

## SCIENTIFIC OPINION

### on 5'-deoxyadenosylcobalamin and methylcobalamin as sources for Vitamin B<sub>12</sub> added as a nutritional substance in food supplements<sup>1</sup>

#### Scientific Opinion of the Panel on Food Additives and Nutrient Sources added to food

(Question No EFSA Q-2005-165, Q-2005-173, Q-2006-280)

**Adopted on 25 September 2008**

#### PANEL MEMBERS

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#### SUMMARY

Following a request from the Commission, the Panel on Food Additives and Nutrient Sources added to Food (ANS) has been asked to evaluate the safety and bioavailability of methylcobalamin and 5'-deoxyadenosylcobalamin, as sources for vitamin B<sub>12</sub> when added for nutritional purposes in food supplements.

The present opinion deals only with the safety and bioavailability of two particular sources of vitamin B<sub>12</sub>, intended for the general population, to be added in food supplements. While the safety of cobalamin itself, in terms of amounts that may be consumed, is outside the remit of this Panel, it is necessary for the evaluation of the sources to read across from the safety data on the vitamin, because the sources evaluated are vitamers of vitamin B<sub>12</sub>.

Methylcobalamin may be produced from genetically modified micro-organisms but the Panel concludes that this source is not part of the present opinion because it would require a separate submission under Regulation no 1829/2003.

Vitamin B<sub>12</sub> is the name given to a group of related compounds containing cobalt as the central ion in a corrin ring. The cobalt ion can be coordinated to a methyl, 5'-deoxyadenosyl-,

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hydroxy- or cyano- group. Hydroxycobalamin and cyanocobalamin used in food supplements are transformed in the human body by coordinating with other ligands into methylcobalamin and 5'-deoxyadenosylcobalamin. The latter two are actively involved in endogenous metabolism. Thus, 5'-deoxyadenosylcobalamin and methylcobalamin can be considered to represent naturally occurring endogenous derivatives of vitamin B<sub>12</sub>.

Assuming that in case of vitamin B<sub>12</sub> the ligand will not influence the biological activity of the main molecule significantly, the Panel concludes that methylcobalamin and 5'-deoxyadenosylcobalamin will be bioavailable, and that the metabolic fate and biological distribution of methylcobalamin and 5'-deoxyadenosylcobalamin are expected to be similar to that of other sources of vitamin B<sub>12</sub> in the diet. Furthermore, following absorption, vitamin B<sub>12</sub>, whatever source, is transformed to either methylcobalamin or 5'-deoxyadenosylcobalamin.

The quantity of methylcobalamin or 5'-deoxyadenosylcobalamin proposed to be used in food supplements amounts to levels up to 500 µg vitamin B<sub>12</sub>/day for adults.

The petitioner of a second dossier on methylcobalamin indicates that methylcobalamin is intended to be used in food supplements, e.g. in capsules, tablets, ampoules or powders at a proposed level of use of 2 µg/day.

The Scientific Committee on Food (SCF) concluded that average intakes of vitamin B<sub>12</sub> are about 2-6 µg/day from food and that intakes up to 32 µg/day have been reported for the total intake including supplements in elderly Dutch subjects. For the UK upper intake levels (97.5<sup>th</sup> percentile) from food sources were reported to be 22.9 and 17.8 µg/day for males and females respectively. The SCF concluded that upper intake levels from all sources were hardly higher, i.e. 23.0 and 18.2 µg/day respectively.

The proposed levels of use amounting up to 500 µg vitamin B<sub>12</sub>/day will substantially increase normal daily intake which has been estimated to amount on average to less than 10 µg/day.

According to the SCF, the majority of vitamin B<sub>12</sub> that enters the body via nutrition is stored in the liver. Normally the liver stores a vitamin B<sub>12</sub> supply of several milligrams of which only 0.1 - 0.2% is lost per day, and therefore is sufficient to cover the daily need for a longer period without supplementation.

The SCF has issued an opinion on the Tolerable Upper Intake level of vitamin B<sub>12</sub> and concluded that it is not possible to derive an Upper Intake Level, mainly because no clearly defined adverse effect could be identified. The Panel noted that the proposed levels of use amounting up to 500 µg vitamin B<sub>12</sub>/day are below the guidance value of 2000 µg/day defined by the UK Expert Group on Vitamins and Minerals (EVM).

The Panel concludes that the use of 5'-deoxyadenosylcobalamin and methylcobalamin as a source of vitamin B<sub>12</sub> in food supplements for the general population at the proposed uses and use levels is not of safety concern.

**Key words:**

Food supplements, 5'-deoxyadenosylcobalamin, adenosylcobalamin, methylcobalamin, Vitamin B<sub>12</sub>, CAS Registry numbers: 13422-55-4, 13870-90-1, 68-19-9.

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#### **BACKGROUND AS PROVIDED BY THE COMMISSION**

The European Community legislation lists nutritional substances that may be used for nutritional purposes in certain categories of foods as sources of certain nutrients.

The Commission has received a request for the evaluation of 5'-deoxyadenosylcobalamin and two requests for the evaluation of methylcobalamin added for nutritional purposes to food supplements. The relevant Community legislative measure is:

- Directive 2002/46/EC of the European Parliament and of the Council on the approximation of the laws of the Member States relating to food supplements<sup>2</sup>.

#### **TERMS OF REFERENCE AS PROVIDED BY THE COMMISSION**

In accordance with Article 29 (1) (a) of Regulation (EC) No 178/2002, the European Commission asks the European Food Safety Authority to provide a scientific opinion, based on its consideration of the safety and bioavailability of 5'-deoxyadenosylcobalamin and methylcobalamin added for nutritional purposes in food supplements.

#### **ACKNOWLEDGEMENTS**

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<sup>2</sup> OJ L 183, 12.7.2002, p.51.

## ASSESSMENT

### 1. Introduction

The present opinion deals only with the safety and bioavailability of particular sources of vitamin B<sub>12</sub> intended for the general population, to be used in food supplements. While the safety of cobalamin itself, in terms of amounts that may be consumed, is outside the remit of this Panel, it is necessary for the evaluation of the sources to read across from the safety data on the vitamin, because the sources evaluated are vitamers of vitamin B<sub>12</sub>.

### 2. Technical data

#### 2.1. Chemistry

Vitamin B<sub>12</sub> is the generic name given to a group of related compounds containing cobalt as the central ion in a corrin ring (Figure 1). This group of biologically active cobalt containing corrinoids is also described as cobalamins. The cobalt ion can be coordinated to a methyl, 5'-deoxyadenosyl-, hydroxy- or cyano- group (Wolters *et al.*, 2004). Hydroxycobalamin and cyanocobalamin used in food supplements are transformed in the human body by coordinating with other ligands into methylcobalamin and 5'-deoxyadenosylcobalamin. The latter two are actively involved in endogenous metabolism (Kelly, 1997; Hillman, 1985).

In foods hydroxy-, methyl- and 5'-deoxyadenosyl-cobalamin are the main cobalamins present.

Vitamin B<sub>12</sub> plays a specific role in amino acid metabolism, i.e. in the methionine synthase reaction, and in the rearrangement of methylmalonyl CoA into succinyl CoA (SCF, 2000). In humans these vitamin B<sub>12</sub> dependent reactions have been identified with methionine synthase functioning with methylcobalamin and the methylmalonyl CoA mutase reaction with 5'-deoxyadenosylcobalamin as the active coenzyme (SCF, 2000).

The chemical structure of vitamin B<sub>12</sub> is presented in Figure 1. In vitamin B<sub>12</sub> the cobalt atom is coordinated to a cyanide group, in methylcobalamin to a methyl group and in 5'-deoxyadenosylcobalamin to a 5'-deoxyadenosine at the 5' position (coenzyme B<sub>12</sub>). In hydroxycobalamin (also called hydroxocobalamine) the R group is a hydroxyl moiety.

Cobalamin is a molecule with several asymmetric centers. Natural cyanocobalamin (vitamin B<sub>12</sub>) is a pure enantiomer. When the reactions used in the manufacturing process of the 5'-deoxyadenosylcobalamin and methylcobalamin do not affect the stereochemistry of the chiral centers of the molecule, it may be assumed that 5'-deoxyadenosylcobalamin and methylcobalamin are also single enantiomers of defined stereochemistry at all the chiral positions, corresponding to that of natural vitamin B<sub>12</sub>.

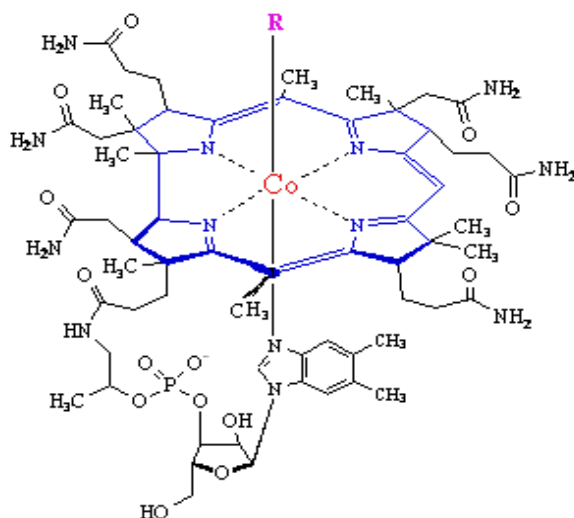


Figure 1. **Chemical structure of vitamin B<sub>12</sub>, with R being a cyano moiety (cyanocobalamin). In methylcobalamin the R group is a methyl moiety, in hydroxycobalamin a hydroxyl moiety and in 5'-deoxyadenosylcobalamin a 5'-deoxyadenosine.**

### ***5'-Deoxyadenosylcobalamin***

The full name of 5'-deoxyadenosylcobalamin is 5,6-dimethylbenzimidazolylcobamide-5'-deoxyadenosine. Synonyms and trade names are adenosylcobalamin, dibenzozide, cobamide, and Coenzyme B<sub>12</sub>.

The molecular formula is C<sub>72</sub>H<sub>100</sub>CoN<sub>18</sub>O<sub>17</sub>P and its molecular weight is 1579.6 g/mol.

5'-Deoxyadenosylcobalamin is a naturally occurring coenzyme form of vitamin B<sub>12</sub> and is commonly present in the human diet (organ meats, fish, eggs and dairy products). This coenzyme form bypasses the body's need to synthesize the active form of vitamin B<sub>12</sub> from cyano- or hydroxycobalamin. 5'-Deoxyadenosylcobalamin is the predominant form of vitamin B<sub>12</sub> in human tissues (up to 70%) (Castle and Hale, 1998).

### ***Methylcobalamin***

The chemical name for methylcobalamin is Coα-[α-(5,6-dimethylbenz-1*H*-imidazolyl)]-Coβ-methylcobamide. Synonyms and trade names are mecobalamin, methylcobalamin, cobalt-methylcobalamin, Algobaz and Cobamet.

The molecular formula is C<sub>63</sub>H<sub>91</sub>CoN<sub>13</sub>O<sub>14</sub>P and its molecular weight is 1344.4 g/mol.

## **2.2. Specifications**

For the cobalamin preparations to which the present opinion refers, the following specifications were provided by the petitioners.

### ***5'-Deoxyadenosylcobalamin***

The petitioner indicates that purity, determined by a spectrometric assay, is >96% on dried product. Impurities are hydroxycobalamin at < 2.0%, acetone as residual solvent at < 0.5% and other impurities at levels < 1.0%. The petitioner also indicates that bacterial endotoxins are at levels < 0.4 IU/ microg.

### ***Methylcobalamin***

Both petitioners for methylcobalamin indicate that the content of the methylcobalamin preparation is not less than 98.0% as determined by HPLC on anhydrous material.

The loss on drying of the anhydrous material is not more than 3% and the content of lead was specified as being not more than 5 mg/kg.

Table 1 summarizes the specifications provided by one of the two petitioners for methylcobalamin:

**Table 1. Specifications for methylcobalamin proposed by one of the petitioners.**

<b>ANALYSIS</b>	<b>SPECIFICATIONS</b>	<b>METHOD OF ANALYSIS</b>
Appearance	Dark red crystals or crystalline powder	JP
Identification		
UV-VIS spectrum pH = 2.0	Conforms to Standard	JP
UV-VIS spectrum pH = 7.0	Conforms to Standard	JP
Color of the reaction	Conforms to Standard	JP
Analytical tests		
Clarity and color of solution	Clear and red color	JP*
Impurities		
Water content (KF)	< 6.0%	JP*
Residual solvents (GC)		
Acetone (GC)	< 5000 ppm	General Test Eu. Ph. 5.4.
Methanol (GC)	< 3000 ppm	General Test Eu. Ph. 5.4.
Related Substances (HPLC)		
Individual impurities	≤ 0.5%	JP
Total impurities	≤ 2.0%	JP
Assay		
Assay (HPLC)	≥ 98.0% on anhydrous product	JP

JP\* The method from Japanese Pharmacopoeia has been slightly modified to adapt it to the available reagents.

## **2.3. Manufacturing Process**

### ***5'-Deoxyadenosylcobalamin***

5'-Deoxyadenosylcobalamin is prepared from vitamin B<sub>12</sub> obtained from a fermentation process using a basis of beet molasses and mineral salts. The petitioner indicates that vitamin B<sub>12</sub> should be stored in the dark.

### ***Methylcobalamin***

The petitioner indicates that methylcobalamin may be manufactured semi-synthetically following extraction of vitamin B<sub>12</sub> from animal material. The petitioner states that alternatively, it may be produced from Genetically Modified Micro-organisms (GMMs). However this source is not part of the present opinion because it would require a separate submission under Regulation no1829/2003 (EC, 2003).

The second petitioner for methylcobalamin indicates that the manufacturing process comprises three steps which are described in detail and include preparation of methylcobalamin from vitamin B<sub>12</sub>, followed by chromatographic purification and crystallization of methylcobalamin.

Because methylcobalamin is highly labile upon exposure to light the whole manufacturing process and the analytical controls require an effective protection from light.

### **2.4. Methods of analysis in food**

One of the two petitioners for methylcobalamin indicates that following appropriate extraction and preparation, high potency preparations may be assayed by UV/visible spectrophotometry.

Alternatively, vitamin B<sub>12</sub> assays may be based on the determination of cobalt, which is quantified by colorimetry with Nitrose-R salt, AA spectroscopy or ICP-MS. The cobalt may also be determined by radiometric methods.

The analysis of vitamin B<sub>12</sub> in food is described by Watanabe and Miyamoto (2002).

Cobalamins can be determined in food by HPLC with mass spectroscopy, radioimmunoassay or a microbiological assay (Lavoisier, 1998).

### **2.5. Reaction and fate in foods to which the source is added**

One of the two petitioners for methylcobalamin indicates that methylcobalamin is relatively stable in foods.

The second petitioner for methylcobalamin provided results from experiments in which methylcobalamin was subjected to a hydrolytic treatment (in acidic and alkaline conditions) and to oxidative stress in solution and its stability was judged based on degradation products and impurities detected. The petitioner indicates that degradation products of methylcobalamin are hydroxycobalamin and cyanocobalamin. These are transformed in the human body by coordinating with other ligands into methylcobalamin and 5'-deoxyadenosylcobalamin. The latter two are actively involved in the metabolism (Kelly, 1997; Hillman, 1985).

Stability studies on hydroxycobalamin or cyanocobalamin under acidic, alkaline or oxidative conditions at elevated temperatures or under light resulted in the formation of several unidentified degradation products.

The main product of photochemical transformation of methylcobalamin in aqueous solution is the hydroxycobalamin (Pratt, 1964; deWitt and Muck, 1973). The rate of photo-degradation is increased by the presence of oxygen, thiol derivatives, quinones and alcohols (Pratt and Whitear, 1971). Other factors that can accelerate the photoreaction are the temperature and the intensity of the incoming light (de Witt and Muck, 1973). The photolysis of methylcobalamin in aqueous medium and in the presence of oxygen gives formaldehyde and aquocobalamin (Kunkely and Vogler, 1993). In neutral media and in the absence of light, the carbon - cobalt



bond in methylcobalamin is very stable to thermal breakdown and the molecule can even stand heating to 100°C during 20 minutes (Lindemans, 1960).

The petitioner for 5'-deoxyadenosylcobalamin indicates that vitamin B<sub>12</sub> is very stable in both crystalline form and aqueous solution. It is stable to heat but decomposes without melting at temperatures above ~210°C.

High levels of vitamin C (ascorbic acid) in the presence of iron can react with vitamin B<sub>12</sub>, resulting in the reduction, subsequent degradation and release of its cobalt atom as the free ion. However, cobalamins with relatively strongly bound ligands (e.g. cyano-, methyl- and 5'-deoxyadenosylcobalamin) are less reactive and are, therefore, more stable in the presence of ascorbic acid.

The cobalamins are unstable upon exposure to light. Cyanocobalamin undergoes a photoreplacement of the cyanide ligand with water; the organocobalamins (methyl- and 5'-deoxyadenosylcobalamin) undergo photoreduction of the cobalt-carbon bond, resulting in the loss of the ligand and the reduction of the corrin cobalt (Lavoisier, 1998).

## **2.6. Case of need and intended levels of use**

### ***Methylcobalamin***

The petitioner of one of the two dossiers for methylcobalamin indicates that methylcobalamin is to be used to provide a source of vitamin B<sub>12</sub> in food supplements, and that methylcobalamin is used by food supplement manufacturers as an ingredient in tablets, caplets, capsules, chewable tablets, effervescent powders and liquids that are food supplements.

This petitioner indicates that the quantity of methylcobalamin to be added to food supplements will be determined by individual formulators but is normally the quantity necessary to supply adults up to 500 µg vitamin B<sub>12</sub>/day, and that this amount would be subject to any restrictions set in a future Commission Directive on maximum permitted levels implementing requirements of the Food Supplements Directive 2002/46/EC.

The petitioner of the second dossier on methylcobalamin indicates that methylcobalamin is intended to be used in food supplements, e.g. in capsules, tablets, ampoules or powders at a proposed level of use of 2 µg /day per os.

### ***5'-Deoxyadenosylcobalamin***

The petitioner proposes to use 5'-deoxyadenosylcobalamin in food supplement capsules or tablets at use levels up to maximum 500 µg 5'-deoxyadenosylcobalamin daily.

## **2.7. Exposure**

Intake of vitamin B<sub>12</sub> may originate from food, from food supplements and from other sources.

The petitioner indicates that exposure to cobalamin vitamers depends on the frequency of consumption of the foods containing these compounds. Kidney (lamb) and liver (lamb, beef, calf, pork) for instance can contain 69-122 µg/100 g, heart (beef) and egg yolk 5-50 µg/100 g, and fish and milk 0.2-5 µg /100 g. 5'-Deoxyadenosylcobalamin accounts for 50-70% of the

total vitamin B<sub>12</sub> in organ meat. In cow's milk vitamin B<sub>12</sub> is mainly present as 5'-deoxyadenosylcobalamin (Castle and Hale, 1998).

Major dietary sources of vitamin B<sub>12</sub>, mainly in the forms of methyl-, deoxyadenosyl- and hydroxycobalamin, include meat (e.g. >0.1 mg/kg in lamb), particularly liver (>0.1 mg/kg) and fish (e.g. 0.03-0.1 mg/kg in salmon, 0.01-0.03 mg/kg in tuna) (EVM, 2003). Dietary vitamin B<sub>12</sub> only comes from animal sources, mainly from dairy products, fish and (red) meat. Therefore individuals consuming large amounts of liver and some types of fish (sardines) may have high intakes in contrast to individuals avoiding animal products.

In 2000 the SCF concluded that average intakes of vitamin B<sub>12</sub> are about 2-6 µg/day from food and that intakes up to 32 µg/day have been reported for the total intake including supplements in elderly Dutch subjects (Asselt *et al.*, 1998). For the UK (Gregory *et al.*, 1990) upper intake levels (97.5<sup>th</sup> percentile) from food sources were reported to be 22.9 and 17.8 µg/day for males and females respectively. The SCF concluded that upper intake levels from all sources were 23.0 and 18.2 µg/day respectively (SCF, 2000).

Hydroxycobalamin and, in particular, cyanocobalamin are forms usually present in vitamin supplements, pharmaceuticals and in fortified food. Methylcobalamin has been used therapeutically outside the UK, for example, in Japan (EVM, 2003).

The petitioner indicates that the quantity of methylcobalamin to be added to food supplements is normally the quantity necessary to supply adults up to 500 µg vitamin B<sub>12</sub>/day. This level of intake from food supplements will add significantly to normal dietary intake.

The petitioner of the second dossier on methylcobalamin indicates that methylcobalamin is intended to be used in food supplements, e.g. in capsules, tablets, ampoules or powders at a propose level of use of 2 µg/day per os.

The petitioner for 5'-deoxyadenosylcobalamin proposes to use 5'-deoxyadenosylcobalamin in food supplement capsules or tablets at use levels up to maximum 500 µg 5'-deoxyadenosylcobalamin daily. This level of intake from food supplements will add significantly to normal dietary intake.

The SCF opinion (SCF, 2000) also reports that data from the USA show 95<sup>th</sup> 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.

## **2.8. Information on existing authorisations and evaluations**

The average dietary requirement for vitamin B<sub>12</sub>, as established by the Scientific Committee on Food (SCF, 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<sub>12</sub> body pool (about 2.5 mg), and to compensate for daily losses (about 0.1% of the total body pool). Recently, recommendations between 2.0 and 3.0 µg/day were established in the Netherlands (Health Council of the Netherlands, 2000; Germany, Austria and Switzerland (D-A-CH 2000), the Nordic countries (NMR, 2004) and the United States (IOM, 2000).

The Scientific Committee on Food has issued an opinion (SCF, 2000) on the Tolerable Upper Intake Level of vitamin B<sub>12</sub> and concluded that it is not possible to derive an Upper Level, mainly because no clearly defined adverse effect could be identified, and also that there is no evidence that the current levels of intake from foods and supplements, estimated by the SCF to amount to <23 µg/day for males and 18 µg/day for females, represent a health risk.

The UK Export Group on Vitamins and Minerals (EVM, 2003) found no evidence for adverse effects of vitamin B<sub>12</sub> in humans and concluded that there was no basis for a Safe Upper Level (SUL) for oral vitamin B<sub>12</sub>, but gave a guidance level of 2 mg/day vitamin B<sub>12</sub> (cyanocobalamin) for adults, based on a clinical trial (Juhlin and Olsson, 1997).

The Food and Nutrition Board (FNB, 1998) also concluded that no adverse effects have been associated with excess B<sub>12</sub> intake from food and supplements in healthy individuals. Consequently FNB concluded that there was no basis for an UL value. On the basis of the data reviewed in 2000, involving high-dose intakes of B<sub>12</sub>, there appear to be essentially no risk of adverse effects to the general population even at intake levels of 37 µg/day. In conclusion, the evidence was considered not sufficient for deriving a Tolerable Upper Intake Level (FNB, 2000).

Hathcock (2004) concluded that vitamin B<sub>12</sub> has no observable adverse effect at any level of oral intake even when given parenterally at 1000 µg twice weekly for up to three years. In addition it was concluded that there is considerable experience and clinical evidence of safety at oral intakes of 3000 µg per day.

The petitioner indicates that 5'-deoxyadenosylcobalamin has been on the market as a human food supplement (doses up to 1000 µg/day) for over 15 years in some EU countries and the USA and no toxic effects have been reported. The select Committee on Generally Recognized as Safe (GRAS) Substances of the Federation of American Society of Experimental Biology has concluded that "the addition of vitamin B<sub>12</sub> to food in amounts far in excess of need or of absorbability appears to be without hazard." According to the FDA, there is no evidence in the available information on cobalamin that demonstrates, or suggests reasonable grounds to suspect a hazard to the public when it is used at levels that are now current and in the manner now practiced, or that might reasonably be expected in the future (Ellenbogen and Cooper, 1991).

### **3. Biological and toxicological data**

#### **3.1. Bioavailability of the source**

Intestinal uptake of vitamin B<sub>12</sub> occurs in the terminal ileum (Ermens *et al.*, 2003). Vitamin B<sub>12</sub> is absorbed and transported across cellular plasma membranes by two mechanisms, including (Castle and Hale, 1998; Andrès *et al.*, 2004):

- 1) Endocytosis of dietary vitamin B<sub>12</sub> bound to gastric intrinsic factor (IF) by ileal enterocytes, which is a receptor-mediated process with a specific IF-cobalamin receptor.
- 2) Passive diffusion. Significant amounts of the vitamin can be absorbed with this process when large quantities of vitamin B<sub>12</sub> are ingested. The rate of absorption by the passive process has been reported to be 1% of the ingested amount of vitamin B<sub>12</sub> (Scott, 1997; Baik and Russell, 1999).

Bioavailability of vitamin B<sub>12</sub> was also evaluated by EVM (EVM, 2003). It was concluded that the absorption of physiological doses of vitamin B<sub>12</sub> is limited to approximately 1.5 – 2 µg/dose or meal, due to saturation of the uptake system. Regardless of dose, approximately 1.2% of vitamin B<sub>12</sub> is absorbed by passive diffusion and consequently this process becomes quantitatively important at high levels of exposure.

The fractional absorption of radiolabeled cyanocobalamin when given at different doses was reported to amount to 50% of a 1 µg dose, 20% of a 5 µg dose, and just over 5% of a 25-µg dose (Adams *et al.*, 1971). Thus, although total amount of vitamin B<sub>12</sub> absorption increases with

increasing intake, the fractional absorption decreases as the oral dose is increased (Baik and Russell, 1999).

Protein binding in certain foods may reduce the bioavailability of the vitamin, particularly in individuals with impaired gastric acid and/or digestive enzyme secretion. The different forms of crystalline cobalamin appear to be absorbed or retained to different extents, depending on the dose. Differences are most apparent at low doses (EVM, 2003).

The cellular uptake of vitamin B<sub>12</sub> involves a transcobalamin (TC) receptor mediating the pinocytotic entrance of the vitamin B<sub>12</sub>-TC complex into the cell. The uptake of vitamin B<sub>12</sub> bound to transcobalamins by the tissues is a rapid process. After cellular uptake, the TC-receptor complex is degraded in the lysosome to yield the free vitamin. Virtually all of the vitamin B<sub>12</sub> within the cell is bound to protein, mainly vitamin B<sub>12</sub>-dependent enzymes.

According to the SCF (2000), the majority of vitamin B<sub>12</sub> that enters the body via nutrition is stored in the liver. Normally the liver stores a vitamin B<sub>12</sub> supply of several milligrams of which only 0.1 - 0.2% is lost per day, and therefore is sufficient to cover the daily need for a longer period without supplementation.

Vitamin B<sub>12</sub> is distributed not only to the liver, but also to bone marrow and virtually all other tissues, including the placenta and breast milk of nursing mothers.

The liver releases vitamin B<sub>12</sub> to the plasma. Plasma vitamin B<sub>12</sub> is bound to specific binding proteins: transcobalamins (TC II) and haptocorrins (HC). The amount of free cobalamin in the circulation is negligible. The physiologic role of HC may be limited as a deficiency of HC has no obvious adverse effects. Vitamin B<sub>12</sub> is released by proteolysis after endocytosis of the vitamin B<sub>12</sub> - transcobalamin complex (Ermens *et al.*, 2003).

Once inside the tissues/cells, the complex is degraded by the lysosomes, and the released cobalamin is metabolised either to methylcobalamin in the cytosol, where it binds to methionine synthase, or to 5'-deoxyadenosyl-cobalamin in the mitochondria, where it binds to methylmalonyl CoA mutase (EVM, 2003). The coenzymatically active forms of vitamin B<sub>12</sub> are 5'-deoxyadenosylcobalamin and methylcobalamin (Wolters *et al.*, 2004). Methylcobalamin predominates in blood plasma and certain other body fluids, such as cerebral spinal fluid, and, in cells is found in the cytosol (Kelly, 1997).

Vitamin B<sub>12</sub> is secreted by the liver in the bile. Because only vitamin B<sub>12</sub> binds selectively to IF that is present in the duodenal lumen, approximately 90% of the secreted cobalamin is reabsorbed. The total amount of cobalamin participating in this enterohepatic circulation is about 2 to 5 times the normal daily intake of vitamin B<sub>12</sub> (Ermens *et al.*, 2003).

Loss of vitamin B<sub>12</sub> occurs mostly through the faeces. When present in amounts exceeding the plasma vitamin B<sub>12</sub> binding capacity, vitamin B<sub>12</sub> is also lost through urine. Other routes of vitamin B<sub>12</sub> loss are through other body secretions and skin. The amount of vitamin B<sub>12</sub> excreted from the body (turnover rate) is fixed at 0.1%-0.2% of total body stores daily, regardless of the size of the pool. Although the rate of vitamin B<sub>12</sub> excretion is not directly proportional to the intake, increased intake of vitamin B<sub>12</sub> results in greater liver storage and, thus, increased excretion (Baik and Russell, 1999).

### **3.2. Toxicological data**

The data-base on the oral toxicity of vitamin B<sub>12</sub> in laboratory animals is limited.

One of the petitioners argues that in case of vitamin B<sub>12</sub> the ligand will not influence the biological activity of the main molecule significantly, and that for these reasons methylcobalamin can be considered a form of vitamin B<sub>12</sub> and therefore it seems reasonable to use existing data on vitamin B<sub>12</sub>, when no specific data on methylcobalamin are available.

### ***Acute toxicity***

The petitioner on 5'-deoxyadenosylcobalamin indicates that vitamin B<sub>12</sub> (including 5'-deoxyadenosylcobalamin) has no appreciable toxicity and refers to a paper on vitamin B<sub>12</sub> in which it is concluded that results of studies with mice suggest that vitamin B<sub>12</sub> is innocuous when administered intraperitoneally or intravenously in relatively high doses (Anonymous, 1987; Winter and Mushett, 1951).

### ***Sub-acute and subchronic toxicity***

No data from sub-acute or subchronic animal studies were available.

### ***Reproductive and developmental toxicity***

There is no evidence relating to vitamin B<sub>12</sub> and teratogenicity or adverse effects on fertility or postnatal development (EVM, 2003).

### ***Genotoxicity***

EVM stated that there is no evidence suggesting that vitamin B<sub>12</sub> is genotoxic (EVM, 2003).

### ***Long term studies***

EVM stated that there is no evidence suggesting that vitamin B<sub>12</sub> is carcinogenic.

In one study a tumor promoting effect was reported in a rat model (Day *et al.*, 1950) but this study was not considered relevant for safety assessment in humans (SCF/NDA, 2006).

### ***Human studies***

In 2000 the SCF concluded that no adverse effects have been associated with excess vitamin B<sub>12</sub> intake from food or supplements in healthy individuals, and that vitamin B<sub>12</sub> has a history of safe long-term use as a therapeutic agent given orally in high dosages. In vitamin B<sub>12</sub> replacement therapy oral dosages between 1-5 mg vitamin B<sub>12</sub> are used, with no supportive evidence of adverse effects (SCF, 2000; Mangiarotti *et al.*, 1986; Maeda *et al.*, 1992; Takahashi *et al.*, 1999; Kuzminski *et al.*, 1998).

Andrès *et al.* (2001) reported a study with the aim to show that oral doses of cyanocobalamin can be used to treat food-cobalamin malabsorption patients. The authors prospectively studied 10 patients with cobalamin deficiency and well-established food-cobalamin malabsorption who

received 3000 µg or 5000 µg of oral crystalline cyanocobalamin once a week for at least 3 months. Complete blood counts and serum cobalamin, homocysteine, and folate levels were determined at baseline and after 3 months of treatment. Patients were re-examined after 6 months.

After 3 months of treatment, all patients had increased hemoglobin levels (mean increase, 1.9 g/dL; 95% confidence interval: 0.9 to 3.9 g/dL;  $p < 0.01$  compared with baseline) and decreased erythrocyte cell volume (mean decrease, 7.8 fL; 95% confidence interval: 0.9 to 16.5 fL;  $p < 0.001$ ). Two patients had only minor, if any, responses. Serum cobalamin levels were increased in all 8 patients in whom it was measured. There was no report of any adverse effects.

Clinical studies have reported no adverse effects following administration of up to 6.0 mg/day of methylcobalamin for several weeks and up to 1.0 mg/day cyanocobalamin for several years (EVM, 2003).

There are a few case reports of adverse effects associated with ingestion of vitamin B<sub>12</sub>, either as a supplement, or following the consumption of yeast extract products, which also contain cyanocobalamin. Five cases of allergic reactions were reported, three of which were recurrences of symptoms in individuals who had been previously exposed to cobalamin by the parenteral route. One further case reported the occurrence of a skin eruption that resembled acne rosacea (EVM, 2003).

#### **4. Discussion**

The present opinion deals only with the safety and bioavailability of 5'-deoxyadenosylcobalamin and methylcobalamin as particular sources of vitamin B<sub>12</sub> intended for the general population, to be used in food supplements. While the safety of cobalamin itself, in terms of amounts that may be consumed, is outside the remit of this Panel, it is necessary for the evaluation of the sources to read across from the safety data on the vitamin, because the sources evaluated are vitamers of vitamin B<sub>12</sub>.

Bioavailability of vitamin B<sub>12</sub> was evaluated by EVM (EVM, 2003). It was concluded that the absorption of physiological doses of vitamin B<sub>12</sub> is limited to approximately 1.5 – 2 µg/dose or meal, due to saturation of the uptake system. Regardless of dose, approximately 1.2% of vitamin B<sub>12</sub> is absorbed by passive diffusion and consequently this process becomes quantitatively important at high levels of exposure.

Assuming that in case of vitamin B<sub>12</sub> the ligand will not influence the biological activity of the main molecule significantly, the Panel concludes that methylcobalamin and 5'-deoxyadenosylcobalamin will be bioavailable, and that the metabolic fate and biological distribution of methylcobalamin and 5'-deoxyadenosylcobalamin are expected to be similar to that of other sources of vitamin B<sub>12</sub> in the diet. Furthermore, following absorption, vitamin B<sub>12</sub>, whatever source, is transformed to either methylcobalamin or 5'-deoxyadenosylcobalamin.

The quantity of methylcobalamin or 5'-deoxyadenosylcobalamin proposed to be used in food supplements amounts to levels up to 500 µg vitamin B<sub>12</sub>/day for adults.

The petitioner of a second dossier on methylcobalamin indicates that methylcobalamin is intended to be used in food supplements, e.g. in capsules, tablets, ampoules or powders at a proposed level of use of 2 µg/day.

The petitioner of 5'-deoxyadenosylcobalamin proposes to use 5'-deoxyadenosylcobalamin in food supplement capsules or tablets at use levels up to maximum 500 µg 5'-deoxyadenosylcobalamin daily.

In 2000 the SCF concluded that average intakes of vitamin B<sub>12</sub> are about 2-6 µg/day from food and that intakes up to 32 µg/day have been reported for the total intake including supplements in elderly Dutch subjects (Asselt *et al.*, 1998). For the UK (Gregory *et al.*, 1990) upper intake levels (97.5<sup>th</sup> percentile) from food sources were reported to be 22.9 and 17.8 µg/day for males and females respectively. The SCF concluded that upper intake levels from all sources were 23.0 and 18.2 µg/day respectively (SCF, 2000).

Therefore the Panel concludes that the proposed levels of use amounting up to 500 µg vitamin B<sub>12</sub>/day will substantially raise normal daily intake which has been estimated to amount on average to less than 10 µg/day.

According to the SCF (2000), the majority of vitamin B<sub>12</sub> that enters the body via nutrition is stored in the liver. Normally the liver stores a vitamin B<sub>12</sub> supply of several milligrams of which only 0.1 - 0.2% is lost per day, and therefore is sufficient to cover the daily need for a longer period without supplementation.

In 2000 the SCF already concluded that no adverse effects have been associated with excess vitamin B<sub>12</sub> intake from food or supplements in healthy individuals, and that vitamin B<sub>12</sub> has a history of safe long-term use as a therapeutic agent given in high dosages per os. In vitamin B<sub>12</sub> replacement therapy oral dosages between 1-5 mg vitamin B<sub>12</sub> are used, with no supportive evidence of adverse effects (SCF, 2000).

The mechanism to limit the absorption when the concentration in the intestine increases is probably one of the reasons that no adverse effects were reported during high dose oral studies. The current available high oral dose studies are however of limited toxicological value. A systematic examination of side-effects was in none of these studies part of the experimental protocol.

The Scientific Committee on Food has issued an opinion (SCF, 2000) on the Tolerable Upper Intake level of vitamin B<sub>12</sub> and concluded that it is not possible to derive an Upper Level, mainly because no clearly defined adverse effect could be identified, and also that there is no evidence that the current levels of intake from foods and supplements, estimated by the SCF to amount to <23 µg/day for males and 18 µg/day for females, represent a health risk.

Hathcock (2004) concluded that vitamin B<sub>12</sub> has no observable adverse effect at any level or oral intake even when consumed parenterally at 1000 µg twice weekly for up to three years. In addition it was concluded that there is considerable experience and clinical evidence of safety at oral intakes of 3000 µg per day. The EVM derived a guidance value of 2000 µg/day.

The Panel notes that the proposed levels of use amounting up to 500 µg vitamin B<sub>12</sub>/day are below this guidance value.

## CONCLUSIONS

The present opinion deals only with the safety and bioavailability of 5'-deoxyadenosylcobalamin and methylcobalamin as particular sources of vitamin B<sub>12</sub> intended for the general population, to be used in food supplements. While the safety of cobalamin itself, in terms of amounts that may be consumed, is outside the remit of this Panel, it is necessary for the evaluation of the sources to read across from the safety data on the vitamin, because the sources evaluated are vitamers of vitamin B<sub>12</sub>.

Methylcobalamin may be produced from genetically modified micro-organisms but the Panel concludes that this source is not part of the present opinion because it would require a separate submission under Regulation no 1829/2003 (EC, 2003).

Assuming that in case of vitamin B<sub>12</sub> the ligand will not influence the biological activity of the main molecule significantly, the Panel concludes that methylcobalamin and 5'-deoxyadenosylcobalamin will be bioavailable, and that the metabolic fate and biological distribution of methylcobalamin and 5'-deoxyadenosylcobalamin are expected to be similar to that of other sources of vitamin B<sub>12</sub> in the diet.

The proposed levels of use amounting up to 500 µg vitamin B<sub>12</sub>/day will substantially increase normal daily intake which has been estimated to amount on average to less than 10 µg/day.

The Panel noted that the Scientific Committee on Food has issued an opinion on the Tolerable Upper Intake level of Vitamin B<sub>12</sub> and concluded that it is not possible to derive an Upper Level, mainly because no clearly defined adverse effect could be identified. The Panel also noted that the proposed levels of use amounting up to 500 µg vitamin B<sub>12</sub>/day are below the guidance value of 2000 µg/day defined by EVM.

The Panel concludes that the use of 5'-deoxyadenosylcobalamin and methylcobalamin as a source of vitamin B<sub>12</sub> in food supplements for the general population at the proposed uses and use levels is not of safety concern.

#### **DOCUMENTATION PROVIDED TO EFSA**

1. Dossier on Methylcobalamin Proposed for Addition to Annex II of Directive 2002/46/EC of the European Parliament and of the Council Relating to Food Supplements. June 2005. Submitted by Health Food Manufacturers Association UK.
2. Dossier on Methylcobalamin Proposed for Addition to Annex II of Directive 2002/46/EC of the European Parliament and of the Council Relating to Food Supplements. July 2005. Submitted by Natuur- & GezondheidsProducten Nederland.
3. Dossier on Adenosylcobalamin (Dibencozide) for consideration by the European Food Safety Authority as a nutritional substance for use in the manufacture of food supplements in view of the amendment of the Commission Directive 2002/46/EC. June 2005. Submitted by Biovitaal, The Netherlands.

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**GLOSSARY / ABBREVIATIONS**

ANS	Scientific Panel on Food additives and nutrient sources added to food
EVM	Expert Group on Vitamins and Minerals
GMM	Genetically Modified Micro-organisms
GRAS	Generally Recognized As Safe
HC	Haptocorrin
IF	Intrinsic Factor
IU	International Units
JECFA	Joint FAO/WHO Expert Committee on Food Additives
NDA	Scientific Panel on Dietetic Products, Nutrition and Allergies
NOAEL	No-Observed-Adverse-Effect Level
PRI	Population Reference Intake
SCF	Scientific Committee on Food
SUL	Safe Upper Level
TC	Transcobalamin
UL	Upper Level