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Opinion of the Scientific Committee on Food on the Tolerable Upper Intake Level of Vitamin B_{12}

(expressed on 19 October 2000)

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FOREWORD

This opinion is one in the series of opinions of the SCF on the upper levels of vitamins and minerals. The terms of reference given by the European Commission for this task, the related background and the guidelines used by the Committee to develop tolerable upper intake levels for vitamins and minerals used in this opinion, which were expressed by the SCF on 19 October 2000, are available on the Internet at the pages of the SCF, at the address: http://www.europa.eu.int/comm/food/fs/sc/scf/index_en.html.

1. INTRODUCTION

Vitamin B_{12} is the generic name for a specific group of cobalt-containing corrinoids with biological activity in humans. This group of biologically active corrinoids is also described as cobalamins. Cyanocobalamin is the commercialy available form used in food supplements and food fortification. In foods, hydroxo-, methyl- and 5'-deoxyadenosyl-cobalamins are the main cobalamins present. Sulphitocobalamin, with a sulphite ligand chelated to the central cobalt atom in the corrin ring, may occur is some processed foods.

Vitamin B_{12} functions primarily as a coenzyme in intermediary metabolism. Only two vitamin B_{12} dependent reactions have been identified thus far for humans: 1) the methionine synthase reaction with methylcobalamin, and 2) the methylmalonyl CoA mutase reaction with 5-deoxyadenosylcobalamin as the active coenzyme, respectively.

A dietary vitamin B_{12} deficiency can occur in strict vegetarians or after gastrectomy, and other diseases affecting cobalamin absorption. About two-thirds of patients with vitamin B_{12} deficiency have pernicious anemia (PA), an autoimmune disorder associated with gastric atrophy and absence of Intrinsic Factor (IF) which results in vitamin B_{12} malabsorption. The key symptom in vitamin B_{12} deficiency is macrocytic megaloblastic anemia. These haematological abnormalities are indistinguishable from those seen in folate deficiency, because of the interrelated function of both vitamins (Herbert, 1986). Another key symptom of vitamin B_{12} deficiency are neurological complications, such as paraesthesia, leg weakness, memory loss, etc, due to progressive lesions in the lateral and posterior columns of the spinal cord (subacute combined degeneration of the spinal cord). Neurological symptoms occur in about 75-90% of all individuals with (untreated) vitamin B_{12} deficiency, and appear generally at a later stage. In about 25% of all cases neurological symptoms are the only symptoms, i.e. without haematological abnormalities (for review see Bower & Wald, 1995; Lindenbaum *et al.*, 1988).

2. NUTRITIONAL BACKGROUND

Vitamin B_{12} plays a specific role in amino acid metabolism, i.e. in methylation reactions, together with folate, in the methionine synthase reaction, and in the rearrangement of methylmalonyl CoA into succinyl CoA (for review see Herbert, 1984; Ellenbogen & Cooper, 1991).

The average dietary requirement for vitamin B_{12}, as established by the Scientific Committee for Food (Nutrient and Energy Intakes for the European Community, Reports of the SCF, 31th Series, 1993) is 1.0 µg/day, with a population reference intake (PRI) for adults of 1.4 µg/day. This is approximately the amount needed to maintain an adequate vitamin B_{12} body pool (about 2.5 mg), and to compensate for daily losses (about 0.1% of the total body pool). Dietary vitamin B_{12} only comes from animal sources, mainly from dairy products, fish and (red) meat. Daily intakes between 2 and 6 µg have been reported for omnivores. Individuals consuming large amounts of liver and some types of fish (sardines) may have high intakes; in contrast, individuals avoiding animal products need supplemental sources of vitamin B_{12}.

The prevalence of marginal cobalamin deficiency in elderly, characterized by low serum cobalamin and increased plasma methylmalonic acid (MMA) levels, has been estimated at about 25% (van Asselt, 1998). This is likely due to a decreased bioavailability of vitamin B_{12} from dietary sources, but not from synthetic vitamin B_{12}. In the US the use of vitamin B_{12} supplements is encouraged for elderly because of their compromised absorption (FNB DRI Report, IOM, 1998).

Other groups at risk for a marginal intake of vitamin B_{12} are those avoiding animal products, such as vegans and individuals on a macrobiotic diet, and subjects with an undiagnosed pernicious anemia.

Detailed intake data on vitamin B_{12} in EU countries are scarce. Recently, average intakes of 4.9 and 3.9 µg/day were reported for adult men and women, respectively, from a representative household survey in The Netherlands, using a two-day dietary record method (Blokdijk *et al.*, 2000). The mean vitamin B_{12} intake in Dutch elderly subjects was about 5 µg/day, with a range of 0.5-16.9 µg/day from dietary intake, and up to 32 µg/day for the total intake (including supplements) (van Asselt *et al.*, 1998).

Data from Ireland (IUNA, 2000) indicate a mean vitamin B_{12} intake from all sources (food + supplements) of 5.4 and 4.1 µg/day for men and women, respectively. The upper 97.5th percentiles were 15.0 and 15.1 µg/day, respectively. Mean intakes from food sources only were 5.2 and 3.6 µg/day, for males and females, with upper 97.5th percentiles of 13.1 and 11.8 µg/day, respectively.

Data from the United Kingdom (HMSO, 1990) indicate a mean vitamin B_{12} intake from all sources (food + supplements) of 7.3 and 5.4 µg/day for men and women, respectively. The upper 97.5th percentiles were 23.0 and 18.2 µg/day, respectively. Mean intakes from food sources only were 7.2 and 5.2 µg/day, for males and females, with upper 97.5th percentiles of 22.9 and 17.8 µg/day, respectively.

Data from the Boston Nutritional Status Survey on vitamin B_{12} supplement use among elderly show that the median (percentile 50) intake from supplements is 5 µg/day for males and 6

µg/day for females (total intake from diet + supplement: 9.7 and 9.0µg/day, respectively); the percentile 95 intake from supplements was 77 µg/day in men and 100 µg/day in women, and the corresponding values for total intake were 83 µg/day in men and 106 µg/day in women, respectively. Data from NHANES III (USA) give a highest mean intake from diet + supplements for males (31-50 years) of 17 µg/day, the percentile 95 intake in pregnant females was 37 µg/day (data taken from FNB DRI Report, IOM, 1998).

Dietary vitamin B_{12} is absorbed through a receptor mediated mechanism in the ileum. Food-bound vitamin B_{12} has first to be liberated through peptic digestion and gastric acid secretion in the stomach. The 'free' vitamin B_{12} becomes then bound to haptocorrins (or R-proteins) secreted by the salivary glands and the gastric mucosa. In the small intestine the R-binders are degraded by pancreatic protease action and the cobalamins are subsequently bound to the Intrinsic Factor (IF), a glycoprotein secreted by the parietal cells of the stomach. Uptake in the ileum is specific for the IF-cobalamin complex. Fractional absorption decreases as the oral dose is increased. Ileal receptors are saturated with dosages between approximately 1.5 and 2.5 µg of vitamin B_{12} per meal. At intakes around 1 µg about 50% is absorbed, at dosages around 25 µg only 5% is absorbed. Very small amounts (ca 1%) can be absorbed by passive diffusion, in the absence of IF.

In blood the cobalamins are transported by specific binding proteins, called transcobalamins. Although normally about 80% of the plasma cobalamins (mainly methyl-, adenosyl- and hydroxocobalamin) are bound by the glycoproteins TC I and TC III, the other 20% is bound to TC II, which is the essential B_{12} carrier in the delivery of the vitamin to the non-hepatic, metabolically active tissues such as the bone marrow and the brain.

After parenteral administration of hydroxycobalamin a rapid decline in plasma levels has been observed in the first 7 hours, followed by a slower decline with a half-life elimination of 21-29 h (Loew, 1988).

Vitamin B_{12} is an exceptional B-vitamin as it can be stored in significant amounts, especially in the liver and the kidney. The average concentration in human liver is between 0.5-1 µg/g; the total body pool size is estimated between 2-3 mg (Grasbeck *et al.*, 1958; Adams *et al.*, 1972). The main excretion is through the bile, but there is a considerable reabsorption of these biliary cobalamin losses in the ileum (enterohepatic circulation). Average daily losses *via* the stool are estimated at ca 0.5 µg. In PA patients these losses are higher, estimated at about 0.2% of the total body pool size, because of a lack of (IF mediated) reabsorption. Urinary excretion is minimal, and increased only if the plasma binding capacity is exceeded, e.g. following parenteral or intravenous administration. Total daily losses are estimated at about 0.1% of the total body pool (for review see Scott, 1997; Ellenbogen & Cooper, 1991).

3. HAZARD IDENTIFICATION

No adverse effects have been associated with excess vitamin B_{12} intake from food or supplements in healthy individuals. Vitamin B_{12} has a history of safe long-term use as a therapeutic agent given in high dosages per os, or *via* intramuscular injections, for treatment of disorders associated with impaired vitamin B_{12} absorption, such as in gastrectomy and malabsorpion. In vitamin B_{12} replacement therapy oral or intramuscular dosages between 1-5 mg vitamin B_{12} are used, with no supportive evidence of adverse effects. The usual treatment

in PA patients is 1 mg administered intramuscularly once every 1 to 3 months, but oral dosages of 300-1000 µg daily could also provide adequate treatment (Berlin *et al*., 1968; Hathcock & Troendle, 1991). At these dosage rates the cobalt and cyanide contributions are toxicologically insignificant (see Hathcock & Troendle, 1991).

Mangiarotti *et al*. (1986) studied the effect of massive supplementation with vitamin B_{12} in a group of dialysis patients. One group of 106 patients received a multivitamin preparation containing 2.5 mg vitamin B_{12} plus 0.7 mg folic acid, 12 mg niacin and 150 mg vitamin C at the end of each dialysis period during 3 years. Serum vitamin B_{12} levels at the end of the treatment period were 4 times greater than normal, but no adverse effects were reported.

High dosages have also been used in other experimental studies, mostly short term, such as for the treatment of sleep-waking rhythm disorders, in which study vitamin B_{12} (no form specified) was given in dosages between 1.5 and 3 mg/day for 8 weeks (n = 13 cases) , with no adverse effects recorded (Maeda *et al*., 1992).

High dose vitamin B_{12} (1 mg cyanocobalamin intramuscular weekly for 1 months, followed by monthly injections for a minimum of 6 months), has also been used to improve cognitive functions in geriatric patients (Martin *et al*., 1992). Cobalamin therapy resulted in cognitive recovery in some patients, and no adverse effects were reported.

In some studies intravenous (i.v.) dosing has been associated with dermal abnormalities, e.g. acne formation in some cases. Ten cases were reported by Puissant *et al*. (1967) after series of up to 12 injections with 5 mg of hydroxocobalamin, but not with cyanocobalamin. The authors suggest that degradation products, formed from the less stable hydroxocobalamin, might have been responsible for the acne formation, rather than the intact compound (but no further data provided).

One case of acneiform eruption, described as acne rosacea, was reported for a 53 yr old women who used vitamin supplements containing 100 mg vitamin B_6 and 100 µg vitamin B_{12} and 10,000 IU vitamin A and an unknown amount of zinc. Upon discontinuation of the supplement a 'dramatic' improvement was observed. The author ascribe the acne to the vitamins B_6/B_{12} without further testing (Sheretz, 1991).

Hydroxocobalamin is used as a cyanide antidote and has also a history of safe and effective use. For this purpose intravenous dosages up to 5 g are given (Forsyth *et al*., 1993).

Foulds *et al*. (1969) described 4 PA patients presenting with tobacco amblyopia (optic neuropathy) who were treated parenterally with 0.25-1 mg cyanocobalamin per month. This treatment restored the haematological, but not the visual abnormalities. When the treatment was changed to hydroxocobalamin the visual impairment also improved. Cyanide, derived from tobacco smoke, has been implicated in the pathogenesis of tobacco amblyopia, and the positive effect of hydroxocobalamin is likely explained by cyanide detoxification.

One case has been reported of a 32 year old man handling animal feed who developed contact dermatitis to vitamin B_{12} and developed skin plaques (Rodrigues *et al*., 1994). However, this anecdotal evidence without follow up is not considered relevant in deriving an UL.

3.1. Carcinogenicity

A tumour promoting effect of vitamin B_{12} has been reported in one study in rats. Rats kept on a methionine deficient diet supplemented with 5 µg/100 g vitamin B_{12} (trademark: Rubramin) and treated with the carcinogen *p*-dimethylaminobenzene (DAB) had a higher incidence of hepatomas compared to the group without supplemental vitamin B_{12}. A control group receiving the supplemented diet without DAB showed no hepatic tumours (Day *et al*., 1950).

Kalnev (1977) studied the effect of methylcobalamin and cyanocobalamin on the growth of Walker's carcinosarcoma and on the longevity of rats with implanted Zajdela ascites hepatoma cells, and reported reduced survival of rats upon treatment with both compounds.

4. DOSE RESPONSE. DERIVATION OF A TOLERABLE UPPER INTAKE LEVEL

No systematic toxicological studies have been reported for vitamin B_{12}. There are no reports attributing carcinogenic or mutagenic or teratogenic potential to cyanocobalamin (see Ellenbogen & Cooper, 1991). In one study a tumour promoting effect was reported in a rat model, but this study is not considered relevant for safety assessment in humans.

There are also no adverse effects known for vitamin B_{12} from foods, or from supplements in amounts far in excess of needs. Some studies suggested acne formation after high parenteral doses of hydroxocobalamin, but not with cyanocobalamin, or after a combination of vitamins A, B_6 and B_{12} given orally.

Oral and parenteral supplementation with dosages between 1-5 mg every fortnight or month have been given for long periods, up to at least 5 years, to patients with compromised vitamin B_{12} absorption, without any identified adverse effects. It should be noted, however, that these studies were not designed to find adverse effects.

Therefore there are no clearly defined adverse effects produced by vitamin B_{12} that can be used to define a LOAEL or NOAEL, which can be used as a basis for deriving an UL.

5. CHARACTERISATION OF RISK

Average intakes of vitamin B_{12} are about 2-6 µg/day from food; intakes up to 32 µg/day have been reported for the total intake (including supplements) in elderly Dutch subjects (van Asselt *et al*., 1998). For the UK (HMSO, 1990) upper intake levels (97.5^{th} percentile) from food sources only were reported to be 22.9 and 17.8 µg/day, for males and females, respectively. Upper intake levels from all sources were hardly higher, i.e. 23.0 and 18.2, respectively.

Data from the USA (see Section 2) show 95^{th} percentile intakes from food and supplements of 83 µg/day in elderly men, 106 µg/day in elderly women, and 37 µg/day in pregnant women. Although it is not possible to derive an UL, there is no evidence that the current levels of intake from foods and supplements represent a health risk.

In addition, adverse effects have not been reported in the treatment of patients with compromised B_{12} absorption who received dosages up 1000 µg/day orally for prolonged periods; however, there was no systematic assessment of adverse effects in these patients.

Supplements available on the market usually contain dosages between 1-5 µg, but higher dose supplements with 50 µg or more are available.

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