

## EXPERT GROUP ON VITAMINS AND MINERALS

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### REVISED REVIEW OF VITAMIN B<sub>12</sub>

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The attached review of vitamin B<sub>12</sub> is a revised version of the paper presented to the Expert Group on Vitamins and Minerals at the meetings on 1 November 2000 and October 2001.

The following annexes are also attached:

- Annex 1 Tables referred to in the paper
- Annex 2 Figures referred to in the paper (for copyright reasons not all the figures in this annex can be reproduced).
- Annex 3 Intakes of vitamin B<sub>12</sub> from food and supplements in the UK
- Annex 4 Summary table of selected nutrition related information and existing guidance on intakes

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VITAMIN B<sub>12</sub> (COBALAMIN)

## GLOSSARY

AdoCbl	Deoxyadenosylcobalamin
AHFS	American Hospital Formulary Service
Cbl	Cobalamin (all forms)
CNS	Central nervous system
CSF	Cerebro-spinal fluid
cyanoCbl	Cyanocobalamin
DH	Department of Health (UK)
FAO	Food and Agriculture Organization of the United Nations
Hcy	Homocysteine
HSDB	Hazardous Substances Data Bank (US)
IF	Intrinsic factor
IFCR	Intrinsic factor-wall receptor
LNRI	Lower reference nutrient intake
methyl Cbl	Methyl cobalamin
NRC	National Research Council (US)
PNS	Peripheral nervous system
RDA	Recommended dietary allowance (US)
RNI	Reference nutrient intake (UK)
SAM	S-adenosyl methionine
TC	Transcobalamin
THF glu	Tetrahydrofolate (glycosylated)
WHO	World Health Organization

**VITAMIN B<sub>12</sub> (COBALAMIN)****Chemistry and nomenclature**

1. Vitamin B<sub>12</sub> (also referred to as cobalamin or Cbl) is a member of the corrinoid family of molecules that contain a planar corrin nucleus made from a tetrapyrrolic ring structure. The centre of the tetrapyrrole ring contains a chelated cobalt atom that can be attached to a methyl, deoxyadenosyl (as shown in Figure 1), hydroxy or a cyano group (the reader is also referred to the review of cobalt prepared for this Committee). The methyl (methyl Cbl) and the deoxyadenosyl (adoCbl) forms of Cbl are the naturally active forms while the cyano and hydroxo forms require metabolic conversion *in vivo* (Weir and Scott, 1999 and references therein). The cobalt atom in the vitamin B<sub>12</sub> molecule may be in the uni-, di- or trivalent state, having 8, 7 or 6 electrons, respectively. There may be 2, 1 or 0 axial ligands present so that the molecule is 6, 5 or 4 co-ordinate. Cyanocobalamin is 6 co-ordinate and is the most stable form of the vitamin (Basu and Dickerson 1996). Methyl Cbl is unstable and readily undergoes photolysis to form hydroxo Cbl (Dolphin *et al* 1964).

Chemical/physical properties	cyanoCbl	hydroxoCbl
CASRN:	68-19-9	13422-51-0
Molecular formula:	C <sub>63</sub> H <sub>88</sub> CoN <sub>14</sub> O <sub>14</sub> P	C <sub>62</sub> H <sub>89</sub> CoN <sub>13</sub> O <sub>15</sub> P
Molecular wt:	1355.4	1346.4
odour	odourless	odourless/slight acetone odour
taste	tasteless	-
colour/form	dark red crystals or amorphous crystalline powder	dark red, orthorhombic crystals or dark red crystalline powder
pH:	4.5-7	8-10, 2% solution
solubility:	~1g/80mL water soluble in alcohol insoluble in acetone, ether, chloroform	moderately soluble in water and lower aliphatic alcohols insoluble in acetone, ether, petroleum
other:	hygroscopic in air	hygroscopic in air

**Natural occurrence**

2. Vitamin B<sub>12</sub> is present in all or most animal tissues but is ultimately derived from bacteria, fungi or algae, which provide the only natural synthetic source. Yeasts, plants, fruit and vegetables do not contain vitamin B<sub>12</sub> (Weir and Scott 1999 and references therein; NRC 1989).

## Occurrence in food, food supplements and medicines

### *Foods*

3. Vitamin B<sub>12</sub> occurs only in animal products and micro-organisms including yeast. The principal forms found in food are the methyl, deoxyadenosyl and hydroxo forms, with cyanoCbl only in trace amounts. Liver is the richest source but useful amounts are found in other animal products and also fortified breakfast cereals. The main contributors to dietary intake are milk and milk products and meat and meat products. Fortified breakfast cereals provide about 5% of intake. Other rich dietary sources include fish, eggs and shellfish. The highest levels of dietary vitamin B<sub>12</sub> occur in animal liver (> 1 mg/kg), a reflection of the fact that this organ acts as the major body store (> 50%) of this vitamin. In contrast, levels present in cows milk are relatively low (in the order of µg/L). Vegetable products are free of vitamin B<sub>12</sub> unless they are contaminated with Cbl synthesising microorganisms (Weir and Scott 1999 and references therein; Marcus and Coulston 1996). Some algae nori (*Porphyra tenera*) and spirulina (*Spirulina*) are rich in vitamin B<sub>12</sub>. However, the bioavailability of the vitamin from these sources appears to be low (Dagnelie *et al* 1991). Cooking tends to liberate vitamin B<sub>12</sub> from its protein binding and may serve to increase the bioavailability of the vitamin in food. However, heating can also result in 10 - 90% loss of vitamin B<sub>12</sub> activity (Joint FAO/WHO Expert Consultation 1988 and references therein).

4. To prevent the masking of symptoms of vitamin B<sub>12</sub> deficiency, foods that are fortified with folic acid may also be fortified with Cbl (Weir and Scott 1999 and references therein; Scott 1997 and references therein; the reader is also referred to the review of Folic Acid prepared for this committee).

### *Food supplements*

5. Both cyanoCbl and hydroxoCbl are used therapeutically and are prepared commercially as fermentation products of organisms such as *Streptomyces olivaceus*, *Streptomyces griseus* and *Bacillus megatherium* (Basu and Dickerson 1996). CyanoCbl is highly water-soluble, heat stable, and when given orally or parenterally, is made metabolically active by the removal of cyanide (NRC 1989; AHFS Drug information 1994).

6. Other forms of Cbl available include Cbl concentrate (which is a dried partially purified preparation from a bacterial or other Cbl-producing microorganism culture), mammalian liver extract and a vitamin B<sub>12</sub> and intrinsic factor mixture derived from the gut of hog or other animal. (NRC 1989; AHFS Drug information 1994).

7. The major form used in food fortification is cyanoCbl. (NRC 1989, AHFS Drug information 1994). Hydroxocobalamin is also permitted but is less commonly used.

8. Methyl Cbl has been used in a number of studies, particularly to assess possible beneficial effects in sleep-wake disorders (see paragraph 69). However, this form is generally not available for therapeutic purposes in the UK.

*Licensed medicinal products for oral use*

9. Multi-nutrient products with recommended daily doses of up to 10 µg vitamin B<sub>12</sub> are available on general sale in supermarkets and other retail outlets, without the supervision of a pharmacist.

*Other uses*

10. Radiolabelled cyanoCbl tracer and large doses (1000 µg) of unlabelled cyanocobalamin may be used in the “Schilling test” for diagnosis of syndromes of vitamin B<sub>12</sub> malabsorption (see paragraph 18).

**Intake and exposure**

11. Vitamin B<sub>12</sub> intakes differ widely among populations, the highest being in those consuming high levels of animal protein. Populations on strict vegetarian diets have the lowest dietary intakes.

*UK*

12. Average daily intakes from food and supplements for adults aged 16-64 years were 7.3 µg for men and 5.4 µg for women in 1986/87. The contribution of dietary supplements to average intakes was small in all age groups.

*US*

13. The average dietary intake of vitamin B<sub>12</sub> in adult men in the US in 1985 was 7.84 µg/day. Corresponding intakes for adult women and children (1-5 years) were 4.85 and 3.80 µg/day, respectively. However, reported levels of vitamin B<sub>12</sub> in foods may have included 5-30% microbically active non-Cbl corrinoids (NRC, 1989).

*Vegetarians*

14. Vegan diets contain very low levels of vitamin B<sub>12</sub>, resulting in intakes of probably less than 0.5 µg/day (Chanarin, 1990).

**Recommended amounts**

15. The UK (DH 1991), US (NRC 1989, Food and Nutrition Board 1998) and FAO/WHO (Joint FAO/WHO Expert Consultation 1988) recommended intakes of vitamin B<sub>12</sub> are shown in Tables 1a-c. The average requirement to prevent or cure megaloblastic anaemia of vitamin B<sub>12</sub> deficiency in adults appears to be less than 1 µg/day. The Reference Nutrient Intake for adults in the UK is 1.5 µg/day. There is no increment for women during pregnancy but there is an increment of 0.5 µg/day recommended throughout lactation (DH 1991).

**Analysis of tissue levels and assessment of status**

16. Plasma levels of vitamin B<sub>12</sub> are routinely measured (by radioisotopic assay or microbiological assay) to determine deficiency. The normal range is wide but concentrations below 150 pg/mL are generally considered indicative of deficiency. However, this is not always the best indication of vitamin status. For example, in healthy pregnancy, tissue levels of vitamin B<sub>12</sub> are normal but serum levels are low, and in patients with myeloproliferative disorder or hepatic disease, plasma Cbl may be greatly elevated due to the presence of high levels of plasma R binder (Hc) binding proteins (Weir and Scott 1999 and references therein).

17. The widely used radioimmunoassay may not be as specific or sensitive as the microbiological assay because the assay also measures Cbl bound to TC I, which is thought to be biologically inactive and thus may give a falsely high impression of vitamin B<sub>12</sub> status (Weir and Scott 1999 and references therein).

18. Methods have been devised to distinguish different malabsorption and functional deficiency syndromes. Until recently, diagnoses largely relied on total serum Cbl measurement and the "Schilling test" for determination of absorption. The Schilling test involves oral administration of a low (physiologic) dose of isotopically labelled vitamin B<sub>12</sub> closely followed by administration of a very large dose (1000 µg) of unlabelled vitamin by intramuscular injection. The unlabelled material saturates the transport system and tissue binding sites. Absorbed labelled material is therefore displaced and may be quantified in the urine collected over the 24 hours after dosing. The test assumes that the large parenteral dose will always "flush" out one third of any radioactive vitamin B<sub>12</sub> that has been absorbed within the 24 hour collection period.

19. More recent diagnostic approaches have included the analysis of methylmalonic acid, homocysteine, holotranscobalamin and anti-intrinsic factor antibodies in plasma and/or urine, the serum gastrin deoxyuridine suppression test, the assessment of gastric biopsy samples, the performance of a Cbl absorbance test, a check for antibodies against intrinsic factor (IF) and determination of response to Cbl therapy (e.g. erythropoiesis) which aid the identification of less obvious causes of deficiencies (Chanarin 1990, Markle 1996, Marcus and Coulston 1996).

20. Normal tissue/body fluid Cbl levels are summarised below (from Chanarin 1990, Weir and Scott 1999 and references therein):

serum/plasma	150-1000 pg/mL
red blood cells, washed/packed	85-225 (mean 155) pg/mL
CSF	5-60 (mean 20) pg/mL
Liver	0.6 – 1.5 (mean 1) µg/g wet wt
	0.70-79 (mean 10) ng/mg protein

## Bioavailability

22. Absorption of vitamin B<sub>12</sub> occurs via two processes, one mediated by intrinsic factor (IF) and the other by non-IF mediated diffusion (see paragraphs 37-44). The latter route accounts for a small but constant proportion (~1.2% of exposure) of vitamin B<sub>12</sub> absorbed and as a consequence, this process becomes quantitatively more important at high (pharmacological) doses.

23. The bioavailability of physiological levels of vitamin B<sub>12</sub> from food is therefore limited by the capacity of the IF-mediated mechanism to ~1.5-2 µg/meal. This mechanism takes a few hours to recover and can then mediate the absorption of a similar amount as a subsequent event. A similar pattern of absorption is observed with crystalline vitamin B<sub>12</sub>, with saturation of the IF system occurring also at 1.5-2.0 µg/test (Scott 1997 and references therein). When a physiological dose of radioactive cyanoCbl is given orally, healthy individuals absorb ~ 70% of a 0.5 µg dose and ~55% of a 1 µg dose (Daller *et al* 1961, Mollin *et al* 1957).

24. Berlin *et al* (1968) found that, on average, the proportion of cyanoCbl dose absorbed by the non IF-mediated route was of the same magnitude whether an individual had completely normal absorption, was suffering from pernicious anaemia (lack of IF) or had some other form of disturbed absorption due to idiopathic malabsorption, ileitis, total gastrectomy, ileectomy or jejunectomy. This finding has been confirmed by others. For example, Altay and Cetin (1999) found that oral administration of 1000 µg vitamin B<sub>12</sub> to children with selective vitamin B<sub>12</sub> malabsorption resulted in similar increases in serum Cbl to those observed in age-matched controls.

25. There are few data concerning the bioavailability of vitamin B<sub>12</sub> from different natural dietary sources. In healthy individuals, radiolabelled vitamin B<sub>12</sub> incorporated into liver, mutton and chicken appears to be absorbed as completely as the equivalent amount of cyanoCbl administered in aqueous solution (Heysell *et al* 1966, Doscherholman *et al* 1978). Vitamin B<sub>12</sub> has also been shown to be well absorbed from milk and fortified bread (Russell *et al* 2001). In contrast, vitamin B<sub>12</sub> in eggs appears to be less well absorbed (Doscherholman *et al* 1976, Schade and Schilling 1967). In individuals suffering from gastritis or who have undergone gastric surgery, the availability of Cbl bound to protein in food may be reduced due to the lack of gastric acid and/or digestive enzymes. The bioavailability of free Cbl in these individuals, however, is not affected. There have been conflicting reports as to the effect of simultaneous administration of food or stimulants of gastric secretion on the absorption of free Cbl in healthy individuals. Some reports have claimed enhanced uptake with food while others have found no effect (Scott 1997 and references therein).

26. Co-administration of IF enhances absorption of Cbl in individuals who lack functional IF, such as pernicious anaemia patients. However, in doses that would be far higher than physiologically normal, IF impairs uptake by direct diffusion due to a reduction in availability of free Cbl (Scott 1997 and references therein).

27. Some early papers reported differences in the extent of absorption of the different crystalline forms of vitamin B<sub>12</sub> that were dependent upon dose (Farquharson

and Adams 1976 and references therein). Adams *et al* (1971) used a double radiolabel tracer technique and whole body monitoring in 63 individuals to compare the retention of different doses of orally administered crystalline forms of ado, methyl, hydroxo and cyano Cbl. The authors made the assumption that the whole body radioactivity 16 days after administration of a radioactive cobalamin was a measure of the amount adsorbed. Subjects received the same oral dose of two cobalamins, one incorporating  $^{58}\text{Co}$  and the other  $^{57}\text{Co}$  at intervals of 24 hours. Whole body monitoring was carried out shortly after each dose and again 16 days after the first dose. Results indicated that following the ingestion of 1  $\mu\text{g}$  doses, the greatest amount of radioactivity was retained when either hydroxoCbl (~56%), cyanoCbl (~49%) or methylcobalamin (~44%) was taken. These levels of radioactivity were significantly higher than that retained following the ingestion of adoCbl (~34%). Following doses of 5  $\mu\text{g}$ , the percentage of radioactivity retained for both cyano and methyl Cbls (~20%) was significantly higher than the level for adoCbl (13%). The percentage of radioactivity retained for hydroxoCbl was similar to that for adoCbl (~16%). At doses of 25  $\mu\text{g}$ , the retention of radioactivity was similar for each of the forms at around 6-8%, although the difference between adoCbl (7.9%) and cyanoCbl (5.6%) was significant.

28. More recently, Okuda *et al* (1992) reported Schilling test data from reportedly healthy individuals using either  $^{57}\text{Co}$  labelled methyl Cbl (n=14) or  $^{57}\text{Co}$  labelled cyano Cbl (n=25). Radioactivity recovered in 24 hour urine samples from the methyl Cbl-tested individuals was found to be approximately one third of that in the cyanoCbl-tested people. This was thought to reflect a longer body retention of methyl Cbl and/or its Cbl conversion products compared to those of cyanoCbl. Okuda *et al* (1973) reported that the absorption of physiological doses of  $^{57}\text{Co}$  labelled methyl Cbl and  $^{57}\text{Co}$  labelled cyanoCbl in the rat, as calculated by faecal recovery, were similar, although hepatic uptake of radioactivity was greatest from methyl Cbl.

29. Following intramuscular injection, plasma levels of vitamin B<sub>12</sub> produced by hydroxocobalamin are higher and more prolonged than those produced by a similar dose of cyanoCbl. This increased retention may be due to a difference in affinity for the cobalamin-binding proteins (Scott 1997 and references therein, HSDB 2000b).

30. The supply of vitamin B<sub>12</sub> available to the tissues is directly related to the size of the hepatic storage pool and the amount in plasma bound to the protein transcobalamin II (TC II), which is required for transport into cells. However, bioavailability to tissues may be decreased in certain disorders where there is an absence of TC II or an increase in Hc plasma binding proteins. Transcobalamin I (TC I) and transcobalamin III (TC III), which when bound to Cbl, has a much slower turnover rate than TC II-Cbl complex (see paragraph 40).

31. The half-life of cyanocobalamin in serum following intravenous administration is ~6 days (as cited by HSDB 2000a).

## Interactions

### *Prednisone-related increase in vitamin B<sub>12</sub> absorption*

32. Prednisone has been reported to increase the absorption of the vitamin in pernicious anaemia patients (Krstic and Radojicic 1977, English abstract only; Jaross and Fleisher 1965, article in German, Ardeman and Chanarin 1965).

### *Drug and other related decreases in vitamin B<sub>12</sub> absorption*

33. Absorption of vitamin B<sub>12</sub> from the gastrointestinal tract may be decreased by a number of drugs that include proton pump inhibitors, aminoglycoside antibiotics, colchicine, extended release potassium preparations, aminosalicic acid and its salts, phenytoin, primidone and phenobarbital (AHFS Drug Information 1994).

34. Cobalt irradiation of the small bowel and excessive alcohol consumption may also decrease vitamin B<sub>12</sub> absorption. Co-oral administration of ascorbic acid may result in destruction of vitamin B<sub>12</sub> (AHFS Drug Information 1994).

### *Drug-related antagonism of vitamin B<sub>12</sub>-haematopoietic response*

35. Concurrent administration of chloramphenicol and vitamin B<sub>12</sub> is reported as antagonising the haematopoietic response to vitamin B<sub>12</sub> in vitamin B<sub>12</sub> deficient patients (AHFS Drug Information 1994).

## Absorption, distribution, metabolism and excretion

**Absorption** (From Seetharam and Li 2000 Seetharam 1999 and references therein, Seetharam *et al* 1999 Scott 1997 and references therein, Weir and Scott 1999 and references therein, Russell-Jones and Alpers 1999 and references therein).

36. Uptake of vitamin B<sub>12</sub> is illustrated in Figure 2 and involves the binding of Cbl with high affinity to a number of glycoproteins that include the so-called intrinsic factor (IF), haptocorrins (Hc, also referred to as R binders, transcobalamin I [TC I] and transcobalamin III [TC III], or cobalaphilin) and transcobalamin II (TC II).

37. Vitamin B<sub>12</sub> is unusual among nutrients and micronutrients in that it requires an additional factor to enable its absorption from the gut, namely the intrinsic factor, IF. In humans, acid stable IF is secreted mainly by the gastric parietal cells but is also present and probably synthesised by the fundal chief cells and antral G cells of the gastric mucosa and in the salivary glands. Normally, secretion of IF is in excess to requirements and in normal subjects, administration of excess IF does not increase absorption of vitamin B<sub>12</sub>.

38. There are species differences in the tissue and cell-specificity of IF expression. Rats and mice differ from humans in that IF is mainly localised in the chief cells, with only a small percentage of parietal cells staining positively for IF. In dogs, IF transcription is not confined only to the stomach but extends to the pancreatic duct cells. Stimulants of gastric hydrochloric acid secretion e.g. histamine, gastrin and

acetylcholine also result in stimulation of IF secretion. It has been found in the rat that IF expression is also regulated by cortisone and growth hormone.

39. Although all body fluids contain Hc glycoproteins, no specific role has been confirmed for them. The formation of Hc-Cbl complexes may provide protection for vitamin B<sub>12</sub> against acid hydrolysis in the stomach and/or prevent the vitamin from being scavenged by intestinal fauna and flora. In contrast to IF and TC II, which are unable to bind non-Cbl corrinoids, the Hc glycoproteins bind to the corrin ring of all corrinoids. As a consequence of this lower specificity, Hc may act to mop up any free Cbl released from damaged tissues and/or remove Cbl analogues produced by food processing or the action of the intestinal flora which may be toxic or have the potential to interfere with IFCR (intrinsic factor cell-wall receptor) uptake of true Cbl.

40. TC II serves as the prime transporter of Cbl into and out of cells. It is synthesised constitutively in many cell types including liver, macrophages, ileal cells, endothelial cells and epithelial cells and is secreted into the plasma at a constant rate. Levels of TC II mRNA are highest in the kidney. The half life of TC II-bound Cbl is relatively short (~ 1 hour) as it is rapidly taken up into cells. In comparison, the turnover of Cbl bound to TC I is much slower with a half-life in humans of 9-10 days. In humans, most (~80-90%) Cbl in the general circulation is bound to TC I.

41. In the stomach, ingested Cbls, present mainly as methyl Cbl and adoCbl, are first released from the food matrix by the actions of gastric acid and pepsin and are then bound by saliva derived Hc. At low gastric pH, B<sub>12</sub> binding affinity is higher for Hc than for IF. However, further along the gut in the jejunum, the combined effects of the higher pH and proteolytic digestion of the Hc by pancreatic enzymes result in the release and transfer of vitamin B<sub>12</sub> from Hc to IF. When complexed with Cbl, IF is relatively resistant to proteolytic degradation. The intestinal uptake of IF-bound Cbl requires the presence of Ca<sup>2+</sup>, a pH of >6 and bile components and takes place in the ileum. Complex formation serves to protect B<sub>12</sub> from catabolism by intestinal bacteria and from hydrolytic attack by pepsin and chymotrypsin as it passes into this part of the gut. Absorption of vitamin B<sub>12</sub> occurs via interaction with a specific IF ileal enterocyte cell-wall receptor (IFCR) also referred to as cubulin. IF-mediated import is limited to the apical membranes of the epithelial cells. The receptors consist of two b units situated within the wall of the cell and two flanking units that bind to the IF glycoprotein and lock the IF-Cbl complex into the receptor. Only IF-bound Cbl is absorbed by the IFCR. Hc-bound Cbl is not absorbed and is excreted in the faeces.

42. The IF-mediated uptake is a saturable process. However, a small proportion of vitamin B<sub>12</sub> is absorbed by slow non-saturable simple diffusion. This mechanism becomes biologically important only in the event of dosing with concentrations having pharmacological effects (30 µg or more) (NRC 1989 and references therein). Berlin *et al* (1968) have shown that receptor-mediated uptake is limited to ~2 µg. However, within a wide dose range of 100-100,000 µg, a constant percentage, ~1.2%, of the dose is absorbed directly by diffusion. Consequently, by increasing dose, the absolute amount absorbed can be increased despite the percentage of the total dose absorbed decreasing. Furthermore, Berlin *et al* (1968) found that, on average, direct uptake of Cbl was of the same magnitude whether an individual had completely normal absorption, was suffering from pernicious anaemia (lack of IF) or had some other form of disturbed absorption due to idiopathic malabsorption, ileitis, total

gastrectomy, ileectomy or jejunectomy. Co-administration of IF, providing doses of IF that would be far higher than physiologically normal, hamper uptake by direct diffusion.

43. The IF-Cbl complex is internalised by the process of endocytosis. Once inside the ileal mucosa cell, the IF is degraded and the liberated Cbl is converted to either the methyl Cbl or the adoCbl form and is then bound to TC II. The TC II-bound Cbl then is exported from the cell (Figure 3, left panel). Ninety-five percent is transported in the portal veins and 5% is carried by the lymphatics. Appearance of Cbl in portal blood occurs approximately three hours post absorption. Although TC II is required for the transport of Cbl between cells and plasma, most Cbl in humans is actually found bound to TC I.

### ***Distribution and metabolism***

44. TC II-Cbl complex enters the tissues/cells by the process of receptor-mediated endocytosis involving specific cell wall receptors (TC II-R), which are known to be expressed in most, if not all, tissues. The intracellular metabolism of Cbl is illustrated in Figures 3 (right panel) and 4. Once internalised, the TC II part of the complex is degraded in the lysosomes. The released Cbl is either converted to methyl Cbl in the cytosol, where it binds to methionine synthase, or to adoCbl in the mitochondria, where it binds to methylmalonyl CoA mutase (Seetharam 1999 and references therein; Weir and Scott 1999 and references therein; Russell-Jones and Alpers 1999 and references therein).

45. Although TC II-R receptor mediated uptake is the most widely distributed mechanism for uptake of Cbl into tissues, it is not the only mechanism available. For example, hepatocytes contain cell-surface receptors for asialoglycoproteins which interact with TC I (and perhaps TC III)-bound Cbl. There is also some evidence to suggest that some tissues can take up free (unbound) Cbl if plasma levels are very high (Fenton and Rosenberg 1995 and references therein).

46. Vitamin B<sub>12</sub> is distributed into the liver, bone marrow and virtually all other tissues, including the placenta. The liver is the major vitamin B<sub>12</sub> handling and storage site in the body. At birth, the full-term infant has 25-30 µg of the vitamin in the liver and a total body content of 30-40 µg. Blood concentration in the neonate is 3-5 times that of the mother. Vitamin B<sub>12</sub> is distributed into the milk of nursing women in concentrations that approximate, and are influenced by, maternal blood concentrations. Several studies have shown that there is no increase or only a limited increase in vitamin B<sub>12</sub> concentration in the breast milk following supplementation (multi-vitamin containing ~2 x the RDA) in well-nourished women (Thomas *et al* 1980 and references therein). The effect of supplementation with much larger doses on breast milk concentrations in well-nourished women does not appear to have been investigated.

47. Vitamin B<sub>12</sub> body concentrations increase with age and, in healthy omnivorous adult humans, the total body content of vitamin B<sub>12</sub> is ~3-5 mg, of which 50-80% is contained within the liver. In liver, erythrocytes, brain and kidney >70% of vitamin B<sub>12</sub> is present in the form of adoCbl with only ~1-3% in the form of methyl Cbl. In contrast, plasma Cbl is mainly in the form of methyl Cbl (60-80%), with the

remainder being OH-Cbl and adoCbl. Since 90% of circulating Cbl is bound to TC I, most methyl Cbl must be Hc-bound. The significance of the different distributions of the different forms is unclear (Seetharam 1999 and references therein, Weir and Scott 1999 and references therein; Russell-Jones and Alpers 1999 and references therein; Joint FAO/WHO Expert Consultation, 1988 and references therein). In the liver, the biological half-life of vitamin B<sub>12</sub> is estimated to be >400 days (Basu and Dickerson 1996).

**Excretion** (From NRC 1989 and references therein, McLaren 1981 and references therein, Weir and Scott 1999 and references therein; HSDB 2000 *a* and *b* and reference sources therein, AHFS Drug Information 1994, Basu and Dickerson 1996).

48. Excretion of Cbl occurs mainly via the faeces and urine. Between 0.05-0.2% (~ 1.5-10 µg, depending on the size of the storage pool) of the total body Cbl is secreted in bile per day. However, much of the Cbl released into the gut is subject to enterohepatic cycling and is reabsorbed following the proteolytic removal of Hc and recombination with IF.

49. A proportion of Cbl that is taken up from the gut lumen but not yet released systemically is also re-deposited into the gastrointestinal tract due to apoptosis of the intestinal mucosal cells. However, this may be subject to reabsorption. Excretion via cellular apoptosis also occurs in skin and kidney. Relatively large amounts of vitamin B<sub>12</sub> may be found in the faeces but most of this is formed by *de novo* synthesis by colonic bacteria and is not thought to be available for absorption. In healthy individuals receiving dietary vitamin B<sub>12</sub> only, typically all but ~1µg secreted in the bile is reabsorbed and <1 µg is usually excreted in the urine. However, following intravenous or intramuscular administration of >50 µg, when the amount of vitamin B<sub>12</sub> present in the plasma exceeds the binding capacity of plasma, liver and other tissues, the excess is eliminated by glomerular filtration. Overall, loss of vitamin B<sub>12</sub> from the body is slow, occurring exponentially at a rate of 0.05-0.2% of total body pool, regardless of pool size. The slow rate of Cbl turnover is well illustrated by the fact that total gastrectomy virtually halts any physiologic absorption of Cbl yet it takes as long as 4-7 years to produce a deficiency severe enough to induce megaloblastic anaemia.

## Function

(From Weir and Scott 1999 and references therein).

### *Cofactor to methionine synthase*

50. In the form of methyl Cbl, vitamin B<sub>12</sub> participates as cofactor to the enzyme methionine synthase in the methylation of homocysteine (Hcy) which involves transfer of the methyl group from N<sup>5</sup>-methyltetrahydrofolate (N<sup>5</sup>-methyl-THF-glu<sub>1,5</sub>) (Figure 5). As such, vitamin B<sub>12</sub> plays a pivotal role in one-carbon (methyl donor) metabolism, vital to many aspects of cellular metabolism, including the synthesis of the building block precursors to DNA and RNA.

*Synthesis of the universal carbon donor S-adenosylmethionine*

51. The methionine formed may be converted to S-adenosylmethionine (SAM). SAM acts as the universal methyl donor in more than 100 methylation reactions within the cell, all of which are essential for internal metabolism. In particular, SAM is the major direct donor of methyl groups in the synthesis of polyamines (e.g. spermidine and putrescine).

*Creation of functional folate, involvement in purine and pyrimidine synthesis, regeneration of methionine from homocysteine*

52. The  $N^5$ -THF-glu<sub>1</sub> formed in the methionine synthase reaction is converted to the polyglutamated form  $N^5$ -THF-glu<sub>5</sub> by folyl- $\gamma$ -glutamate synthetase which is the central folate acceptor molecule in the folate one-carbon cycle. In turn,  $N^5$ -THF-glu<sub>5</sub> receives the  $\beta$ -carbon moiety from serine, via serine hydroxymethyltransferase, to give glycine and  $N^5,N^{10}$ -methylene-THF-glu<sub>5</sub>.  $N^5,N^{10}$ -methylene-THF-glu<sub>5</sub> either acts as a methyl donor in the conversion of deoxyuridylate monophosphate to thymidylate monophosphate (the precursor to the pyrimidine base thymidine) in a reaction catalysed by thymidine synthetase, is converted to  $N^{10}$  formyl-THF-glu<sub>5</sub>, which provides carbons 2 and 8 in the synthesis of the purine bases, or is reduced to methyl-THF-glu<sub>5</sub>, which can serve to re-methylate homocysteine to methionine.

*Cellular uptake of folate*

53. Methionine synthase also acts as gatekeeper for the entry of folate into the cell. Folate enters in the form of  $N^5$ -methyl-THF-glu<sub>1</sub> and can only remain inside the cell following demethylation via methionine synthase. Consequently, the uptake of folate into the cell is also dependent on the methyl Cbl form of vitamin B<sub>12</sub>.

*Cofactor to methylmalonyl CoA mutase*

54. As deoxyadenosylCbl (adoCbl), vitamin B<sub>12</sub> has the role of obligate cofactor for the enzymatic conversion of L-methylmalonyl CoA to succinyl CoA by methylmalonyl CoA mutase.

*Cofactor to leucine 2,3-aminomutase*

55. Poston (1976) has described an AdoCbl-dependent interconversion of leucine and  $\beta$ -leucine by leucine 2,3-aminomutase. However, this function has not been referred to in recent reviews on Cbl function.

**Deficiency**

56. Development of vitamin B<sub>12</sub>-deficiency through dietary inadequacy is rare in the developed world. However, vegans who eat no animal-derived foods are at risk of a deficiency that can develop insidiously over several years. More than 95% of Cbl deficiencies are attributable to inadequate absorption or transport as a result of inherited defects in Cbl transport proteins or passage of Cbl through ileal cells, or acquired disorders due to ageing, surgery or other diseased states (Table 2) (Weir and Scott 1999 and references therein).

57. Vitamin B<sub>12</sub> deficiency results in macrocytic, megaloblastic anaemia, neurological effects due to demyelination of the spinal chord and brain, optic and peripheral nerves and in other less specific symptoms such as sore tongue and weakness. Neuropsychiatric manifestations of deficiency may be seen in the absence of anaemia, especially in the elderly. Vitamin B<sub>12</sub> deficiency is also associated with hyperhomocysteinaemia (a positive risk factor for atheroma, coronary thrombosis, strokes and peripheral vascular disease), neural tube defects and hepatic steatosis. Vitamin B<sub>12</sub> and folate deficiency give similar symptoms. The reason for this probably relates to the functional interaction between the two vitamins and involves impairment of one-carbon metabolism (Weir and Scott 1999 and references therein).

#### *Megaloblastic anaemia*

58. Megaloblasts are large, abnormal, nucleated cells that are precursors of erythrocytes. In vitamin B<sub>12</sub> deficiency they accumulate and are found in bone marrow. These cells arise from a failure of red cell precursors to divide normally. Megaloblastic anaemia is also characterised by a decrease in white cell and platelet counts and general impairment of cell division in fast turn-over tissues. Morphological changes are similar, if not identical, to those seen in folic acid deficiency. This is not surprising since vitamin B<sub>12</sub>-deficiency actually induces functional folate deficiency (Chanarin, 1990 & 1999 and references therein).

59. Vitamin B<sub>12</sub> deficiency leads to the inhibition of methionine synthase, which results in the impairment of THF-related one-carbon metabolism and consequently purine and pyrimidine synthesis. Ultimately, this has a “knock on” effect on DNA and RNA synthesis and the cell replication process as a whole. Inhibition of methionine synthase also inhibits the formation of SAM and consequently SAM-related activity (Chanarin, 1990 & 1999 and references therein).

60. Initially, it was proposed that vitamin B<sub>12</sub>-deficiency led to an intracellular deficiency in THFglu<sub>5</sub> within the cell due to impairment of the demethylation of N<sup>5</sup>-methylTHFglu<sub>1</sub>, the so called “methylfolate trap” hypothesis. However, this theory is not supported by experiment. Cbl deficiency can be by-passed not only by provision of N<sup>5</sup>-formylfolate but also by the provision of methionine. The pathway by which methionine by-passes Cbl deficiency appears to be via SAM and its utilisation in polyamine synthesis in which methionine gives up three of its carbons (Figures 6 & 7). The methylthioribose residue from this reaction regenerates methionine using the carbon atoms from ribose (Figure 7) leaving a surplus carbon as formate. Radiolabel studies using <sup>14</sup>C-methylthioribose have shown that the residual formate provides carbon atoms 2 and 8 of the purine nucleus. Therefore, it is currently suggested that defective folate-mediated one-carbon metabolism arises from an intracellular deficiency of “active” formate required to convert folate, in the form of tetrahydrofolate, to N<sup>10</sup>-formyltetrahydrofolate (Chanarin 1990 & 1999 and references therein).

#### *Vitamin B<sub>12</sub> deficiency-associated neuropathy*

61. The clinical manifestations of vitamin B<sub>12</sub> deficiency-associated neuropathy are related to disorders of myelin in both the CNS and PNS (demyelination and axon

loss in the white matter of the CNS and in the peripheral nerves). Manifestations include myelopathy, peripheral neuropathy, dementia and neuropsychiatric disorders. Early symptoms include tingling and paraesthesias of the lower extremities accompanied by “pins and needles” and perception of muscle weakness. As the disease progresses, patients develop spastic paraparesis, hyperactive reflexes, muscle weakness and impaired bowel and bladder function. There may also be visual loss (Jeffery 1998).

62. The biochemical processes leading to neuropathologic changes are not completely understood. Vitamin B<sub>12</sub> deficiency results in impairment of methymalonyl CoA mutase resulting in an accumulation of propionyl CoA. It has been suggested that propionyl-CoA may displace succinyl-CoA in the synthesis of even-chain fatty acids and result in the insertion of abnormal fatty acids into myelin. However, it is more likely that inhibition of methionine synthase activity, the consequent impairment of one-carbon metabolism involving SAM and the decreased methylation ratios in the brain and nervous tissues form the underlying basis of Cbl-deficiency-induced neuropathy (Chanarin 1999 and references therein, Weir and Scott 1999 and references therein).

#### *Hyperhomocysteinaemia*

63. Epidemiological studies have shown an association between mild hyperhomocysteinaemia and increased risk of occlusive vascular disease (Cravo and Camilo 2000 and references therein). Homocysteinuria, a metabolic disorder resulting from defective homocysteine metabolism, is also associated with skeletal abnormalities and mental retardation (Perry 1999 and references therein). Furthermore, it has been suggested that maternal homocysteine levels may play a role in the aetiology of foetal malformations such as neural tube defects (Bronstrup *et al* 1998 and references therein).

64. Removal of homocysteine occurs either by trans-sulphuration or transmethylation. The former pathway involves vitamin B<sub>6</sub> (pyridoxal-5'-phosphate)-dependent metabolism of homocysteine to cystathionine by cystathionine- $\beta$ -synthase and ultimately glutathione formation. The latter pathway involves remethylation of homocysteine to methionine via methionine synthase and is dependent upon vitamin B<sub>12</sub> and folate. Deficiency of B vitamins and acquired (frequently observed in chronic alcoholics) or genetic defects in folic acid, methionine or homocysteine metabolism (involving cystathionine- $\beta$ -synthase, methionine synthase or methyl tetrahydrofolate reductase) thus can all lead to the accumulation of homocysteine. Both hyperhomocysteinaemia and hyperhomocysteinuria may be responsive to folic acid, vitamin B<sub>12</sub> and possibly vitamin B<sub>6</sub> supplementation (Cravo and Camilo 2000, Perry 1999, Clarke 1998 and references therein). Folic acid and Vitamin B<sub>12</sub> have been established as important determinants of fasting homocysteine, however a recent paper identified vitamin B<sub>6</sub>, riboflavin, alcohol, caffeine intakes, smoking and hypertension as other adjustable determinants of fasting homocysteine (Jacques *et al* 2001).

*Hepatic steatosis*

65. The pathogenesis of hepatic steatosis has been associated with methionine and choline deficiency. Ethanol, which also inhibits methionine synthase, causes hepatic steatosis (Weir and Scott 1999 and references therein).

*Vitamin B<sub>12</sub> and Breast cancer*

65b. A prospective epidemiological study found a threshold level for serum vitamin B<sub>12</sub> below which an increased risk of breast cancer among postmenopausal women was observed. However, as is true of most observational studies, it is not known whether the low vitamin B<sub>12</sub> status in these subjects is a factor that enhances breast cancer development or whether it is a natural consequence of breast cancer. This is the first observation to suggest that vitamin B<sub>12</sub> status may influence breast carcinogenesis and more studies to examine possible mechanisms are needed (Wu *et al* 1999).

**Overview of reported beneficial effects**

66. Vitamin B<sub>12</sub> therapy is indicated in patients with vitamin B<sub>12</sub> malabsorption, such as occurs with sprue, gastrectomy, regional enteritis, gastroenterostomy, ileal resection, malignancy, granuloma, strictures or anastomoses involving the ileum. Therapy is also indicated when secretion of IF is decreased by lesions that destroy the gastric mucosa (as occurs with gastric atrophy secondary to multiple sclerosis, in certain endocrine disorders, or in iron deficiency), or when antibodies to IF are present in gastric juice (as occurs in juvenile and autoimmune pernicious anaemia). CyanoCbl or hydroxoCbl may also be recommended in the event of bacterial overgrowth or infestation with fish tapeworm (AHFS Drug Information, 1994). CyanoCbl has also been used in the management of methylmalonic aciduria in infants and in pregnant women when amniocentesis has shown methylmalonic acidemia in the foetus (AHFS Drug Information 1994). HydroxoCbl acts as a potent cyanide antagonist and may be effective in the treatment of cyanide toxicity. It has been suggested that certain optic neuropathies, such as Leber's optic atrophy or tobacco amblyopia, may be related to cyanide toxicity and therefore may respond to massive doses of hydroxoCbl but not cyanoCbl (Freeman 1992 and references cited therein).

67. Vitamin B<sub>12</sub> is frequently administered parenterally by intracutaneous or intramuscular injection. CyanoCbl is generally considered the preparation of choice although hydroxoCbl may be used initially as it produces a more sustained increase in plasma vitamin B<sub>12</sub>. However, oral administration of vitamin B<sub>12</sub> has been shown to be effective for the treatment of nutritional deficiency, as occurs in strict vegetarians (and particularly their breast-fed offspring). Moderate doses of Cbl, in combination with intrinsic factor (IF), have been used in the treatment of pernicious anaemia. Larger oral doses (up to 2000 µg/day), without co-administration of IF, have also been used successfully in the treatment of pernicious anaemia (McIntyre *et al* 1960; Berlin *et al* 1978 & 1968, Kuzminski *et al* 1998). Orally administered cyanoCbl supplements (up to 400 µg/day), particularly in combination with folic acid, have also been shown to be effective in the treatment of hyperhomocysteinaemia (Clarke 1998).

68. Some studies have shown that ingestion of supplements combining vitamin B<sub>12</sub> and folic acid may be beneficial in certain other disorders. A small controlled,

double blind, cross-over study in 26 patients diagnosed with idiopathic osteoarthritis suggested that daily ingestion of a combined cyanoCbl + folate (20 µg + 6400 µg) supplement was more beneficial than folic acid (6400 µg) alone in improving hand-grip (Flynn *et al* 1994). A study by Juhlin and Olsson (1997) suggested that oral supplements of vitamin B<sub>12</sub> (1 mg cyanoCbl) and folic acid (5 mg), given twice daily for 3 months or more, combined with sun exposure, was more effective than either the vitamins or sun exposure alone in inducing repigmentation in 100 vitiligo patients.

69. Some studies have suggested that large doses of vitamin B<sub>12</sub>, particularly methyl Cbl, may influence biological rhythms by shortening the length of the sleep-wake cycle and improving the entrainment of the endogenous sleep-wake cycle to the environmental 24 hour rhythm, and thereby have benefit in the treatment of sleep-wake disorders (Okawa *et al* 1990, Takahashi *et al* 1999, Mayer *et al* 1996 and references cited therein). In addition, vitamin B<sub>12</sub> has been reported to increase light sensitivity by affecting melatonin secretion (Honma *et al* 1992). It has been suggested that large doses of methyl Cbl may be beneficial in the treatment of oligozoospermia (Moriyama *et al* 1987, Kumamoto *et al* 1988).

70. Vitamin B<sub>12</sub> and folic acid are required for genomic stability. Folic acid is required for the prevention of chromosomal damage caused by uracil being misincorporated into DNA and acts by converting dUMP to dTMP. Vitamin B<sub>12</sub> deficiency leads to decreased methionine synthase activity and thus decreased SAM concentration, which is required for DNA methylation and therefore for dUMP to be converted to dTMP. It has therefore been theorised that adequate intakes of vitamin B<sub>12</sub> and folic acid may decrease cancer risk by increasing genomic stability (discussed, Fenech 2001).

## Toxicity

### *Human*

#### *Case Reports*

71. It is generally accepted that vitamin B<sub>12</sub> has very low toxicity in humans. There have been reports of the development of folliculitis (acne) and/or anaphylactic reactions (erythematous skin rash, severe urticaria, oedema, anaphylactic shock and death) following parenteral administration of Cbls. However, these have been relatively rare. On occasion, skin patch tests have failed to demonstrate hypersensitivity to Cbl and, in these cases, anaphylaxis may have been attributable to impurities retained during the biosynthesis of vitamin B<sub>12</sub> in older preparations or substances added in solution preparation (Guillevin 1998 and references therein). Intravenous administration of megadoses (2,500 µg) of vitamin B<sub>12</sub> to dialysis patients after each dialysis resulted in a four-fold elevation of plasma vitamin B<sub>12</sub> and was not associated with any toxic effect (Mangiarotti *et al* 1986).

72. There have been a small number of case reports of adverse effects associated with ingestion of vitamin B<sub>12</sub> (Denis *et al* 1996, Fisher 1973, James and Warin 1971, Higson 1989, Price and Macdonald 1981, Sherertz 1991 - summarised in Table 3). Five cases experienced allergic reactions, three of which reported the recurrence of symptoms in individuals who had been previously exposed to Cbl by the parenteral

route. In three cases where skin patch tests were performed, two were negative for cyanoCbl but one of these was positive for cobalt. One further case reported the occurrence of a skin eruption that resembled acne rosacea. Vitamin B<sub>12</sub> exposures were generally not specified in these reports.

#### *Single dose study*

73. In an experiment designed to determine the uptake of large doses of the vitamin B<sub>12</sub>, preliminary to a study of the treatment of pernicious anaemia with high oral doses of the vitamin without IF, no adverse effects were reported in individuals administered single oral doses of up to 100,000 µg (Berlin *et al* 1968). Only a few individuals were administered the highest doses (≥10,000 µg, n=15; 100,000 µg, n=3).

#### *Clinical trials and supplementation studies*

74. Several studies have been conducted to assess the beneficial effects of high doses of orally administered Cbl in vitamin B<sub>12</sub> deficiency-related diseases including pernicious anaemia, in otherwise healthy patients exhibiting moderate hyperhomocysteinaemia and in vitiligo patients. One study has also investigated the effect of supplementation on plasma homocysteine levels in healthy females of child-bearing age. Other studies have investigated high oral doses of vitamin B<sub>12</sub>, mainly in the form of methyl Cbl, as a possible beneficial treatment for male infertility and also the effect of vitamin B<sub>12</sub> upon circadian rhythm as a possible beneficial treatment for sleep-wake disorders. These studies are summarised in Tables 4, 5 and 6. In an uncontrolled clinical study reported by Moriyama *et al* 1987<sup>1</sup>, clinical adverse reactions were observed in one of 16 oligozoospermia patients administered 6000 µg/day and in one of 23 patients administered 12000 µg/day and an apparent drug-related “laboratory” adverse reaction was observed in one of 23 patients given 12000 µg/day methyl Cbl for 16 weeks (the route of administration is not clear from the information available but is presumed to be by the oral route). With the exception of this study, there have been no reports of adverse effects related to cobalamin treatment, including studies in which individuals received 6000 µg/day methyl Cbl for up to 12 weeks (Takahashi *et al* 1999, Kumamoto *et al* 1988), 4500 µg /day for 2 weeks (Oren *et al* 1994), 2000 µg/day cyanoCbl for up to a year or 1000 µg/day cyanoCbl for several years (Kuzminski *et al* 1998, Juhlin and Olsson 1997, Berlin *et al* 1968 & 1978).

### **Vulnerable groups**

#### *Genetic variations*

75. There are no known genetic traits that result in an increased risk of Cbl toxicity. However, there are a number of inherited disorders that result in deficiency or functional deficiency of Cbl (summarised in Figure 8 and Table 7).

<sup>1</sup> Article in Japanese with English abstract. No further detail available.

*Individuals with tobacco amblyopia*

76. Normally, cyanoCbl is present as less than 8% of total plasma Cbl. However, individuals with conditions such as tobacco amblyopia, Leber's optic atrophy and dominantly inherited optic atrophy, have levels raised to as much as 35% of total. In smokers, cyanide intake is increased due its presence in tobacco smoke and it has been suggested that tobacco amblyopia may be related to elevated cyanide levels in these smokers (Baumeister *et al*, 1975 and references therein) and may play a role in optic neuritis sometimes observed in vitamin B<sub>12</sub> deficiency. HydroxoCbl acts as a potent cyanide antagonist by conversion to cyanoCbl and some optic neuropathies appear to respond to massive doses of hydroxoCbl but not cyanoCbl (Freeman, 1992 and references cited therein). It has been claimed that pre-existing cyanide toxicity in these individuals may be exacerbated by administration of cyanoCbl (Sawyer 1982). However, there are no data in the literature to support this.

*Adverse drug reactions*

77. Suspected adverse reactions to medicinal products are reported to the Committee on Safety of Medicines/Medicines Control Agency. Many factors influence the number of reports received, and in most situations there is considerable "under-reporting" of reactions. Most of the adverse reactions reported for products containing vitamin B<sub>12</sub> relate to multiconstituent products, and may not, therefore, be directly attributable to the vitamin. Single constituent vitamin B<sub>12</sub> products are associated with a low number of adverse reactions, with no trend or pattern to indicate a particular problem.

**Animal toxicity**

78. Data concerning the toxicity of vitamin B<sub>12</sub> in experimental animals are limited but it has generally been shown that vitamin B<sub>12</sub> has low toxicity.

*Acute toxicity (non-oral)*

79. Winter and Mushett (1951) reported an absence of toxic effects in mice injected intravenously and intraperitoneally with very high doses (up to 1600 mg/kg) of crystalline vitamin B<sub>12</sub>. This corresponds to ~10,000 times the daily requirement in adult humans. Tsao and Myashita (1993) reported LD<sub>50</sub> values of 1800 mg/kg and >2000 mg/kg for methyl Cbl and adenosylCbl, respectively, administered to CDF1 mice via intraperitoneal injection. In contrast, convulsions followed by cardiac or respiratory failure, were described in albino mice (n=10/group) following the administration of 1.5 – 3 mg/kg vitamin B<sub>12</sub> concentrate by intraperitoneal (*i.p.*) injection or 3.0 mg/kg by subcutaneous injection. However, no adverse reactions were observed in animals administered 0.75 mg/kg by the *i.p.* route (Traina 1950). Winter and Mushett (1951) suggested that the toxic effects observed by Traina (1950) may have been due to impurities or contaminants in the preparation of vitamin B<sub>12</sub> concentrate employed in this study. Tuberculin-type cutaneous sensitisation to crystalline vitamin B<sub>12</sub> (cyanoCbl) has been demonstrated in guinea pigs following intracutaneous injection (Lipton and Steigman 1963).

80. Little is known of the potential for delayed toxicity related to cobalt accumulation. One study (Pery-Man *et al* 1996) evaluated the toxicity of equimolar doses of cobalt from hydroxoCbl (70 mg/kg/day *i.p.*, n=14) as compared with that of cobalt salts (cobalt chloride hexahydrate, 12 mg/kg/day *i.p.*, n=14) on rat cardiac and diaphragmatic muscles. Control rats were injected with saline. Deposits of cobalt in the diaphragm and the myocardium of cobalt-treated animals were significantly higher than in hydroxoCbl treated animals. Neither treatment had a significant effect on the mechanical properties of cardiac or diaphragmatic muscle nor caused any histological change. The authors concluded that repeated administration of hydroxoCbl was devoid of significant diaphragmatic and cardiac muscle toxicity.

81. Subcutaneous administration of vitamin B<sub>12</sub> to young chicks ( $\geq 0.01$   $\mu\text{g}/\text{chick}$ , dose on body weight basis not stated) has been shown to have an adverse effect on memory function. There was no apparent adverse effect on neurological function, as assessed by righting reflex (Crowe and Ross 1997). The authors suggested that the effect on memory in chicks was attributable to an underdeveloped/rudimentary blood-brain barrier, resulting in an unprotected central nervous system.

#### *Acute toxicity (oral)*

82. As a preliminary test to determine a maximum tolerated dose for an investigation into the antinociceptive properties of cyanoCbl, no toxic effects were observed in female NMRI mice (21-28 g) when the vitamin was administered in 0.8% aqueous hydroxypropyl methylcellulose gel at doses up to 5000 mg/kg *p.o.* (Leuschner 1992). The number of animals tested was not stated.

#### *Chronic/carcinogenicity*

83. As far as can be established there are no studies to suggest that vitamin B<sub>12</sub> alone is directly carcinogenic in animals. However, there is some evidence to suggest that the vitamin may have some tumour promotional properties under certain circumstances.

84. Day *et al* (1950) reported that an average daily intake  $\sim 0.35$   $\mu\text{g}$  of vitamin B<sub>12</sub> (Rubramin) per day per animal<sup>2</sup> enhanced the incidence of dimethylaminobenzene (DAB)-induced hepatomas from 17 to 78% in rats maintained on a methionine deficient purified diet and from 11 to 33% in rats maintained on a methionine sufficient purified diet for 170 days. However, a procarcinogenic effect of vitamin B<sub>12</sub> was not observed in animals fed a similar methionine deficient diet in the absence of dietary DAB, where hepatoma incidence was zero. It should be noted that of the nine animals per group, there were two non-hepatoma related premature deaths in the unsupplemented group and two, one and one hepatoma-related premature deaths in the vitamin B<sub>12</sub>, methionine and vitamin B<sub>12</sub> plus methionine groups, respectively. Poirier (1975) reported that dietary supplementation with vitamin B<sub>12</sub> (0.5 ppm ;  $\sim 25$   $\mu\text{g}/\text{kg}/\text{day}$ <sup>3</sup>) significantly lowered survival time of rats with hepatocellular carcinoma induced by diethylnitrosamine. Kal'nev *et al* (1977 – Russian with abstract in English, cited by Miller and Hayes 1982) found that treatment with methyl Cbl or cyanoCbl

<sup>2</sup>  $\sim$  equivalent to 1.75 - 2.5  $\mu\text{g}/\text{kg}$  - present authors conversion

<sup>3</sup> present author's conversion

(route and dose uncertain) reduced the survival of rats with implanted hepatoma or carcinosarcoma.

85. It has been suggested that the promotional property of vitamin B<sub>12</sub> may relate to the role of vitamin B<sub>12</sub> in nucleic acid synthesis and cell replication and hence represents a non-specific effect. The human significance of these findings is uncertain.

86. In contrast, Tsao and Myashita (1993) showed that intraperitoneally administered methyl Cbl and deoxyadenosylCbl, but not cyanoCbl, increased survival time of mice implanted with P388 leukemia tumour cells. Shimizu *et al* (1987) demonstrated increased survival and reduced growth of inoculated ascites tumour cells in mice treated with methyl Cbl.

#### *Reproductive toxicity*

87. As far as can be established there have been no reports of any adverse effects relating to the administration of vitamin B<sub>12</sub> on male and female fertility and pre- and post-natal development.

#### *Genotoxicity*

88. There is a dearth of literature concerning the assessment of the mutagenicity of vitamin B<sub>12</sub> and as far as it can be established, there are no reports that this vitamin is mutagenic in the bacterial Ames test or any other test of mutagenicity. However, ethanol-chloroform extracts of pyrolysates of cyanoCbl prepared at temperatures of 300-600°C for 5 min were found to be mutagenic in *Salmonella typhimurium* strains TA98 and TA100 but only in the presence of a metabolic activating system (Demura *et al* 1990).

#### **Mechanisms of toxicity**

89. No data identified.

#### **Regulatory considerations**

90. The report of the American Academy of Pediatrics Committee on Drugs in Pediatrics [(1994), Vol 93 (1) p140 – as cited by HSDB 2000 *a* and *b*] suggests that maternal medication with vitamin B<sub>12</sub> is usually compatible with breast-feeding and that there have been no reported signs or symptoms in infants or any effects on lactation. The Recommended Daily Allowance in the Food Labelling Regulations for vitamin B<sub>12</sub> is 1µg. The Infant Formula and Follow-on Formula Regulations (1995) recommend a minimum vitamin B<sub>12</sub> content of 0.1 µg/100 kcal. The Processed Cereal-based Foods and Baby Foods for Infants and Young Children Regulations (1999) recommend a maximum vitamin B<sub>12</sub> content of 0.35 µg/100 kcal. The Foods Intended for Use in Energy Restricted Diets for Weight Reduction Regulations (1997) recommend that whole diet products should provide 1.4 µg vitamin B<sub>12</sub> and meal replacements 0.42 µg.

**Existing recommendations on maximum intake levels**

91. A report from the Committee on Medical Aspects of Food Policy (DH 1991) stated that “Vitamin B<sub>12</sub> has extremely low toxicity; it is toxic at only g/kg intakes in experimental animals, and no toxic effects have been encountered in man. Injections of as much as 3 mg/day have been used in attempts to treat fatigue and various neurological disorders, and 1 mg/day has been used to treat vitamin B<sub>12</sub>-responsive inborn errors of metabolism”. However the report fails to cite any data sources.

92. The NRC (1989) stated that no clear toxicity had been reported from daily absorption of up to 100 µg, although no supporting data were cited. In a review of the workshop on folate, vitamin B<sub>12</sub> and choline sponsored by the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board, one speaker was quoted as saying “no toxic effects have been attributed to vitamin B<sub>12</sub>, even after intakes as high as 1000 µg/day for a full year” (Glade 1999). A Report of the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes and its Panel on Folate, Other B Vitamins, and Choline and Subcommittee on Upper Reference Levels of Nutrients concluded that data were not sufficient for deriving a Tolerable Upper Intake Level (Food and Nutrition Board 2000).

**Existing recommendations on maximum supplementation levels**

93. The European Federation of Health Product Manufacturers Associations (EHPM 1997) recommended an Upper Safe Level for long term consumption of 3000 µg/day. They did not set an upper level for short term consumption.

**Summary**

94. Vitamin B<sub>12</sub> (Cobalamin, Cbl) is a water-soluble vitamin and a member of a family of related molecules known as the corrinoids which contain a corrin nucleus made up of a tetrapyrrolic ring-like structure. The centre of the tetrapyrrolic ring nucleus contains a cobalt ion that can be attached to methyl, deoxyadenosyl, hydroxy or cyano groups.

95. Vitamin B<sub>12</sub> is present in virtually all animal tissues, mainly in the forms of methyl, deoxyadenosyl- (ado) and hydroxo- cobalamin. However, all vitamin B<sub>12</sub> originates from certain types of bacteria, fungi and algae. Plants contain no vitamin B<sub>12</sub> beyond that derived from microbial contamination. Major dietary sources include meat and fish, particularly liver. HydroxoCbl and, in particular, cyanoCbl are synthetic forms used in vitamin pills, pharmaceuticals and in the fortification of food. Outside of the UK, methyl cobalamin has been used or tested therapeutically in countries such as Japan.

96. The Reference Nutrient Intake (RNI) for vitamin B<sub>12</sub> in adults in the UK is 1.5 µg/day. In the developed world, dietary intake generally exceeds requirement, the exception being those individuals adhering to strict vegan diets.

97. Measurement of vitamin B<sub>12</sub> in plasma is routinely used to determine deficiency, but may not be a reliable indication in all cases. Various other plasma markers

have been identified and methods devised to distinguish different causes of deficiency.

98. The absorption of physiologic doses of crystalline vitamin B<sub>12</sub> or that present in food is limited to ~1.5 - 2 µg/dose or meal due to saturation of the receptor-mediated uptake system which predominates at these exposure levels. Regardless of dose, approximately 1.2% of vitamin B<sub>12</sub> is absorbed by passive diffusion and consequently this process becomes quantitatively more important at pharmacological levels of exposure. 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 crystalline forms (cyano, methyl, ado, hydroxo) of Cbl may be absorbed with different efficiencies, but this appears to be most apparent at low doses.
99. Ingested vitamin B<sub>12</sub> is released from the food matrix by the action of digestive enzymes and gastric acid and becomes bound to salivary haptocorrin. As the pH rises further along the gut, and under the influence of pancreatic enzymes, vitamin B<sub>12</sub> is released from the haptocorrin protein and becomes complexed with intrinsic factor (IF). The Cbl-IF complex binds to specific ileal enterocyte cell wall receptors and is internalised by endocytosis. Once inside the cell, the IF is degraded and liberated vitamin is converted to the methyl or the deoxyadenosyl form which is bound to transcobalamin II (TC II) binding protein and then exported into the portal blood. In the general circulation, most Cbl is bound to transcobalamin I (TC I) but the majority of Cbl available for uptake into the tissues is that bound to TC II. Uptake into cells occurs through receptor mediated endocytosis involving specific TC II cell wall receptors. Once inside the tissues/cells, the complex is degraded by the lysosomes, and metabolised either to methyl Cbl in the cytosol, where it binds to methionine synthase, or to deoxyadenosyl Cbl in the mitochondria, where it binds to methylmalonyl CoA mutase. Excretion occurs mainly via the faeces and urine, but also through the shedding of skin cells. Excretion is very slow, with much of that secreted into the gut in bile undergoing enterohepatic cycling. In addition to IF-mediated absorption, ~1.2% of vitamin B<sub>12</sub> is absorbed by passive diffusion.
100. Vitamin B<sub>12</sub> serves as cofactor to at least two enzymes, methionine synthase and methylmalonyl CoA mutase. Methionine synthase plays a pivotal role in one-carbon metabolism, being crucial both in the synthesis of the universal methyl donor S-adenosyl methionine and in the cellular import and metabolism of folate. Methylmalonyl CoA mutase converts L-methylmalonyl CoA to succinyl CoA and is important in the even-chained fatty acid synthesis.
101. Dietary deficiency is a rare occurrence in the developed world although those individuals adhering to vegan diets may be at risk. Most vitamin B<sub>12</sub> deficiency is, therefore, attributable to inherited or acquired defects resulting in malabsorption or the impairment of transport of the vitamin within the body. Deficiency impacts on the haematopoietic and nervous systems. Associated diseases include megaloblastic anaemia and neuropathies related to disorders of myelin in both the central and peripheral nervous systems. Vitamin B<sub>12</sub> deficiency can lead to moderate hyperhomocysteinaemia, a risk factor in occlusive vascular disease.

102. Oral supplementation is indicated prophylactically mainly where there is a likelihood of deficiency in those whose gastrointestinal function is normal e.g. in individuals who are strict vegetarians. Inherited and acquired disorders relating to vitamin B<sub>12</sub> malabsorption are usually treated by repeated parenteral injection. However, oral administration of very high doses of vitamin B<sub>12</sub> has been shown to be effective in the treatment of pernicious anaemia and other types of B<sub>12</sub> deficiency.
103. The toxicity of vitamin B<sub>12</sub> is generally accepted as being very low. Clinical studies have reported no adverse effects following administration of up to 6000 µg/day methylCbl for several weeks and up to 1000 µg/day cyanoCbl for several years. However, there may be a very small number of individuals who are hypersensitive to cobalamin.
104. The data-base on the oral toxicity of cobalamin in laboratory animals is limited. The vitamin is acutely toxic at dose levels of the order of g/kg. There is some limited evidence to suggest that high doses of vitamin B<sub>12</sub> may have some tumour promoting activity, but data are not consistent.
105. There is no evidence to suggest that vitamin B<sub>12</sub> is mutagenic or otherwise genotoxic.

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**ANNEX 1 to EVM/00/20**

**TABLES REFERRED TO IN THE PAPER**

Table 1a. (adapted from DH, 1991)

Dietary Reference Values for Vitamin B<sub>12</sub> (µg/day)

Age	Lower Reference Nutrient Intake	Estimated Average requirement	Reference Nutrient Intake
0-3 months	0.1	0.25	0.3
4-6 months	0.1	0.25	0.3
7-9 months	0.25	0.35	0.4
10-12 months	0.25	0.35	0.4
1-3 years	0.3	0.4	0.5
4-6 years	0.5	0.7	0.8
7-10 years	0.6	0.8	1.0
<i>males</i>			
11-14 years	0.8	1.0	1.2
15-18 years	1.0	1.25	1.5
19-50 years	1.0	1.25	1.5
50+ years	1.0	1.25	1.5
<i>females</i>			
11-14 years	0.8	1.0	1.2
15-18 years	1.0	1.25	1.5
19-50 years	1.0	1.25	1.5
50+ years	1.0	1.25	1.5
Pregnancy	No increment		
Lactation:			
0-4 months			+0.5
4+ months			+0.5

**Table 1b. (adapted from Weir and Scott, 1998)****Recommended Dietary Intakes for vitamin B<sub>12</sub> (µg/day)**

<b>Age</b>	<b>RNI UK</b>	<b>USA (1989)</b>	<b>USA (1998)</b>
0-6 months	<b>0.3</b>	<b>0.3</b>	<b>0.4</b>
6-12 months	0.4	0.5	0.5
1-3 years	0.5	0.7	0.9
4-6 years	0.8	1.0	1.2 (4 to 8 years)
7-10 years	1.0	1.4	-
<b>Males</b>			
11-14 years	1.2	2.0	1.8 (9 to 13 years)
15-18 years	1.5	2.0	2.4
19-50 years	1.5	2.0	2.4
50+ years	1.5	2.0	2.4 (51 to >70 years)
<b>Females</b>			
11-14 years	1.2	2.0	1.8 (9 to 13 years)
15-18 years	1.5	2.0	2.4
19-50 years	1.5	2.0	2.4
50+ years	1.5	2.0	2.4 (51 to >70 years)
<b>Pregnancy</b>	No increment	2.2	2.6
<b>Lactating</b>	-	2.6	2.8

[UK figures from DH, 1991]

**Table 1c. FAO/WHO recommendations for safe levels of vitamin B<sub>12</sub> intake**

	<b>recommended daily intake</b>
<b>adults*</b>	<b>1.0 µg/day</b>
<b>adults with achlorhydria</b>	<b>2.0 µg/day</b>
<b>pregnancy</b>	<b>additional 0.4 µg/day</b>
<b>lactation</b>	<b>additional 0.3 µg/day</b>
<b>Infants</b>	<b>0.1 µg/day in breast milk</b>
<b>children</b>	<b>0.04 µg/kg/day to a maximum of 1.0 µg in total</b>

**\*with normal gastrointestinal function**

**[Data from Joint FAO/WHO Expert Consultation, 1988]**

**Table 2. Inherited and acquired causes of cobalamin malabsorption**

Disorders		Pathophysiology
Inherited	Lack of luminal IF e.g. as occurs in pernicious anaemia	Cbl remains bound to Hc and not recognised by IFCR
	Lack of IFCR at brush border	Lack of endocytosis of Cbl
	Lack of intracellular TC II	Cbl cannot exit enterocytes
	Defective lysosomal transport?	Cbl retained in lysosomes
Acquired	Gastric surgery or gastritis	Cbl release from food proteins impaired due to lack of acid/pepsin
	Zollinger-Ellison syndrome	Impaired transfer of Cbl from Hc to IF due to low luminal pH competition for Cbl
	Bacterial overgrowth/tape worm	Competition for Cbl uptake
	Pancreatic insufficiency	Impaired transfer of Cbl from Hc to IF due to lack of pancreatic proteases
	Surgical resection (Crohn's disease)	Loss of IFCR

IF = intrinsic factor; Hc = haptocorrins; IFCR = IF-cobalamin receptor; TC II = transcobalamin II; Cbl = cobalamin

**Table 3. Case reports of adverse effects associated with ingestion of vitamin B<sub>12</sub>**

case	form	dose	Duration	effect/comments	reference
male, 69 years who had 1 month previously suffered an allergic reaction following parental administration of cyanocobalamin for B <sub>12</sub> deficiency	marmite, which contains cynaocobalamin	“three thickly spread sandwiches”	consumed within a single day	recurrence of symptoms , subsequent skin patch test to cyanocobalamin was negative	Denis <i>et al</i> , 1996
female who had previously received vitamin B <sub>12</sub> by injection	NS	NS	Single	inflammatory reaction at injection site, positive patch test to vitamin B <sub>12</sub>	Fisher, 1973
female, 50 years, pernicious anaemia patient, who had previously received cyanocobalamin parentally for 8 years without adverse effect, then developed shivering, bronchospasm, utricaria, dyspnoea, aphonia following injection	vitamin B <sub>12</sub> peptide, cyanocobalamin	NS	100 µg, 2x/day	recurrence of symptoms, subsequent skin patch test was negative	James and Warin, 1971
15 months old Down’s syndrome child	marmite, which contains cynaocobalamin	NS, “thinly spread” on sandwiches		angio-odema of the mouth and periorbital tissues	Higson, 1989
woman aged 60, shown to be allergic to cobalt by patch test	NS	habitual ingestion of vitamin B <sub>12</sub> tablets	NS	recurrent cheilitis, subsequent patch test was negative with hydroxycobalamin. However, symptoms improved on cessation of ingestion of B <sub>12</sub> tablets	Price and Macdonald, 1981
				eruption resembling acne rosacea	Sherertz, 1991

**NS – not stated**

**Table 4. Oral \*supplementation as treatment for pernicious anaemia and other vitamin B<sub>12</sub> deficiency states**

study type	dose	treatment duration/follow up	adverse effects related to supplementation	reference
uncontrolled supplementation trial in patients with pernicious anaemia (n=12)	up to 500 µg/day	6 to 50 months	none reported	Marshall Chalmers and Shinton, 1958
uncontrolled supplementation trial in patients with pernicious anaemia (n=8)	1000 µg/day	?	none reported	McIntyre <i>et al</i> , 1960
uncontrolled supplementation trial in patients with pernicious anaemia (n=37)	100 µg/day	up to 3.5 years	none reported	Thompson <i>et al</i> , 1962
uncontrolled supplementation trial in patients with pernicious anaemia (n=27)	300 µg/day <sup>s</sup>	up to 1 year	none reported	Waife <i>et al</i> , 1963
uncontrolled supplementation trial in patients with pernicious anaemia and other types of B <sub>12</sub> deficiency (n=64)	500 rising to 1000 µg/day	10 to 70 months (42 patients for over 4 years)	it was reported that no negative reactions were attributable treatment	Berlin <i>et al</i> , 1968;
study of supplementation in patients with pernicious anaemia (n=11)	1000 µg/day	mean 8.4 years	none reported	Berlin <i>et al</i> 1978

<b>study type</b>	<b>dose</b>	<b>duration</b>	<b>adverse effects related to supplementation</b>	<b>reference</b>
randomised controlled study comparing oral (n=18) and parenteral (n=15) treatment in newly diagnosed cobalamin deficiency cases	2000 µg/day	120 days	none reported	Kuzminski <i>et al</i> , 1998

\*Supplement was cyanocobalamin otherwise and was without coadministration of intrinsic factor (IF) with the exception of <sup>\$</sup> where supplementation was either in the form of cyanocobalamin or hydroxocobalamin or a mixture of the two. Study end points included any or all of the following; haematological and neurologic response, measurement of plasma vitamin B<sub>12</sub>, homocysteine and/or methylmalonic acid.

**Table 5. Studies assessing the effect of oral vitamin B<sub>12</sub> supplementation on plasma homocysteine levels**

<b>study type</b>	<b>* cobalamin dose</b>	<b>duration</b>	<b>Adverse effects related to supplementation</b>	<b>reference</b>
supplementation trial in moderately hypercysteinaemic males(n=22)	50 µg/day combined with 1000 µg folic acid and 12.2 mg pyridoxine	6 weeks	None reported ; 2 participants withdrew from the study but for reasons that were unrelated to any cobalamin-induced adverse effects	Ubbink <i>et al</i> , 1993
randomised, factorial-design, blind, placebo-controlled study in hypercysteinaemic males (n=100)	400 µg/day alone (n=17) or combined with 650 µg folic acid and 10 mg pyridoxine (n=20)	6 weeks	None reported; data from 2 participants were withdrawn through lack of compliance, seven other participants withdrew during the study for reasons that were not stated	Ubbink <i>et al</i> , 1994
randomised, placebo-controlled, cross-over study in healthy female volunteers of child bearing age (n=156)	400 µg/day (combined with 400 µg folic acid)	4 weeks per treatment period	None reported although 6 participants withdrew during the study for reasons that were not stated	Bronstrup <i>et al</i> , 1998
randomised double-blind placebo-controlled factorial-design study in male volunteers with mild hyperhomocysteinaemia (n=132)	20 µg/day (combined with 1 mg folic acid and 7.2 mg pyridoxine with (n=28) or without (n=22) antioxidants)	8 weeks	None reported although 20 participants did not complete the study for reasons that were not stated	Woodside <i>et al</i> , 1998

study type	* cobalamin dose	duration	Adverse effects related to supplementation	reference
randomised, placebo-controlled study in healthy volunteers (n=227, mean age 53) and patients with history of venous thrombosis (n=89, mean age 62).	\$400 µg/day alone (n=107) or combined with 5 mg folic acid and 50 mg pyridoxine (n=103 )	8 weeks	None reported, although 6 participants withdrew during the study for reasons that were not stated	Den Heijer <i>et al</i> , 1998
Groups were stratified for plasma homocysteine levels				

\*Supplement was cyanocobalamin with the exception of § where supplementation was with hydroxocobalamin. In each case, the study endpoint was the measurement of plasma total homocysteine

**Table 6. Miscellaneous supplementation studies**

<b>study type</b>	<b>* cobalamin dose</b>	<b>duration</b>	<b>adverse effects related to supplementation</b>	<b>reference</b>
uncontrolled study in infertile males (n=26)	1500 µg/day**	4-24 weeks	none reported	Isoyama <i>et al</i> , 1984 [abstract]
study in infertile males (n=26)	1500 µg/day with (n=40) or without (n=12) Clomid (25 mg/day)**	8-60 weeks	none reported	Isoyama <i>et al</i> , 1986 [abstract]
preliminary study to assess effect on sperm count and motility rate	12000 µg/day (n=23)** 6000 µg/day (n=16)**	16 weeks	clinical adverse reactions were observed in one patient in each group; drug-related laboratory adverse reactions were observed in the higher dose group; no further detail as only abstract was available in English	Moriyama <i>et al</i> , 1987[abstract]
double-blind, multicentred, placebo-controlled comparative study (n=375) to assess effect on sperm count and motility rate	6000 µg/day (n=125)** 1500 µg/day (n=124)** placebo (n=126)**	12 weeks	none reported	Kumamoto <i>et al</i> , 1988 [abstract]
blind placebo-controlled cross-over study in healthy individuals (n=9) to investigate effect on plasma melatonin rhythm	3000 µg/day	4 weeks	none reported	Honma <i>et al</i> , 1992
Randomised double-blind placebo-controlled parallel study to investigate as treatment for seasonal affective disorder in 27 patients	1500 µg/day 3x/day cyanocobalamin (n=14) placebo (n=13)	2 weeks washout followed by 2 weeks treatment	none reported	Oren <i>et al</i> , 1994

study type	*cobalamin dose	duration	adverse effects related to supplementation	reference
multi-centred double-blind study in Japan to investigate effect of patents with sleep-wake disorders (n=51)	30 (n=21) or 6000 (n=27) µg/day **	up to 8 weeks	none reported; all patients were accounted for at the 4 week follow up, 5 patients were not accounted for after 8 weeks	Takahashi <i>et al</i> , 1999
preliminary randomised study with no control group in healthy adults (6 F, 14 M) to investigate effect on performance and circadian rhythm	3000 µg/day methylcobalamin (n=9) or 3000 µg/day cyanocobalamin (n=10)	9 d pretreatment observation then 14 d treatment	none reported; one male excluded through non-compliance; the route of administration was not made clear but the article referred to “intake”	Mayer <i>et al</i> , 1996
multi-centre double-blind, placebo-controlled study to assess effect on delayed sleep phase syndrome	3000 µg/day (n=27)** placebo (n=23)**	4 weeks	none reported	Okawa <i>et al</i> , 1997
uncontrolled study to investigate the effect of supplementation on repigmentation in vitiligo patients (n=100)	1000 µg (+ 5mg folic acid), twice daily	up to 12 months	none reported although 27/100 and 48/100 participants had stopped taking the supplements after 1-2 months and 3-6 months, respectively. Reasons for withdrawal were not stated	Juhlin and Olsson, 1997

\*Supplement was methyl cobalamin unless stated otherwise. \*\* The route of administration was not clear from the information available.

**Table 7. Salient biochemical features of cultured fibroblasts from patients with various defects in cellular cobalamin metabolism\* (adapted from Fenton and Rosenberg, 1995)**

Biochemical Parameter	Mutant Class						
	<i>cbIA</i>	<i>cbIB</i>	<i>cbIC</i>	<i>cbID</i>	<i>cbIE</i>	<i>cbIF</i>	<i>cbIG</i>
<b>Studies with intact cells</b>							
[ <sup>14</sup> C] propionate oxidation	-	-	-	-	+	-	+
[ <sup>14</sup> C] MeH <sub>4</sub> F fixation	+	+	-	-	-	-	-
MeCbl synthesis	+	+	-	-	-	-	-
AdoCbl synthesis	-	-	-	-	+	-	+
Conversion of Cn-Cbl to OH-Cbl	+	+	-	±	+	-	+
Lysosomal efflux of free Cbl	+	+	+	+	+	-	+
<b>Enzyme activities in cell extracts*</b>							
Mutase holoenzyme	-	-	-	-	NT	NT	NT
Mutase total enzyme	+	+	+	+	NT	NT	NT
Methyltransferase holoenzyme	+	+	-	-	+	-	±
Methyltransferase total enzyme	+	+	±	±	+	±	±
Cob(I)alamin adenosyl transferase	+	-	+	+	NT	+	NT

\* Holoenzyme is defined as the enzyme activity measured in the absence of added cofactor, total enzyme is the activity measured in the presence of saturating concentrations of cofactor.

**ANNEX 2 to EVM/00/20**

**FIGURES REFERRED TO IN THE PAPER**

**Figure 1. The structure of deoxyadenosylcobalamin (AdoCbl)**

**Figure 2. Mechanism of vitamin B<sub>12</sub> absorption.**

This figure cannot be reproduced for copyright reasons but can be consulted in Weir and Scott, 1999.

**Figure 3.**

**Proposed cellular sorting of cobalamin (Cbl) imported into a polarised epithelial cell bound to intrinsic factor (IF) from the apical plasma membrane (left panel) and to transcobalamin II (TC II) from the basolateral membranes (right panel). The broken lines represent incompletely defined pathways. The dark oval on the periphery of the endosomes or prelysosomes (left) or the lysosomes (right) represent the Cbl transporter; the dark rectangle represents a block in Cbl exit from these acidic vesicles due to a potential defect in the Cbl transporter**

This figure cannot be reproduced for copyright reasons but can be consulted in Seetharam, 1999.

**Figure 4. Intracellular Cbl metabolism**

This figure cannot be reproduced for copyright reasons but can be consulted in Markle, 1996.

**Figure 5. The role of methionine synthase and Cbl as cofactor in one-carbon metabolism.**

This figure cannot be reproduced for copyright reasons but can be consulted in Weir and Scott, 1999.

**Figure 6. (adapted From Chanarin, 1990)**  
**The pathway by which methionine donates a formate unit is via methylthioribose formation in regeneration of methionine following the synthesis of polyamines from S'adenosyl methionine**

**CHO**

**Methionine**

**S-adenosyl  
methionine**

**Methylthioribose**

**Ado**

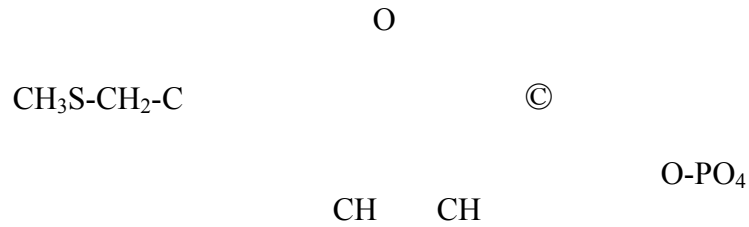
**Decarboxylated  
S-adenosyl-methionine**

**Methyladenosine**

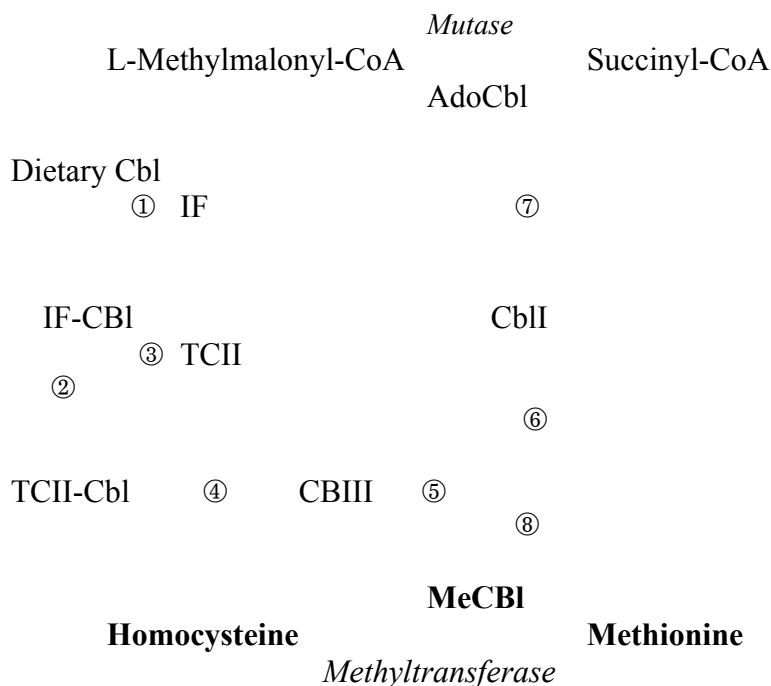
**Polyamines**

Figure 7. (adapted from Chanarin, 1990)

Methylthioribose is regenerated into methionine (boxed area). The  $\text{CH}_3\text{-S}$  moiety is derived from the original methionine molecule with the rest of the molecule coming from ribose. The regeneration of methionine releases formate (from the circled carbon)



**Figure 8. (adapted from Fenton and Rosenberg, 1995)**  
**Summary scheme of inherited defects of cobalamin metabolism. The circled numbers and their key signify the general sites at which abnormalities have been identified and the affected protein or process at each site. Cbl III = cob(III)alamin (e.g. OH-Cbl); Cbl I = cob(I)alamin; AdoCbl = adenosylcobalamin; MeCbl = methylcobalamin**



## Key

Site	Mutant Class	Localisation of defect
①	-	Intrinsic Factor
②	-	Intestinal Cbl absorption
③	-	Transcobalamin II
④	<i>cbIF</i>	Lysosomal Cbl efflux
⑤	<i>cbIC, cbID</i>	Cytosolic Cbl metabolism
⑥	<i>cbIA</i>	Mitochondrial Cbl reduction
⑦	<i>cbIB</i>	Cob(I)alamin Adenosyl-transferase
⑧	<i>cbIE, cbIG</i>	Methyltransferase-associated Cbl utilisation

## ANNEX 3 to EVM/00/20.REVISED SEPT2001

INTAKES OF VITAMIN B<sub>12</sub> FROM FOOD AND SUPPLEMENTS

The data presented on vitamin B<sub>12</sub> intakes are obtained from dietary surveys of specific population age groups in Britain carried out over the last 15 years<sup>45678</sup>. In each survey food consumption data were collected by means of a dietary record (usually weighed) kept for 4 or 7 consecutive days. Nutrient intakes were calculated using a set of nutrient composition data contemporaneous with the time of the survey. Therefore some apparent differences in intakes between population age groups may be due to changes in the nutrient composition data and reflect changes in the nutrient composition of manufactured foods over time.

**Total intakes of Vitamin B<sub>12</sub>**

Table 1 provides information on the absolute intakes of vitamin B<sub>12</sub> by the British population, classified by age and sex. Mean and median intake and the upper and lower end of the intake distribution (defined as upper and lower 2.5 percentiles respectively) are given.

Average intake of vitamin B<sub>12</sub> was lowest for pre-school children and highest in males aged 16-64 years. Average intakes of vitamin B<sub>12</sub> from food increased with age for boys between 7-10 and 11-14 years. For males and females aged 16-64 years intake was higher for those aged 35 and over than in the 16-24 year group. Average intakes decreased steadily with age for males and females aged 65 years and over free living in the community. Intakes from food only and from food and supplements at the 97.5%ile were between two and six times the median in most groups. Mean and median vitamin B<sub>12</sub> intakes (from food and all sources) were well above the Reference Nutrient Intakes for each age /gender group.

Table 2 provides information on vitamin B<sub>12</sub> intakes adjusted for body weight and classified by age and sex. Vitamin B<sub>12</sub> intakes adjusted for body weight displayed a trend to decrease with age for pre-school children and young people, and to a lesser extent for free-living older adults. For adults aged 16-64 years there was a trend towards an increase in vitamin B<sub>12</sub> intake per unit body weight.

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<sup>4</sup> Food and nutrient intakes of British infants. 1986

<sup>5</sup> National Diet and Nutrition Survey of children aged 1½-4½ years. 1992/3

<sup>6</sup> National Diet and Nutrition Survey of young people aged 4-18 years. 1997/8

<sup>7</sup> Dietary and nutritional survey of British adults. 1986/7

<sup>8</sup> National Diet and Nutrition Survey of people aged 65 years and over. 1994/5

### Sources of Vitamin B<sub>12</sub> in the diet

Table 3 indicates the contribution made by different types of food to average intakes of vitamin B<sub>12</sub> by young people aged 15-18 years. This data set was collected in 1997 and so most closely reflects current eating habits.

Milk and milk products were the main source of vitamin B<sub>12</sub> in this age group, providing 39% of total intake, of which 21% came from semi-skimmed milk. This was followed by meat and meat products (28%), of which a quarter came from beef, veal and dishes, and cereals and cereal products (12%) of which nearly half was from breakfast cereals. Some manufacturers of breakfast cereals voluntarily fortify their products with varying quantities of vitamin B<sub>12</sub>.

The main sources of vitamin B<sub>12</sub> were the same for school children, infants and children aged 1<sup>1</sup>/<sub>2</sub> – 4<sup>1</sup>/<sub>2</sub> years. Milk and milk products provided just under half of total intake for pre-school children and 40% for infants, with infant formula providing 18% of total intake in this age group. However for adults and older people meat and meat products was the main source of vitamin B<sub>12</sub>, providing half the total intake for adults and over 40% for older people.

### Vitamin B<sub>12</sub> intakes from supplements

Dietary supplements containing vitamin B<sub>12</sub> provided 2% of the population average intakes of vitamin B<sub>12</sub> for adults, for free-living older people and for females aged 85 and over in institutions. The contribution was negligible for other age groups in institutions. Dietary supplements containing vitamin B<sub>12</sub> taken by toddlers and young people had a negligible effect on population average intakes.

If we consider consumers of supplements containing vitamin B<sub>12</sub> separately we can expect the proportion of intake from supplements to be much higher. Table 4 depicts the number of consumers of dietary supplements containing vitamin B<sub>12</sub> in each age group. The table also provides information on the mean, median and range of intakes of vitamin B<sub>12</sub> from supplements for those who consumed them. Use of supplements containing vitamin B<sub>12</sub> was low in all the population groups. The highest prevalence of vitamin B<sub>12</sub> supplement use was in females aged 65 years and over living in the community, at 5%.

In most age groups intake of vitamin B<sub>12</sub> from supplements was no more than 6.6µg/day. The exception was males and females aged 16-64 years whose intakes from this source ranged from 0.1µg/day up to 50µg/day. This high intake of vitamin B<sub>12</sub> from supplements was partly due to the use of high dose multivitamin and chelated mineral tablets (containing 25µg vitamin B<sub>12</sub>).

Diet and Nutrition Surveys Branch  
Nutrition Division  
October 2000

**Table 1: Total intakes of Vitamin B<sub>12</sub>**

Age/sex	Absolute Vitamin B <sub>12</sub> intake (µg/day)							
	Food Only				Food and Supplements			
	2.5% ile	Mean	Median	97.5% ile	2.5% ile	Mean	Median	97.5% ile
<b>Infants (1986)</b> 6-12mths M&F	1.0	3.2	3.0	6.1	*	*	*	*
<b>Pre-school children (1992/3)</b>								
1½-2½ yrs/M&F	0.8	2.9	2.5	6.7	0.9	2.9	2.5	6.7
2½-3½ yrs/M&F	0.9	2.8	2.4	7.2	0.9	2.8	2.4	7.2
3½-4½ yrs/M	0.8	2.8	2.6	6.2	0.8	2.8	2.6	6.3
3½-4½ yrs/F	0.9	2.8	2.4	6.2	0.9	2.8	2.5	6.2
<b>Young people (1997/8)</b>								
4-6 yrs/M	1.6	4.0	3.8	8.3	1.6	4.0	3.8	8.5
4-6 yrs/F	1.6	3.6	3.4	7.6	1.6	3.6	3.4	7.6
7-10 yrs/M	1.6	3.9	3.7	7.8	1.6	4.0	3.8	7.8
7-10 yrs/F	1.2	3.5	3.4	6.9	1.2	3.5	3.4	6.9
11-14 yrs/M	1.4	4.5	4.0	9.3	1.4	4.5	4.0	9.3
11-14 yrs/F	1.1	3.2	3.0	7.1	1.1	3.3	3.0	7.2
15-18 yrs/M	2.1	5.0	4.6	9.5	2.1	5.0	4.6	10.0
15-18 yrs/F	1.1	3.4	3.0	7.3	1.1	3.4	3.1	7.6
<b>Adults (1986/7)</b>								
16-24 yrs/M	1.7	6.2	5.1	19.8	1.9	6.3	5.1	19.7
16-24 yrs/F	1.3	4.4	3.4	17.4	1.3	4.4	3.4	17.4
25-34 yrs/M	2.7	7.1	5.7	24.7	2.7	7.1	5.7	24.7
25-34 yrs/F	1.1	4.5	3.5	16.4	1.1	4.6	3.5	16.4
35-49 yrs/M	2.4	7.6	5.9	26.7	2.4	7.7	6.0	26.7
35-49 yrs/F	1.5	5.6	4.1	18.6	1.6	5.9	4.1	19.6
50-64 yrs/M	2.6	7.8	5.9	23.6	2.6	8.0	5.9	23.7
50-64 yrs/F	1.6	5.8	4.3	16.4	1.6	5.9	4.4	17.2
<b>Older people free-living in the community (1994/5)</b>								
65-74yrs/M	2.0	6.4	4.9	19.4	2.0	6.5	5.0	19.4
65-74yrs/F	1.2	4.6	3.5	17.7	1.2	4.7	3.7	17.7
75-84 yrs/M	1.6	5.5	4.0	20.3	1.6	5.6	4.0	21.1
75-84 yrs/F	1.3	4.5	3.3	19.9	1.3	4.7	3.4	19.9
85 & over/M	1.7	4.8	3.8	11.6	1.7	4.9	3.8	11.8
85 & over/F	0.7	3.5	2.9	10.9	0.7	3.6	2.9	10.9
<b>Older people living in institutions (1994/5)</b>								
65-84 yrs/M	2.1	4.9	4.4	13.1	2.1	4.9	4.4	13.1
65-84 yrs/F	2.0	4.5	3.8	12.5	2.0	4.5	3.8	12.5
85 & over/M	1.7	5.0	4.3	13.8	1.7	5.0	4.3	13.8
85 & over/F	1.4	4.6	3.7	14.4	1.4	4.7	3.7	14.4

\*Data unavailable

**Table 2: Bodyweight adjusted Vitamin B<sub>12</sub> intake**

Age/sex	Bodyweight adjusted Vitamin B <sub>12</sub> intake (µg/kg bwt /day) <sup>9</sup>		
	<i>intakes from food and supplements</i>		
	Mean	Median	97.5% ile
<b>Infants (1986)<sup>10</sup></b> 6-12mths/M&F	0.330	0.320	0.630
<b>Pre-school children (1992/3)</b> 1½-2½ yrs/M&F	0.236	0.209	0.546
2½-3½ yrs/M&F	0.192	0.169	0.450
3½-4½ yrs/M	0.170	0.159	0.365
3½-4½ yrs/F	0.174	0.153	0.388
<b>Young people (1997/8)</b> 4-6 yrs/M	0.192	0.184	0.429
4-6 yrs/F	0.179	0.166	0.358
7-10 yrs/M	0.134	0.125	0.265
7-10 yrs/F	0.114	0.101	0.235
11-14 yrs/M	0.099	0.089	0.202
11-14 yrs/F	0.070	0.064	0.149
15-18 yrs/M	0.076	0.070	0.147
15-18 yrs/F	0.059	0.052	0.116
<b>Adults (1986/7)</b> 16-24 yrs/M	0.090	0.072	0.290
16-24 yrs/F	0.075	0.055	0.289
25-34 yrs/M	0.096	0.075	0.332
25-34 yrs/F	0.075	0.058	0.249
35-49 yrs/M	0.101	0.079	0.345
35-49 yrs/F	0.094	0.066	0.315
50-64 yrs/M	0.103	0.076	0.325
50-64 yrs/F	0.094	0.069	0.308
<b>Older people free-living in the community (1994/5)</b> 65-74 yrs/M	0.086	0.064	0.260
65-74 yrs/F	0.073	0.056	0.212
75-84 yrs/M	0.076	0.056	0.251
75-84 yrs/F	0.074	0.052	0.301
85 and over/M	0.072	0.054	0.170
85 and over/F	0.063	0.051	0.273
<b>Older people living in institutions (1994/5)</b> 65-84 yrs/M	0.073	0.064	0.180
65-84 yrs/F	0.076	0.063	0.198
85 and over/M	0.075	0.065	0.246
85 and over/F	0.081	0.058	0.282

<sup>9</sup> Body weights measured for each subject for all age groups except infants aged 6-12 months where reported body weights were used.

<sup>10</sup> Intakes for infants aged 6-12 months are from food only.

**Table 3: Sources of Vitamin B<sub>12</sub> in the diet<sup>11</sup>**

Food Type	Contribution of food types to average daily intake of Vitamin B <sub>12</sub>		
	µg/day		% of total
Cereal and cereal products	0.49		12
- of which all breakfast cereals		0.21	5
Milk and milk products	1.64		39
- of which milk "semi-skimmed"		0.88	21
- of which milk "whole"		0.46	11
Egg and egg dishes	0.21		5
Fat spreads	-		-
Meat and meat products	1.17		28
- of which beef, veal & dishes		0.30	7
Fish and fish dishes	0.40		9
- of which oily fish including canned		0.21	5
Vegetables, potatoes and savoury snacks	0.04		1
Fruits and nuts	-		-
Sugar, confectionery and preserves	0.04		1
Beverages	0.16		4
Miscellaneous	0.04		1
<b>Total intake from food</b>	<b>4.19</b>		<b>100*</b>
<i>Intake from dietary supplements</i>	<i>0</i>		<i>0</i>
<b>Total intake from food and supplements</b>	<b>4.19</b>		<b>100</b>

\*Total allows for rounding

<sup>11</sup> NDNS: young people aged 4-18 years. 1997/8. 15-18 year group.

**Table 4: Vitamin B<sub>12</sub> intake from supplements**

<i>Age/sex</i>	<b>Consumers of vitamin B<sub>12</sub> supplements</b>		<b>Vitamin B<sub>12</sub> intake from supplements (consumers only) (µg/day)</b>		
	<i>Number</i>	<i>%</i>	<i>Mean</i>	<i>Median</i>	<i>Range</i>
<b><i>Infants (1986)</i></b> 6-12 mths/M&F	*	*	*	*	*
<b><i>Pre-school children (1992/3)</i></b> 1½-4½ yrs/M&F	28	2	1.3	1.0	0.2 - 2.5
<b><i>Young people (1997/8)</i></b> 4-6 yrs/M&F	15	4	0.8	0.5	0.1 – 2.5
7-10 yrs/M&F	12	2	0.8	0.8	0.1 – 2.0
11-14 yrs/M	4	2	0.6	0.4	0.1 – 1.0
11-14 yrs/F	5	2	1.8	1.7	0.1 – 4.6
15-18 yrs/M	5	3	1.8	1.4	0.7 – 2.9
15-18 yrs/F	7	3	1.6	0.7	0.1 – 5.0
<b><i>Adults (1986/7)</i></b> 16-64 yrs/M	24	2	4.4	1.9	0.1 – 50.0
16-64 yrs/F	45	4	3.7	2.0	0.1 – 50.0
<b><i>Older people free-living in the community (1994/5)</i></b> 65 and over/M	27	4	1.9	2.0	0.2 – 6.6
65 and over/F	31	5	2.3	2.0	0.0 – 6.3
<b><i>Older people living in institutions (1994/5)</i></b> 65 and over/M	2	1	0.6	0.1	0.1 – 1.4
65 and over/F	5	2	0.7	0.3	0.2 – 2.3

\* Data unavailable

## ANNEX 4 to EVM/00/20.REVISED SEPT2001

**Vitamin B12 : Summary table of selected nutrition related information and existing guidance on regulations**

Unit of usage	µg/day		µg/100 kcal
	Male	Female	
<i>UK DRV<sup>12</sup> for adults (19-50+)</i>			
LRNI	1.0	1.0	
EAR	1.25	1.25	
RNI	1.5	1.5	
<i>Mean adult UK dietary intake from food (all sources)</i>			
Adults (16-64) <sup>13</sup>	7.2 (7.3)	5.2 (5.4)	
65 years and over <sup>14</sup>			
free living	6.1 (6.1)	4.5 (4.6)	
institutionalised	4.9 (4.9)	4.6 (4.7)	
EU labelling RDA <sup>15</sup>	1		
Supplemental doses	5-1000		
<b>Regulations</b>			
Infant foods <sup>16</sup>			0.35
Weight reduction <sup>17</sup>			
whole daily diet replacement	1.4 per day		
meal replacement	0.42 per meal		
<i>Maximum total safe daily intake</i>			
COMA 1991 <sup>1</sup>	1000-3000		
EHPM 1997 <sup>18</sup>	3000		

<sup>12</sup> Committee on Medical Aspects of Food and Nutrition Policy (1991). Dietary Reference Values for Food Energy and Nutrients for the United Kingdom. Report on Health and Social Subjects 41. London: HMSO.

<sup>13</sup> Dietary and nutritional survey of British adults. 1986/7

<sup>14</sup> National Diet and Nutrition Survey of people aged 65 years and over. 1994/5

<sup>15</sup> The Food Labelling Regulations 1996

<sup>16</sup> The Processed Cereal-based Foods and Baby Foods for Infants and Young Children Regulations 1999 (amended)

<sup>17</sup> The Foods Intended for Use in Energy Restricted Diets for Weight Reduction Regulations 1997.

<sup>18</sup> Vitamins and Minerals A Scientific Evaluation of the Range of Safe Intakes. European Federation of Health Product Manufacturers 1997.

