet addressed the harmlessness of riboflavin to humans.

3. Vitamin B6 (a more detailed examination follows in Annex 5b)

The consumption of foods such as vitamin B_6 has never been associated to side effects. Side effects reported in medical literature concern excessive intake of pyridoxine HCl (PN, HCl) prescribed as treatment for carpal tunnel or premenstrual syndrome (PMS). Such doses have been linked to sensory neuropathy and dermatological lesions (Cohen & Bendich, 1986 ; Schaumburg & Berger, 1988). The causal link between high pyridoxine ingestion and neuropathy in animals was established in 1942 (Unna & Antopol, 1940) and in humans, 1983 (Schaumburg *et al.*, 1983).

Sensory neuropathy

The first clinical observation of neurotoxicity induced by pyridoxine in humans was published by Schaumburg *et al.* in 1983. Seven adults (5 women and 2 men) exhibited neuropathy of the extremities after having ingested 2000-6000 mg pyridoxine/day for 2-40 months. Four adults had a severe neurological impairment and were unable to walk. The diagnosis was objective and neurological signs and symptoms regressed as soon as pyridoxine intake was discontinued. Two were however left with sequellae. All had symptoms of Lhermitte-Duclos disease (Hydrocephalus) with affected posterior medulla oblongata. Other cases of sensory peripheral neuropathy associated to high pyridoxine intake were reported in the 1980s

(Baer, 1984; Bredesen & Parry, 1984; de Zigher *et al.*, 1985; Friedman *et al.*, 1986). Its pathogenesis and dose/response relationship have been well documented through animal studies (Phillips *et al.*, 1978; Schaeppi & Krinke, 1985). The data shows that risks of neuropathy are diminished when intake remains below 1g pyridoxine/day (Bernstein & Lobitz, 1998; Del Tredici *et al.*, 1985).

Other side effects

Painful and scarring dermatological lesions have been reported from intakes of 2-4 g pyridoxine/day for more than one year (Friedman *et al.*, 1986; Schaumburg & Berger, 1988). More recently, Tanaka *et al.* found that photodermatosis in a patient whose intake was 30 mg pyridoxine/day (1996). However, a dose-response relationship cannot be established on such insufficient data. Moreover, the mechanism(s) of pyridoxine-induced dermatitis are as of yet unclear (Schaumburg et Berger, 1988). Addiction was observed in a newborn baby whose mother was treated with 50 mg pyridoxine during the last 5 months of pregnancy (Hunt *et al.*, 1954): the child had seizures which were subdued with pyridoxine. Finally, a case of rhabdomyolysis has been seen in a newborn baby with homocystinuria after 7 days of treatment with 500 mg pyridoxine/day (Shoji *et al.*, 1998).

Dose-response relationship and upper intake level

Bernstein & Lobitz (1988) administered a daily dose of 100-150 mg pyridoxine, for up to 5 years, to 70 patients with diabetic neuropathy or carpal tunnel syndrome. A thorough neurological exam showed no side effects. Del Tredici *et al.* (1985) identified no side effects in patients taking 100-300 mg pyridoxine/day over 4 months. Others (Brush *et al.*, 1985; Ellis *et al.*, 1984; Mitawelli *et al.*, 1984; Tolis *et al.*, 1977) also report an absence of neurological side effects in hundreds of patients taking 100-500 mg/day. Two publications suggest that the daily upper limit (to avoid toxicity) is 500 mg/day (Berger & Schaumburg, 1984; Schaumberg *et al.*, 1983). However, Berger & Schaumburg (1984) describe the case of a young woman who ingested 200 mg pyridoxine/day over a two-year period and developed a sensory neuropathy as the daily dose reached 500 mg/day. Several research teams have observed the development of sensory neuropathy for doses inferior to 500 mg/day. Bredesen & Parry (1984) gathered 16 patients with a sensitive neuropathy, specifically axonopathy, provoked by 200 mg-5 g PN HCl/day. A nervous system biopsy showed axonal degeneration in two patients. The authors maintain that the symptoms appear one month to three years after the beginning of the treatment, relative to the dose, and that when the daily dose is equal or above 2g, neuropathy appears after less than a year. The next year, Parry & Bredesen (1985) reported on two patients who had ingested 500 mg/day for 8 or 36 months, and another who had ingested 100-200 mg/day; both developed a sensory neuropathy. Two studies (Dalton 1985 ; Dalton & Dalton, 1987) seem even more significant in that they demonstrate sensory neuropathy in women treated for PMS with less than 500 mg pyridoxine/day. In a letter to the editor, Dalton (1985) describes sensory neuropathy (characterised by hyperesthesia, fasciculations, numbness, paresthesia, ataxia) in 40% of the women (n = 58) treated with doses of 50-300 mg pyridoxine/day. A subsequent publication (Dalton & Dalton, 1987) described how of 173 women treated for PMS with 50-500 mg pyridoxine/day, 103 (60 %) developed neurological symptoms. The posologies were comparable in women *with* neuropathy (117 ± 92 mg/d) and those *without* neuropathy (116 ± 66 mg/d). The amount of exposure and the total absorbed dose differentiated the two groups. This study led to the conclusion that neuropathy can be identified at 50 mg/day, the case in 20 % of detected cases. After three months without pyridoxine, the women noted a marked improvement, and after 6 months, were restored to health.

An intake equal to or greater than 50 mg PN-HCl/day therefore seems potentially toxic. It is therefore plausible that the Lowest Observed Adverse Effect Level (LOAEL) is 50 mg/day in women, and perhaps higher in men. Using a tolerance factor of 10, the upper intake level is thus equal to 5 mg/day. The Scientific Committee on Food fixed the tolerable upper intake level at 25 mg/day based on the average intake of women affected by neuropathy i.e. about 100 mg/day, and by applying a tolerance factor of 4 (2 to take into consideration long-term effects and 2 to factor in the shortcomings of this study). In the US, the Food and Nutrition Board of the Institute of Medicine (FNB, 1998) estimates that the “No Observed

Adverse Effect Level » (NOAEL) is equal to 200 mg/day based on two studies (Bernstein & Lobitz, 1998 ; Del Tridici *et al.*, 1985) and established that the upper intake limit be 100 mg/day by choosing a tolerance factor of 2.

4. Niacin

Data on intestinal absorption of niacin are limited. In humans, 85% of a single 3g-niacin dose have been found in the urine. The capacity for intestinal absorption does not therefore seem limited.

Vasodilating effects

The oral intake of pharmacological doses of niacin can lead to vasodilation. Nicotinic acid stimulates local release of histamine (this is not the case of nicotinamide). It thus possesses a vasodilatory effect on capillaries. A vasodilation reaction is noted for a dose as low as 30 mg (Estep *et al.*, 1977 ; Henkin *et al.*, 1990 ; McKenney *et al.*, 1994).

Effects on the gastro-intestinal system

Niacin could have deleterious effects on the gastro-intestinal tract. In the study carried out by the Coronary Drug Project Research Group, 13.9 % of subjects suffered from gastralgia, 8.5 % from nausea and 4.6 % from diarrhoea (The Coronary Drug Project Research Group, 1975). Moreover, 30-50 % of subjects in this study had high plasma transaminase and alkaline phosphatase. Such an increase in enzymatic activity is a sign of hepatoxicity of niacin. Nausea, vomiting and hepatoxicity are generally noted for intakes of 3 g de nicotinamide/day (Rader *et al.*, 1992) and 1.5 g nicotinic acid/day (McKenney *et al.*, 1994). High dose niacin also has renal, cardiac and dermatological side effects.

Tolerable upper intake level

The hepatoxicity of niacin is a fact: 30-50 % of people treated with 3g niacin/d over several years exhibit biological manifestations of hepatic conditions. The identification of a severe icterus after an intake of 750 mg/d is the only information that allows for the establishment of a LOAEL. If a tolerance factor of 10 is applied, the tolerable upper intake level of niacin would be 75 mg/d.

Cutaneous manifestations are also commonly observed; the least aggravating, erythemia, can appear from 50-100 mg/day. If a tolerance factor of 10 were applied, the tolerable upper intake level of niacin would be 5 mg/day, within the recommended daily intakes. To use a tolerance factor of 3 would make the upper intake level 33 mg/d (approximately twice the RNI). Considering the uncertainty regarding hepatic conditions, this upper intake level that corresponds to 2 RNI (14 mg/d) is actually the best justified: it corresponds to 1/100 of the dose (3 g/d) for which there is ample information on side effects.

5. Folic acid

No important side effects have been associated to high doses of dietary folates (Butterworth & Tamura, 1989). Side effects are only seen with the excessive ingestion of folic acid (peroylglutamic acid). Intestinal absorption of folic acid occurs through an active transport mechanism for doses close to the RNI and via a mechanism of passive diffusion for high doses. In the intestinal mucous membrane folic acid is metabolised into 5-methyl tetrahydrofolate, the principal plasma form of folic acid, and can then be ingested by cells. This activation stage of folic acid could reduce side effects of high doses of folic acid, which are eliminated through the urine without having been metabolised to 5-methyl tetrahydrofolate. Folic acid is not easily toxic.

However, high doses of folic acid could be neurotoxic. The neurotoxicity of folates would be due to 5-methyltetrahydrofolic acid (5-CH_3-H_4PteGlu), the principle transport form of folic acid. In line with this

hypothesis, the increase of 5-CH_3-H_4PteGlu, observed in vitamin B_{12} deficiency, would have a deleterious effect on the central nervous system and could explain the neurological symptoms of those suffering from pernicious anaemia (Ruck *et al.*, 1980). From an experimental point of view, folic acid and epilepsy may be associated. Symptoms of epilepsy can be seen in rats receiving intravenous doses of 25-200 nmol (10-100 mg) (Tremblay *et al.*, 1984). From a clinical point of view, large doses of folic acid increase the frequency of epileptic seizures in patients treated with anti-epileptics (Miller *et al.*, 1982). In a study (Chien *et al.*, 1975) looking at epileptic patients treated with anticonvulsives, it became clear that certain subjects were sensitive to intravenous doses of 7 mg folic acid when in other subjects 75 mg intravenous folic acid had no effect. *In vitro* folic acid on isolated cells or tissues appears to be a neuroexcitor.

Excessive oral intake of folic acid (5 mg/d) can hide haematological signs of megaloblastic anaemia from a due à clinical deficiency of vitamin B_{12}. This is a serious problem as the neurological deterioration can progress despite a normal haematological status and the effects can be irreversible (Schwartz *et al.*, 1950; Editorial, 1947).

Finally, some studies suggest that folic acid supplementation can inhibit intestinal absorption of zinc (Milne *et al.*, 1984; Mukherjee *et al.*, 1984; Simmer *et al.*, 1987). These observations have not been experimentally followed up in either humans or rats. A recent double-blind study showed no difference in plasma and erythrocyte zinc contents between the case group (10 mg folic acid/day for 6 months) and the placebo group (Butterworth Jr *et al.*, 1988). Also, Krebs *et al.* (1988) found that 30 mg folic acid does not alter intestinal absorption of a stable zinc isotope (Zn).

Overall the data indicate that high doses of synthetic folic acid can have side effects. Data from animal or *in vitro* studies show that 10 mg folic acid is neurotoxic and epileptogenic. No data yet points to neutoxicity of folic acid in humans. The upper intake limit was therefore established by taking into consideration the fact that 5 mg folic acid can hide haematological signs and favour the progress of neurological symptoms in individuals who are clinically deficient in vitamin B_{12}. The application of a tolerance factor of 5 brings the upper intake limit to 1 mg/day.

6. Biotin

Biotin toxicity has never been reported in humans. It must indeed be very improbable as children with a congenital enzymatic deficiency curable with biotin tolerate injections of 10 mg/day over several months. Biotin toxicity thus seems inexistent and no upper safety limit has been established for this vitamin.

7. Pantothenic acid

Animals tolerate 200 mg/kg/d over long periods of time. Doses of 10 g/day cause intestinal problems such as diarrhoea. The DL_{50} is 2.5 g/kg for mice and 3.5 g/kg for rats. No particular side effect has been noted in humans when high doses of pantothenic acid were administered. Therefore, no upper safety limit has been established for this vitamin.

8. Vitamin B_{12}

Vitamin B_{12} absorption in the ileum initiates passive diffusion and a mechanism of attachment to a specific receptor after having created a complex with the intrinsic factor. Passive diffusion intervenes only for doses between 10 and 1000 µg. The maximum absorbable quantity is 1.5 µg. Passive absorption of vitamin B_{12} is inefficient (only 1-5 % of ingested doses are absorbed). However, when ingested doses are greater than 100 µg, the absorbed quantity is equal or greater than that being absorbed by the mechanism which is dependent on the intrinsic factor.

Absorption of vitamin B_{12} is therefore regulated by doses < 10 µg (4 RNI) and limited by doses > 100 µg. Vitamin B_{12} toxicity is extremely low as demonstrated in animal experimentation. Animals tolerate doses in

g/kg without exhibiting side effects. High doses (2 mg/kg) do not affect the central nervous system as demonstrated in a neurophysiological test in cats (Holm *et al.*, 1974). In humans, a few rare cases of allergic reaction of anaphylactic shock have been reported in the literature (Faivre *et al.*, 1975).

9. Vitamin C

Human tolerance of ascorbic acid is not well known. The physiological mechanisms controlling ascorbic acid absorption, its tissue levels, its metabolism, and its renal elimination suggest that vitamin C does not accumulate in the body even when high doses are ingested. Large quantities of vitamin C could be ingested on a daily basis and still be well tolerated: 15-30 g/day according to Wintermeyer (1981), 15 g/day orally for several months according to White (1981) and 100 g/day orally according to Pauling (1981). The safety upper intake limit (i.e. the amount of vitamin C that can be consumed over a lifetime without deleterious health effects on a person) has been proposed by Wintermeyer (1981): 15 mg/kg in humans, i.e. 1050 mg/day for a 70-kg male. Although there are few data indicating that even 1g/day vitamin C is toxic, it is suggested that even high doses may for example lead to urolithiasis, interaction with metal ions and vitamin B_{12}, mutagenic capacity, foetal toxicity, oxidation, etc.

Can high doses of ascorbic acid lead to urolithiasis? It is a plausible concern if one considers vitamin C metabolism. In humans, ascorbic acid is excreted through the urine, and oxalic acid is one of the principal metabolites. The absorption of high doses of vitamin C can there fore increase physiological oxaluria so as to initiate precipitation and urolithiasis. With small doses of ascorbic acid, 35-50 % of oxalate excretion through the urine comes from vitamin C metabolism. Studies on oxalic acid excretion after ingestion of several grams/day have discordant conclusions. Regardless of inconsistent experimental conditions and even methodological problems regarding oxalic acid dosage, it seems that an increase in oxaluria is insignificant even with several g/vitamin C/d, and that oxaluria is not proportional to the dose of vitamin C ingested. In subjects with a history of urolithiasis, a rise in oxaluria is noted in certain people only (Briggs, 1976). In another study (Butz & Kohlbecker, 1980) on 41 subjects with lithiasis, the intake of 1 g/day of ascorbic acid for 5 days only increased oxaluria from 17 to 22 mg/d. overall, it seems that the metabolism of ascorbic acid contributes to the urinary excretion of oxalic acid and that even with high doses of vitamin C (> 1 g/day), excretion does not exceed the amount of oxalate considered critical. However, it may be recommended to evaluate the risks of hyperoxaluria in subjects with lithiasis and those ingesting heavy doses of vitamin C.

Urinary excretion of uric acid does not appear to be modified with the ingestion > 1g ascorbic acid. As ascorbic and uric acids are reabsorbed via an active mechanism with common transport system, it seems logical that high doses of ascorbic acid increase urinary excretion of uric acid. An increase in uricosuria has not been reported for oral doses < 2 g, but has for single doses > 4 g (Stein *et al.*, 1976). Hyperuricosuria and acidification of urine by vitamin C could initiate the precipitation of urates and although renal lithiasis (Stein *et al.*, 1976).

In vitro studies (Herbert & Jacob, 1974) report that high doses of vitamin C destroy the vitamin B_{12} found in foods consumed simultaneously. These result seem to stem from methodological issues and have not been confirmed.

Vitamin C could interfere in electrolyte and metal ion metabolism, such as in the case of copper. In guinea pigs, 25 mg ascorbic acid/kg/day reduces by 30-60 % hepatic copper concentrations; this could be due to a reduced binding of copper and metallomethionine. Vitamin C stimulates the absorption of non-haem iron. Cook *et al.* (1984) studied the effects of a daily supplementation of 2 g ascorbic acid (1 g per main meal) on iron metabolism; there was no increase in ferritinaemia, either after 4 or 24 months of supplementation. It was concluded that regulatory mechanisms must counterbalance the increase in ascorbic acid-induced non-haem iron absorption in the intestine. However, high doses of vitamin C could be problematic in patients with hematochromatosis, thalassemia, or sideroblastic anaemia.

Numerous undesirable clinical effects of high dose ascorbic acid have been cited, although these are mostly anecdotal. The most common reactions to mega-doses are intestinal problems such as diarrhoea, nausea and abdominal cramps, possibly linked to direct osmotic effect of vitamin C on the intestine. However, a case-control study of late-stage cancer patients receiving 10g/d showed an identical pattern in the development of diarrhoea in cases and controls. Also, other unwanted clinical effects such as gastro-duodenal ulcers or vascular thrombosis are only based on isolated cases, and coincidences could also be involved. Some authors mention the risk of secondary deficiency with a sudden cessation of high dose ascorbic acid intake (Cochrane, 1965 ; Rhead et Schrauzer, 1971) although this is as yet unsupported by experimental evidence.

As to the oxidative effect of vitamin C, recent studies (Podmore *et al.* 1998; Rehman *et al.* 1998) fail to indicate whether 500 mg/day of ascorbic acid can damage DNA (Halliwell, 1999).

Tolerable upper intake levels

A dose of 15 mg/kg/d (i.e. 1 g/d for adults) as suggested by Wintermeyer, in accordance with scientific data, is proposed.
A tolerable upper intake level of 1 g/day can be established due to the gastrointestinal problems manifested for doses at least equal to 5 g/day when a tolerance factor of 5 is applied. It is noteworthy that regular consumption of supplements containing vitamin C has been associated with a more rapid development of atherosclerosis: in subjects taking in over 500 mg of vitamin C as a supplement, the intima-media thickness of carotid arteries increased 2.7 times more that in subjects that did not consumed supplements containing vitamin C (Anonymous, 2000).

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Annexe 5.c. Developments on the toxicity of vitamin B_6

The consumption of foods rich in vitamin B_6 has never been associated to side effects. Reported side effects (in medical literature) are associated with the consumption of very high doses of pyridoxine prescribed for the treatment of pathologies such as carpal tunnel syndrome or PMS. Such doses have been associated with the development of sensory neuropathy and dermatological lesions (Cohen & Bendich, 1986 ; Schaumburg & Berger, 1988). The cause-and-effect relationship between high intake of pyridoxine and neuropathy was established in animals in 1942 (Unna & Antopol, 1940) and in humans in 1983 (Schaumburg *et al.*, 1983).

Sensory neuropathy

The first clinical observation of neurotoxicity induced by pyridoxine in humans was published by Schaumburg *et al.* in 1983. Seven adults (5 women and 2 men) exhibited neuropathy of the extremities after having ingested 2000-6000 mg pyridoxine/day for 2-40 months. Four adults were unable to walk. The diagnosis was objective and neurological signs and symptoms regressed as soon as pyridoxine intake was discontinued. Other cases of sensory peripheral neuropathy associated to high pyridoxine intake were reported in the 1980s (Baer et Sullivan, 1984; Bredesen et Parry, 1984; de Zigher *et al.*, 1985; Friedman *et al.*, 1986). The pathogenesis of sensory peripheral neuropathy and the dose/response relationship have been well documented through animal studies (Phillips *et al.*, 1978; Schaeppi & Krinke, 1985). The data shows that risks of neuropathy are diminished when intake remains below 1g pyridoxine/day (Bernstein & Lobitz, 1998; Del Tredici *et al.*, 1985).

Other side effects

Painful and scarring dermatological lesions have been reported from intakes of 2-4 g pyridoxine/day for over one year (Friedman *et al.*, 1986; Schaumburg & Berger, 1988). However, a dose-response relationship cannot be established on such insufficient data. Moreover, the mechanism(s) of pyridoxine-induced dermatitis are as of yet unclear (Schaumburg et Berger, 1988). Other side effects have been observed. A baby whose mother was treated with 50 mg pyridoxine during the last 5 months of pregnancy was born addicted (Hunt *et al.*, 1954): the child had seizures which were subdued with pyridoxine. Finally, a case of rhabdomyolysis has been seen in a newborn baby with homocystinuria after 7 days of treatment with 500 mg pyridoxine/day (Shoji *et al.*, 1998).

Dose-response relationship and upper intake level

Bernstein & Lobitz (1988) administered a daily dose of 100-150 mg pyridoxine, for up to 5 years, to 70 patients with diabetic neuropathy or carpal tunnel syndrome. A thorough neurological exam showed no side effects.

Del Tredici *et al.* (1985) identified no side effects in patients taking 100-300 mg pyridoxine/day over 4 months. Others (Brush *et al.*, 1985; Ellis *et al.*, 1984; Mitawelli *et al.*, 1984; Tolis *et al.*, 1977) also report an absence of neurological side effects in hundreds of patients taking 100-500 mg/day. Two publications suggest that the daily upper limit (to avoid toxicity) is 500 mg/day (Berger & Schaumburg, 1984; Schaumberg *et al.*, 1983). However, Berger & Schaumburg (1984) describe the case of a young woman who ingested 200 mg pyridoxine/day over a two-year period and developed a sensory neuropathy as the daily dose reached 500 mg/day. Several teams observed the development of sensory neuropathy for doses inferior to 500 mg/day. Parry et Bredesen Parry & Bredesen (1985) reported on two patients who had ingested 500 mg/day for 8 or 36 months, and another who had ingested 100-200 mg/day; both developed a sensory neuropathy. Two studies (Dalton 1985 ; Dalton & Dalton, 1987) seem even more significant in that they demonstrate sensory neuropathy in women treated for PMS with less than 500 mg pyridoxine/day. In a letter to the editor, Dalton (1985) describes sensory neuropathy (characterised by hyperesthesia, fasciculations, numbness, paresthesia, ataxia) in 40% of the women (n = 58) treated with doses of 50-300 mg pyridoxine/day. A subsequent publication (Dalton & Dalton, 1987) described how of 173 women treated for

PMS with 50-500 mg pyridoxine/day, 103 (60 %) developed neurological symptoms. This observation has several shortcomings: the real dose of consumed pyridoxine + lack of clinical exam by a confirmed neurologist. In the US, the NOAEL is considered equal to 200 mg/day and the tolerable upper intake limit is 100 mg/day by choosing a tolerance factor of 2 based on the fact that data from this area are unreliable. It would have been preferable to choose a higher tolerance factor of!

Annexe 5.d. Table on tolerable upper intake levels of vitamins

Nutritional value	Tolerable upper intake level as suggested by RNI	Tolerable upper intake level as suggested by the SCF	Tolerable upper intake level as suggested in the ILSI document	Tolerable upper intake level as suggested by the FNB
Thiamine RNI = 1.4 mg RNI 2001: M = 1.3mg/day F = 1.1 mg/day 66% RNI = 0.7 - 0.9 mg Beriberi is rare in industrialised countries but does exist in at-risk groups: severe alcoholics, the malnourished, older people in institutions. There are two types of problems: neurological and cardiac	No tolerable upper intake level Daily doses of 500 mg over years have no associated anomalies. No systematic studies in subjects using doses > several RNI Side effects when parenteral (anaphylactic chock) or intramuscular administration (skin reaction from 100 mg) No side effects when oral administration > 5 mg	To be examined by SCF	Tolerable upper intake level 50 mg/day, based on Shrimpton report	No tolerable upper intake level: rapid fall in absorption for doses > 5 mg et rapid urinary excretion of thiamine
Riboflavin RNI = 1.6 mg RNI 2001: M = 1.6 mg/day F = 1.5 mg/day 66% RNI = 1 - 1.1 mg	Non toxin vitamin No tolerable upper intake level No systematic study on toxicity in humans	No tolerable upper intake level Only reported side effect in laboratory animals receiving doses of 50 mg/kg body weight: renal lithiasis = formation of riboflavin crystals In humans: of 55 patients treated with 400 mg riboflavin/day for 3 mos, 2 developed diarrhoea and polyuria (Schoenen *et al.*, 1998)	Tolerable upper intake level based on Shrimpton report: 200 mg/day	No side effects associated to high levels of riboflavin: limited solubility and absorption capacity, and rapid urinary excretion
Niacin RNI : 18 mg RNI 2001 M = 14 mg/day F = 11 mg/day 66% RNI = 7.3 - 9.2 mg	Tolerable upper intake level: 33 mg/day Side effects and toxicity of nicotinic acid must be distinguished from that of nicotinic amide	To be examined by SCF	1500 mg/day	LOAEL at 50 mg nicotinic acid/day based on vasodilation reactions in 4/6 patients treated with this dose for 92 days (Sebrell & Butler, 1938)

Nutritional value	Tolerable upper intake level as suggested by RNI	Tolerable upper intake level as suggested by the SCF	Tolerable upper intake level as suggested in the ILSI document	Tolerable upper intake level as suggested by the FNB
	Nicotinic acid ⇒ cutaneous vasodilation at doses of 30 - 1000 mg/day – no vasodilation effect with nicotinic amide Hepatoxicity, glucose intolerance, problems with vision: observed with administration of high doses of nicotinic acid (> 1 g/day)			Same effect observed in 5/100 patients treated with 50 mg nicotinic acid/day; at 100 mg/day, 50% patients have a vasodilation reaction (Spies *et al.*, 1938) Tolerable upper intake level: 35 mg/day
Pantothenic acid RNI = 6 mg RNI 2001 M et F = 5 mg/day 66% RNI = 3.3 mg	No tolerable upper intake level as no side effects have been reported in the literature	To be examined by SCF	Tolerable upper intake level = 500 mg/day based on Shrimpton report	No tolerable upper intake level

Nutritional value	Tolerable upper intake level as suggested by RNI	Tolerable upper intake level as suggested by the SCF	Tolerable upper intake level as suggested in the ILSI document	Tolerable upper intake level as suggested by the FNB
Vitamin B6 RNI = 2 mg RNI 2001 M = 1.8 mg/day F = 1.5 mg/day 66% RNI = 1.0 - 1.2 mg	Doses of 50 mg/day can cause neuropathies (Dalton & Dalton, 1987) ⇒ tolerable upper intake level = 5 mg/day with tolerance factor of 10	SCF Report (2000): the SCF uses Dalton & Dalton (1987) data but uses a LOAEL of 100 mg, a tolerance factor of 4 and thus puts forth a tolerable intake level of 25 mg (20 mg for 15-17 yrs; 15 mg 11-14 yrs; 10 mg for 7-10 yrs; 7 mg for 4-6 yrs; (5 mg 1-3 yrs)	25 mg/day based on SCF value	Does not take into consideration Dalton & Dalton study (1987) A NOAEL of 200 mg/day and a tolerance factor of 2 = tolerable intake level 100 mg/day
Biotin RNI = 0.15 mg RNI 2001 M and F = 50 µg/day 66% RNI = 33 µg	No tolerable upper intake level	To be examined by SCF	Tolerable upper intake level of 2500 µg based on Shrimpton report	No tolerable upper intake level
Folates RNI = 200 µg RNI 2001: M = 330 µg/day and F = 300 µg/day 66% RNI = 200 - 220 µg	Side effects are only noted with ingestion of synthetic folic acid but not dietary folates Folic acid intake equal or greater to 5 mg ⇒ neurological problems in B12-deficient patients ⇒ tolerable upper intake level= 1 mg with tolerance factor of 5	LOAEL estimated at 5mg, i.e. where haematological problems can be hidden where there is vitamin B12 deficiency Tolerable upper intake level = 1 mg for adults (800 µg for 15-17 yrs; 600 µg for 11-14 yrs; 400 µg for 7-10 yrs; 300 µg for 4-6 yrs; 200 µg for 1-3 yrs)	1000 µg based on SCF value	LOAEL of 5 mg/day based on the fact that: At this dose, over 100 cases of neurological problemshave been reported in the literature A tolerable upper intake level established based on a tolerance factor of 1 mg/day
Vitamine B12 RNI = 1 µg RNI = M and F = 2.4 µg/day 66% RNI = 1.6 µg	No tolerable upper intake level for oral administration, but side effects noted when administration by injection	No tolerable upper intake level as side effects are no known	Tolerable upper intake level = 3000 µg/day based on Tolerable Upper Intake Level of the US National Food Board	No tolerable upper intake level because in 1986, 26% of the American population consumed B12 without signs of toxicity
Vitamin C RNI = 60mg RNI 2001 M and F = 110 mg/day	Tolerable upper intake level based on doses which cause gastrointestinal problems (diarrhoea): 5 mg ⇒ 1 mg/day	To be examined by SCF	2000 mg based on the Tolerable Upper Intake level of the UA National Food Board	No tetarogenic or carcinogenic effects Side effects only report from 3 g or more/day: diarrhoea,

Nutritional value	**Tolerable upper intake level as suggested by RNI**	**Tolerable upper intake level as suggested by the SCF**	**Tolerable upper intake level as suggested in the ILSI document**	**Tolerable upper intake level as suggested by the FNB**
66% RNI = 73 mg	with tolerance factor of 5			gastrointestinal problems, hyperoxaluria, kidney stones, increased uric acid excretion, oxidative effects, rebound phenomenon LOAEL at 3g/day, with tolerance factor of 1.5; tolerable upper intake level of 2g/day

Annex 6. Note on the results of the OCA study on consumers of nutritional supplements

Geneviève Potier de Courcy

The MONICA study demonstrates that 15 % of the French male population between 50-60 years consumes supplements. The unpublished 1998/1999 OCA survey on points of sale, addressing 800 consumers of supplements in France, gave the following results.

General characteristics:

- 2/3 were women;
- 42% were between 15 and 34 years, 44% between 35 and 54 years and therefore few respondents were older;
- 55% are periodic consumers;
- The represented proportions of employees or intermediary professions (38%) and executives and liberal professions (14.6%) were far superior to the national average, respectively 20% and 4.8%.
- IMC was < 24 for 72% among them and only 6% > 29.

Furthermore, for 79% of respondents, the stated reasons for consuming these supplements were tiredness, stress or exams, and only 14% stated nutritional reasons.

Their diet reflected health-consciousness, judging from the high consumption of fruit and vegetables, tea, cereals, and fish, and a lower consumption of meats, fats and oils, compared to representative surveys (ASPCC).

In terms of the specific amounts of consumed nutrients, of the 415 products, 2 main categories emerge: 21% of the vitamin C category, and 19% of the magnesium category. Other important intakes were from multivitamin products with or without minerals.

Conversely, significant intakes of nutrients (>10% RNI) per day and per consumer (M and F average) were:

- Retinol: 99 and 107 µg
- Total vitamin A: 126 and 180 ER (RNI= 800 ER)
- **Vitamin B1**: 5.8 and 0.9 mg (RNI = 1.4 mg)
- Vitamin B2: 0.8 and 0.5 mg (RNI = 1.6 mg)
- **Vitamin B6**: 1.5 and **3.7 mg** (RNI = 2 mg)
- Vitamin B9: 48 and 45.4 µg (RNI = 200 µg)
- vitamin B12 : 0.77 and 0.6 µg (RNI = 1 µg)
- **vitamin C** : **171** and **172 mg** (RNI = 60 mg)
- vitamin D : 0.5 and 0.6 µg (RNI = 5 µg)
- vitamin E : 2.3 and 3.6 mg (RNI = 10 mg)
- iron: 4.9 and 7 mg (RNI = 14 mg)
- Magnesium: 51 and 64 mg (RNI = 300 mg)

The total result of which (including diet) is (average M and F per day):

Beta-carotene: **2.66 and 3.11 mg** (= 1.1 and 1.73 RNI at 50% total vitamin A)
Retinol: 964 and 867 µg
Total vitamin A: **1407 and 1388 ER** (RNI= 800 and 600 ER)
Vitamin B1: 7.3 **and 2 mg** (RNI = 1.3 and 1.1 mg)
Vitamin B2: **2.8 and 2.2 mg** (RNI = 1.6 and 1.5 mg)
Vitamin B3: 24.3 **and 21.4 mg** (RNI = 14 and 11 mg)
Vitamin B5 7.4 and 7.9 mg (RNI = 5 mg)

Vitamin B6: **3.5 and 5.4 mg** (RNI = 1.8 and 1.5 mg)
Vitamin B9: 315 and 286 µg (RNI = 330 and 300 µg)
Vitamin B12: **7.5 and 6.8 µg** (RNI = 2.4 µg)
Vitamin C: **255 and 263 mg** (RNI = 110 mg)
Vitamin D: 3.5 and 3.3 µg (RNI = 5 µg)
Vitamin E: 8.6 and 10 mg (RNI= 12 mg)
Calcium: 1034 and 960 mg (RNI = 900 mg)
Iron: 20 and 19 mg (RNI = 9 and 16 mg)
Magnesium: 328 and 343 mg (RNI = 420 and 360 mg)

These data point to the fact that consumers of dietary complements usually have very high vitamin and mineral intakes, except for certain nutrients that lie slightly under their respective RNI. Nutrients for which there could potentially be excess intake are: retinol and total vitamin A (high dietary intake), niacin, vitamin B6 and iron.

Overall, the population in question does not seem to suffer from inadequate dietary intakes of vitamins and minerals, in light of its socio-professional status, overweight status and dietary patterns.

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Annex 7. Characteristics of the OCA simulation study (9)

- Simulations were carried out on 8 vitamins: vitamin A (retinal and its precursor), beta-carotene, vitamins D, E, C, B2, B6 and B9; and 3 minerals: calcium, iron and magnesium.

- The **list of foods likely to be enriched** has been drawn up by technical bodies based on the Répertoire Général des Aliments and includes 278 products of the 590 in the ASPCC study (see table in document 9).

- Energy is preferred to weight or volume as it is more representative of nutritional density of a food. Four levels of **fortification per 100 kcal** of foods have been tested: 10%, 20%, 40% and 100% of RDA or RNI (Annex 1, document 9).

- 5 levels of market shares, or more precisely of proportion of enriched foods consumed, have been selected: 3%, 5%, 10%, 50% and 100%. The hypothesis that the 278 foods consumed be 100% fortified has been considered in case certain individuals consume *only* fortified foods, out of all potentially fortifiable foods. Although improbable, this hypothesis remains possible for a small proportion of the population.

- In this analysis, maximum intake levels are taken either from CSHPF (September 1995) tolerable upper intake levels (6), or from CEDAP document n° 27 (October 1998) (8).

- Risks of excess were evaluated by making sure that the population with the highest intake levels (97.5^{th} percentile) remains below 10% maximum intake level, in order to take into consideration the remaining 2.5% of the population.

Annex 8. ILSI-Europe study on fortification by micronutrients in Europe (11)

The model proposed by ILSI Europe lays within harmonised regulatory framework in Europe on fortification of foods by vitamins and minerals, and put forth the following calculation to determined how much of each micronutrient can be added to foods:

$$FAn = \frac{(UL\text{-}CI\ 95)}{(0.5 \times 36 \times PFFn)}$$

Where:

- **FAn** (fortification amount) is the quantity of a vitamin or mineral that can be added to a food per 100 kcal.
- **UL** (Tolerable Upper Intake level) is the maximum level of daily chronic intake of a nutrient (regardless of the source) where there is no deleterious health risk (CSAH/SCF 2000a)
- **CI 95** (current intakes) is estimation for Europe of actual micronutrient levels in non-fortified foods (except in voluntary supplementation) (95^{th} percentile)
- **UL-CI 95** represents the maximum quantity of each nutrient that can be added to food with minimum risk to the health of the population.
- **0.5** is the estimated percentage (50%) of foods (or dietary intake of energy) which can definitely be fortified and which incorporate (above and beyond fresh meat, fruit and vegetables), technological, economic and other constraints (organoleptic, regulatory, kind of AOC, and length of conservation of nutrients in foods), which limit the possibility to fortify;
- **36** is the number of 100 kcal portions in individual consumption knowing that the average of the highest energy intakes (95^{th} percentile) in the adult make in Europe is 3600 kcal/d.
- **PFFn** (hypothetical fractions of potentially fortified foods in the market) is the proportion of potential market shares for fortified foods, tested at 5, 10, 25, 50, or 100 % (as per OCA simulations).

Annex 9. Tolerable upper intake limits of micronutrient fortification of commonly eaten foods

Method:

The data used are those from the ASPCC study (1994), which had already been utilised CEDAP simulation exercise (9) : the modified ILSI Europe method is thus based on ASPCC data.

The **list of foods likely to be enriched** has been drawn up by technical bodies based on the Répertoire Général des Aliments and includes 278 products of the 590 in the ASPCC study.

In each of the two models, the hypothesis that the 278 foods consumed be 100% fortified has been considered in case certain individuals *only* consume fortified foods out of all potentially fortifiable foods.

For each vitamin and mineral the intake at all levels of fortification between 1% -100 % RNI are calculated per 100 kcal. In each of the three cases, the population percentile just below the tolerable upper intake level is retained. The figures below therefore correlate the fortification levels and the population proportion for which intakes do not exceed the tolerable upper intake level.

In the most general case, the simulated exposure of an individual *i* for the fortification level *e* is calculated where *pj* is the proportion of fortified products *j*, *cij* is the intake of products *j* by the individual *i* and *tj* is the amount of micronutrient in non-fortified product *j*. *B* is the density by binomial law.

*Expo(i,e)=_j[B(1,pj)e+B(1.1-pj)tj]*cij*

Vitamin C
RNI=60 mg/d LS=1 100 mg/d

The figure on vitamin C illustrates that according to the tolerance level chosen among the highest consumers, (95^{th}, 97.5^{th} or 100^{th} percentile), the possibility to fortify lies respectively at 88% (ILSI model), 62% (modified ILSI model) or 38% RNI/100 kcal.

Vitamin D
RNI=5 µg/d LS=20 µg/d

The figure on vitamin D illustrates that according to the tolerance level chosen among the highest consumers, (95^{th}, 97.5^{th} or 100^{th} percentile), the possibility to fortify lies respectively at 15% (ILSI model), 12% (modified ILSI model) or 2.5% RNI/100 kcal, with 100% proportion of enriched foods consumed.

Calcium
RNI=800 mg/d LS=2 000 mg/d

Finally, for calcium the fortification potential is limited to a range of 2.5 - 5%, regardless of the hypothesis. These discrepancies are due to the distribution curve in the particular zone, and the relative space between actual dietary intake and tolerable upper intake level.

Annex 10. Detailed analysis of proposed optimum fortification contents for 6 vitamins and 3 minerals according to the OCA simulation method (9)

Zone which represents a value for low consumers or zone where the fortification level is inadequate for high consumers.

Zone which has value for low consumers, but where the fortification level but is inadequate for high consumers

Zone wherein the fortification level is adequate for high consumers but has no value for low consumers.

Zone of value= Zone which represents a value for low consumers, and where the fortification level is adequate for high consumers.

	Proportion of enriched foods consumed	**Significance for low consumers**				**Absence of risk for high consumers**			
		AJR				**AJR**			
		10%	**20%**	**40%**	**100%**	**10%**	**20%**	**40%**	**100%**
Vitamine A (totale)	**3%**	no	no	no	no	no	no	no	no
	5%	no	no	no	yes	no	no	no	no
	10%	no	no	yes	yes	no	no	no	no
	50%	no	yes	yes	yes	no	no	no	no
	100%	yes	yes	yes	yes	no	no	no	no

	Proportion of enriched foods consumed	**Significance for low consumers**				**Absence of risk for high consumers**			
		AJR				**AJR**			
		10%	**20%**	**40%**	**100%**	**10%**	**20%**	**40%**	**100%**
rétinol	**3%**	no	no	no	no	no	no	no	no
	5%	no	no	no	yes	no	no	no	no
	10%	no	no	yes	yes	no	no	no	no
	50%	yes	yes	yes	yes	no	no	no	no
	100%	yes	yes	yes	yes	no	no	no	no

	Proportion of enriched foods consumed	Significance for low consumers				Absence of risk for high consumers			
		AJR				AJR			
		10%	20%	40%	100%	10%	20%	40%	100%
bêta carotène	3%	no	no	no	no	yes	yes	yes	yes
	5%	no	no	no	no	yes	yes	yes	yes
	10%	no	no	no	no	yes	yes	yes	yes
	50%	no	no	no	yes	yes	yes	yes	no
	100%	no	no	no	yes	yes	yes	no	no

	Proportion of enriched foods consumed	Significance for low consumers				Absence of risk for high consumers			
		AJR				AJR			
		10%	20%	40%	100%	10%	20%	40%	100%
Vitamine D	3%	no	no	no	no	yes	yes	yes	yes
	5%	no	no	no	no	yes	yes	yes	yes
	10%	no	no	no	no	yes	yes	yes	yes
	50%	no	yes	yes	yes	yes	yes	no	no
	100%	yes	yes	yes	yes	yes	no	no	no

	Proportion of enriched foods consumed	Significance for low consumers				Absence of risk for high consumers			
		AJR				AJR			
		10%	20%	40%	100%	10%	20%	40%	100%
Vitamine E	3%	no	no	no	no	yes	yes	yes	yes
	5%	no	no	no	no	yes	yes	yes	yes
	10%	no	no	no	yes	yes	yes	yes	yes
	50%	no	yes	yes	yes	yes	yes	yes	no
	100%	yes	yes	yes	yes	yes	yes	no	no

	Proportion of enriched foods consumed	Significance for low consumers				Absence of risk for high consumers			
		AJR				AJR			
		10%	20%	40%	100%	10%	20%	40%	100%
Vitamine C	3%	no	no	no	no	yes	yes	yes	yes
	5%	no	no	no	no	yes	yes	yes	yes
	10%	no	no	no	yes	yes	yes	yes	yes
	50%	no	yes	yes	yes	yes	yes	yes	yes
	100%	yes	yes	yes	yes	yes	yes	yes	no

	Proportion of enriched foods consumed	Significance for low consumers				Absence of risk for high consumers			
		AJR				AJR			
		10%	20%	40%	100%	10%	20%	40%	100%
Vitamine B2	3%	no	no	no	no	yes	yes	yes	yes
	5%	no	no	no	no	yes	yes	yes	yes
	10%	no	no	no	yes	yes	yes	yes	yes
	50%	yes	yes	yes	yes	yes	yes	yes	no
	100%	yes	yes	yes	yes	yes	yes	yes	no

	Proportion of enriched foods consumed	Significance for low consumers				Absence of risk for high consumers			
		AJR				AJR			
		10%	20%	40%	100%	10%	20%	40%	100%
Vitamine B6	3%	no	no	no	no	yes	yes	yes	yes
	5%	no	no	no	no	yes	yes	yes	yes
	10%	no	no	no	yes	yes	yes	yes	no
	50%	no	yes	yes	yes	yes	yes	no	no
	100%	yes	yes	yes	yes	yes	no	no	no

	Proportion of enriched foods consumed	Significance for low consumers				Absence of risk for high consumers			
		AJR				AJR			
		10%	20%	40%	100%	10%	20%	40%	100%
Vitamine B9 (*)	3%	no	no	no	yes	yes	yes	yes	yes
	5%	no	no	no	yes	yes	yes	yes	no
	10%	no	yes	yes	yes	yes	yes	yes	no
	50%	yes	yes	yes	yes	yes	no	no	no
	100%	yes	yes	yes	yes	yes	no	no	no

* taking into consideration the tolerable upper intake level for children and the dietary intake measured by the average value of the RNI between values for men and values for women

	Proportion of enriched foods consumed	Significance for low consumers				Absence of risk for high consumers			
		AJR				AJR			
		10%	20%	40%	100%	10%	20%	40%	100%
Calcium	3%	no	no	no	no	yes	yes	yes	no
	5%	no	no	no	no	yes	yes	yes	no
	10%	no	no	no	yes	yes	yes	no	no
	50%	no	yes	yes	yes	yes	no	no	no
	100%	yes	yes	yes	yes	no	no	no	no

	Proportion of enriched foods consumed	**Significance for low consumers**				**Absence of risk for high consumers**			
		AJR				**AJR**			
		10%	**20%**	**40%**	**100%**	**10%**	**20%**	**40%**	**100%**
Fer	**3%**	no	no	no	no	yes	yes	yes	no
	5%	no	no	no	no	yes	yes	yes	no
	10%	no	no	no	yes	yes	no	no	no
	50%	no	yes	yes	yes	no	no	no	no
	100%	yes	yes	yes	yes	no	no	no	no

	Proportion of enriched foods consumed	**Significance for low consumers**				**Absence of risk for high consumers**			
		AJR				**AJR**			
		10%	**20%**	**40%**	**100%**	**10%**	**20%**	**40%**	**100%**
Magnésium*	**3%**	no	no	no	no	yes	yes	yes	no
	5%	no	no	no	no	yes	yes	yes	no
	10%	no	no	no	yes	yes	yes	no	no
	50%	no	yes	yes	yes	yes	no	no	no
	100%	yes	yes	yes	yes	no	no	no	no

* taking into consideration the tolerable upper intake level for children and the dietary intake measured by the average value of the RNI between values for men and values for women