CTD MODULE 2

2.4. NONCLINICAL OVERVIEW

CYANOCOBALAMIN

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ABBREVIATIONS

AUC	Area Under the plasma concentration vs. time Curve
B_{12}	vitamin B_{12} (cobalamin)
CAS	Chemical Abstract Service
CoA	Coenzyme A
CL	Clearance
C_{max}	peak plasma Concentration
EGF	Epidermal Growth Factor
EM(E)A	European Medicines Agency
IV	Intravenous(ly)
LD_{50}	Lethal Dose (50% kill)
MSDS	Material Safety Data Sheet
NOAEL	No Observed Adverse Effect Level
RDA	Recommended Dietary Allowance
THF	Tetrahydrofolate
T_{max}	Time to peak plasma concentration
TNF	Tumour Necrosis Factor
TS	Thymidylate Synthase
UL tolerable	Upper intake Level

2.4.1 Overview of the Nonclinical Testing Strategy

Vitamin B12 is a water-soluble vitamin, also called cobalamin. Cobalamin is the general term used to describe a group of cobalt-containing compounds (corrinoids) that have a particular structure that contains the sugar ribose, phosphate, and a base (5, 6-dimethyl benzimidazole) attached to the corrin ring.

Cyanocobalamin is the most common and widely produced form of the chemical compounds that have vitamin B12 activity. Vitamin B12 is the "generic descriptor" name for any of such vitamers of vitamin B12. Cyanocobalamin usually does not occur in living organisms, but humans, like all animals, can convert commercially produced cyanocobalamin into active (cofactor) forms of the vitamin, that are active in human metabolism: methylcobalamin and 5-deoxyadenosylcobalamin.

Vitamin B12 (cobalamin) functions as a coenzyme for a critical methyl transfer reaction that converts homocysteine to methionine and for a separate reaction that converts L-methylmalonylcoenzyme A (CoA) to succinyl-CoA.

The Recommended Dietary Allowance (RDA) for vitamin B12 is based on the amount needed for the maintenance of haematological status and normal serum vitamin B12 values. An assumed absorption of 50 percent is included in the recommended intake. The RDA for adults is 2.4 µg/day of vitamin B12. Because 10 to 30 percent of older people may be unable to absorb naturally occurring vitamin B12, it is advisable for those older than 50 years to meet their RDA mainly by consuming foods fortified with vitamin B12 or a vitamin B12-containing supplement. Individuals with vitamin B12 deficiency caused by a lack of intrinsic factor require medical treatment. The median intake of vitamin B12 from food in the Western World has been estimated to be approximately 5 µg/day for men and 3.5 µg/day for women. The ninety-fifth percentile of vitamin B12 intake from both food and supplements was approximately 27 µg/day. There is no sufficient scientific evidence to set a Tolerable Upper Intake Level (UL) for vitamin B12 at this time ([redacted]).

This non-clinical overview is based entirely on published scientific literature. Searches were carried out in bibliographic, and factual databases. Specific search criteria were used, adjusted to the specific database terminology, scope and structure, covering all aspects required for this overview. Primarily English language literature was selected initially on the basis of search results including abstracts, and subsequently on the basis of original publications acquired. Where necessary, reference lists of original publications were searched manually for complementary publications.

Cyanocobalamin is indicated for the treatment of haematological, neurological and other symptoms as a result of vitamin B12 deficiency; in malabsorption of vitamin B12, for example as a result of lack of intrinsic factor (pernicious anaemia), ventricular resection or small intestinal disease; and at aminosalicylic therapy which may impair B12 resorption.

The proposed contraindications, precautions and warnings applied to this formulation of cyanocobalamin are the same as those applied to all other preparations used in this indication on the market and are supported by the findings in the published literature.

This overview has been prepared as part of a marketing authorisation application to market a formulation of Cyanocobalamin 1 mg film-coated tablets.

2.4.2 Pharmacology

2.4.2.1 General Properties

Product Name: Cyanocobalamin 1 mg film-coated tablets

Active constituent:

International Non-proprietary Name (INN): Cyanocobalamin

Chemical Name: 5,6-dimethyl-benzimidazolyl cyanocobamide

Synonyms: Vitamin B12; 1H-benzimidazole, 5,6-dimethyl-1-(3-O-phosphonoalpha-d-ribofuranosyl)-, monoester with cobinamide cyanide hydroxide, inner salt; alpha-5,6-dimethyl-1H-benzimidazolyl-, cyanide

Molecular Formula: $C_{63}H_{88}Co\text{-}N_{14}O_{14}$

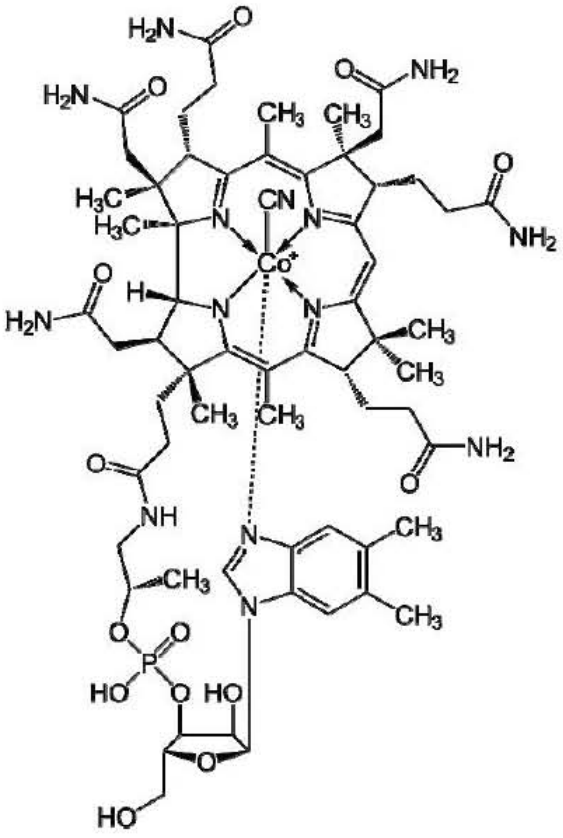
Molecular Weight: 1355.48

CAS Registry: 68-19-9

ATC: B03BA

Physical Properties: Dark red crystals or amorphous or crystalline red powder, odourless, hygroscopic. Soluble in alcohol, insoluble in acetone, chloroform, ether. Water solubility: 0.0384 mg/L at 25°C.

Structural Formula:



2.4.2.2 Pharmacodynamics

Vitamin B12 contains a cobalt-centered corrin nucleus and shows a complex structure. The term vitamin B12 includes all corrinoids qualitatively exhibiting the biological activity of cyanocobalamin.

The coenzymatically active forms of vitamin B12 are adenosylcobalamin and methylcobalamin, which are involved in two enzymatic reactions in human metabolism. One reaction requiring methylcobalamin is the remethylation of homocysteine to methionine catalyzed by the methionine synthase. In this reaction, 5-methyl tetrahydrofolic acid (5-methyl-FH) is involved as a methyl group donor (Fig. 1), whereas cobalamin is just intermediate acceptor of the methyl group. In cobalamin deficiency, methionine synthesis is impaired and homocysteine can accumulate. Furthermore, the methionine synthase reaction provides tetrahydrofolic acid (THF), which is the essential form for other folatedependent reactions.

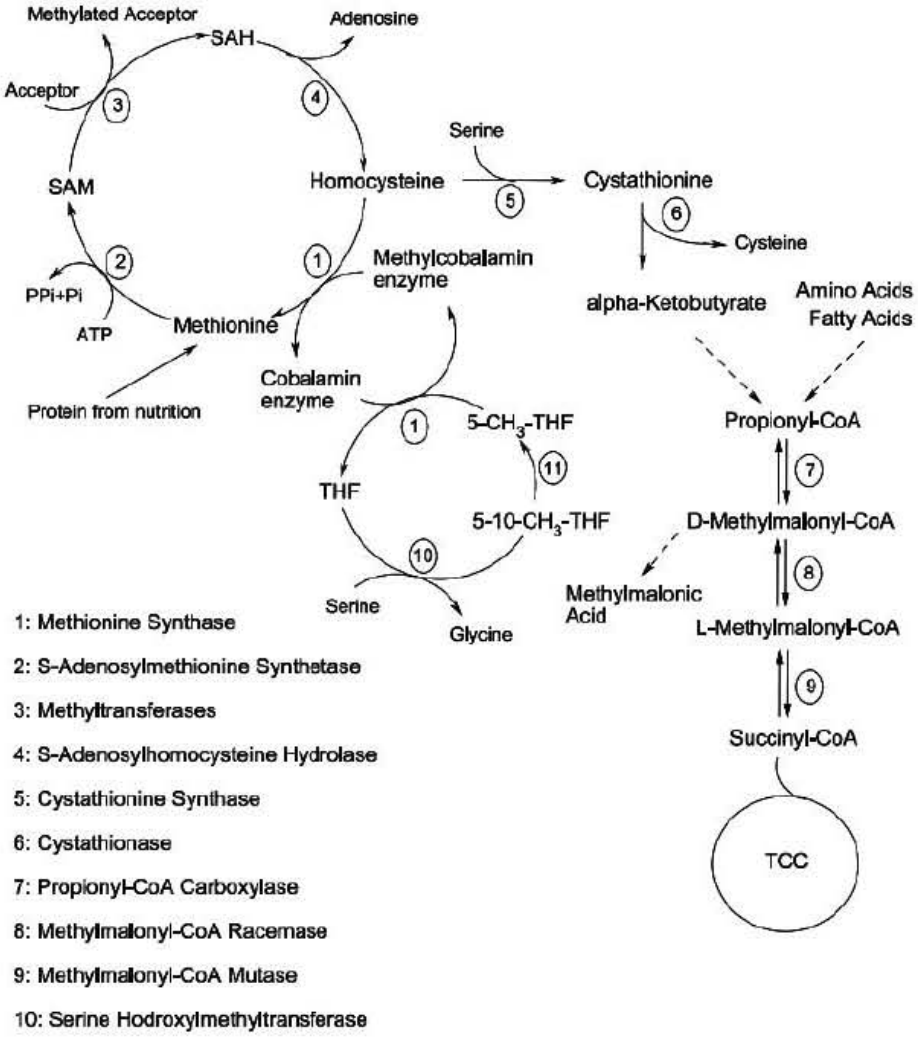


Fig. 1. Role of cobalamin in homocysteine metabolism. Abbreviations: SAH: Sadenosylhomocysteine; SAM: S-adenosylmethionine; THF: tetrahydrofolate; 5-CH3-THF: 5-methyl tetrahydrofolic acid; TCC: tricarboxylic acid cycle (after Wolters et al, 2004).

a) Methionine synthase

In bacteria, methionine synthase catalyzes the terminal step in the de novo biosynthesis of methionine. In mammals, however, methionine is an essential amino acid and so the enzyme has a quite different role. The enzyme functions to recycle homocysteine (forming methionine) and to liberate H_4folate from CH_3-H_4folate, which is the circulating form of the vitamin that is delivered from the bloodstream to the cells. H_4folate is required for purine, pyrimidine and amino-acid biosynthesis. Methionine synthase catalyzes successive transmethylation reactions in which a methyl group is transferred from a tertiary amine, CH_3-H_4folate, to cob(I)alamin, and then to the thiolate of homocysteine to generate H_4folate and methionine [redacted] .

b) Methylmalonyl-CoA mutase

In mammals, methylmalonyl-CoA mutase is a mitochondrial matrix enzyme that converts methylmalonyl-CoA to succinyl-CoA in catabolic pathways leading from branched-chain amino acids, odd-chain fatty acids, and cholesterol. Impaired functioning of methylmalonyl-CoA mutase either caused by genetic defects or induced by cobalamin deficiency leads to methylmalonic acidaemia in which precursors and abnormal metabolites of methylmalonyl-CoA accumulate. Depending on the severity of the condition, the clinical consequences range from benign to neonatal death [redacted] .

Cobalamins are exclusively synthesized by bacteria. Rich sources of cobalamin are of animal origin (e.g. meat, egg, liver).

Causes of Cobalamin Deficiency

a) Dietary deficiency

The average daily requirement for dietary cobalamin is about 2 to 5 µg in humans. More than 2000 µg of cobalamin is normally stored in the human body. Since dietary intake of cobalamin is usually more than 20 µg/day, dietary cobalamin deficiency is very rare. Even if dietary cobalamin deficiency occurs, it requires a few years to develop clinical cobalamin deficiency. cobalamin deficiency. Dietary cobalamin deficiency frequently occurs in strict vegetarians who do not take any animal products.

b) Gastric lesion

In pernicious anaemia the fundamental lesion is the impaired production or inactivation of intrinsic factor. Other causes are: inherited intrinsic factor deficiency and Imerslund-Gräsbeck's disease, total gastrectomy.

c) Intestinal lesion

Cobalamin malabsorption is common in patients with various types of pancreatic exocrine insufficiency such as chronic pancreatitis, cystic fibrosis, and pancreatectomy. Persons infected with Diphyllobothrium latum, a parasite of freshwater fish, may develop cobalamin malabsorption.

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d) Others

Inhalation of nitrous oxide.

Pathology of Cobalamin Deficiency

Cobalamin deficiency causes megaloblastic anaemia and neurocognitive abnormalities but effects on immune function and bone formation have also been described.

Megaloblastic anaemia likely reflects impaired thymidylic acid synthesis and misincorporation of uracil into DNA in haematopoietic precursors. Pernicious anaemia is a classic cause of cobalamin deficiency. Pernicious anaemia is an autoimmune disease characterized by the destruction of the gastric mucosa.Gastric secretions contain little or no intrinsic factor. About 50% of patients with pernicious anaemia have anti-intrinsic factor antibodies.

The spectrum of neurocognitive abnormalities in cobalamin deficiency is broad and the findings on MRI and electrophysiologic examinations are diverse. Moreover, neurologic changes often occur in the absence of haematologic abnormalities. Cobalamin-deficient central neuropathy in the rat is associated with a locally increased expression of neurotoxic tumour necrosis factor-alpha (TNF-α) and a locally decreased expression of neurotrophic epidermal growth factor (EGF). These findings suggest that cobalamin oppositely regulates the expression of TNF-α and EGF, and raise the possibility that these effects might be independent of its coenzyme function. Furthermore, adult cobalamin-deficient patients have high levels of TNF-α and low levels of EGF in the serum and cerebrospinal fluid. Serum levels of TNF-α and EGF of cobalamin-treated patients normalize concomitantly with haematological disease remission. These observations suggest that cobalamin deficiency induces an imbalance in TNF-α and EGF levels in biological fluids that might have a role in the pathogenesis of the damage caused by pernicious anaemia [redacted]

An increased incidence of tuberculosis in vegetarians, impaired antibody responses to pneumococcal vaccine in elderly patients with low cobalamin levels, and abnormal lymphocyte subpopulations in cobalamin-deficient subjects with megaloblastic anaemia suggest a role for cobalamin in immune function.

Low serum cobalamin levels increase the risk of osteoporosis.

Safety Pharmacology

No formal safety pharmacology tests have been performed with cyanocobalamin.

Pharmacodynamic Interactions

Cobalamin-folate biochemistry is interrelated. Tetrahydrofolate (THF) is required for the activity of thymidylate synthase (TS) and DNA synthesis. Other than folate precursor deficiency itself, also cobalamin deficiency contributes to functional intracellular deficiency of THF. Cobalamin indeed is required by methionine synthetase for the generation of methionine from homocysteine; meanwhile, THF is generated from 5-methyl THF. In fact, only THF can ultimately act as a coenzyme of the TS. As a consequence of the deficient TS activity, the synthesis of deoxythymidine monophosphate (dTMP) and deoxythymidine triphosphate (dTTP) from deoxyuridine monophosphate (dUMP) via thymidylate synthase is impaired and dUMP (and, eventually, deoxyuridine triphosphate (dUTP)) accumulates. Since

DNA-polymerase does not distinguish dUTP from dTTP, increased amounts of dUTP are misincorporated into DNA. Appropriate DNArepair enzymes (DNA uracil glycosylase) recognize the faulty incorporation and excise dUTP, but, lacking an adequate dTTP supply, effective repair does not occur, leading to DNA strands breaking. Megaloblastic changes due to cobalamin or folate deficiency are clinically indistinguishable. The cause of cobalamin deficiency is not generally revealed until specific laboratory tests are done; on the contrary, the recent patient's history may give clues to the possible folate deficiency. Likely due to the widespread folate supplementation in Western countries, the haematologic picture of cobalamin deficiency is often attenuated, and neurological presentations may become more common and overlooked. It is currently not generally accepted that folate deficiency may induce neurological manifestations, so that the occurrence of neurological symptoms in the presence of folate deficiency should prompt investigations aimed at ruling out cobalamin deficiency.

The effect of a vitamin B_{12} and folic acid deficient diet on juvenile and adolescent baboons (Papio cynocephalus anubis) was studied [redacted], [redacted] The baboons developed clinical and haematological signs characteristic of folacin deficiency, although they were less severe in juvenile baboons. The signs disappeared when folic acid was replaced in the diet. The serum vitamin B_{12} levels increased in all baboons fed the vitamin B_{12} and folic acid deficient diet. When folic acid was added to the diet, the levels gradually decreased in adolescent baboons, but continued to increase in juvenile baboons. In adolescent baboons, liver vitamin B_{12} levels decreased to a lesser extent when fed a vitamin B_{12} and folic acid deficient diet than when fed a vitamin B_{12}-deficient diet. In juvenile baboons fed a vitamin B_{12} and folic acid deficient diet, for 7 months and a vitamin B_{12}-deficient diet for a further 11 months, liver vitamin B_{12} levels did not decrease at any time but were similar to those in baboons fed a vitamin B_{12} and folic acid supplemented diet.

Vitamin B_{12}-deficient subjects exhibit losses of this functional form of folate since 5-methyl-FH4 is accumulated via the methyl-folate trap. This explains why many symptoms of cobalamin deficiency are similar to folate deficiency.

2.4.3 Pharmacokinetics

The absorption of cyanocobalamin (vitamin B12) from the intestinal tract of man and other animals has been studied by a variety of indirect methods.

Absorption

Small amounts of cobalamin are absorbed via an active process that requires an intact stomach, intrinsic factor (a glycoprotein that the parietal cells of the stomach secrete after being stimulated by food), pancreatic sufficiency, and a normally functioning terminal ileum. In the stomach, food-bound cobalamin is dissociated from proteins in the presence of acid and pepsin.

Vitamin B12 was administered subcutaneously to groups of normal rats at dosages ranging from 10 to 0.31 µg ([redacted]). It was found that at the higher levels the recovery of this vitamin in the urine was almost quantitative. As the dosage decreased, the recovery became poorer. At low levels, no increase of microbiological activity over the basal excretion was observed. The data taken as a whole suggest that the tissues of the body retained about 1 or 2 ng of the vitamin per rat. A large fraction of orally administered vitamin B12 appeared in the faeces; urinary excretion was very low, indicating poor absorption. Nevertheless, some absorption must have taken place, since oral feeding of large doses to dogs was followed not only by appearance of activity in the urine but by occurrence of demonstrable blood levels.

[redacted]) studied the vitamin B_{12} activity in portal and peripheral blood of dogs following oral administration of 1.5 µg/kg cyanocobalamin. In the post-absorptive state, plasma vitamin B_{12} activity of dogs is the same in portal and peripheral blood plasma, but portal plasma contains greater amounts of activity than does peripheral plasma during active absorption of the vitamin from the intestinal tract. Crystalline vitamin B12 placed in ligated segments of the duodenum or in the unligated duodenum of dogs results in the rapid appearance of vitamin B_{12} activity in the plasma of portal and peripheral blood. During absorption, small but definite amounts of vitamin activity also appear in the urine. In dogs subjected to operation but administered saline in place of vitamin B12, plasma and urine activity remained constant. The plasma vitamin B12 activity of dogs with stomachs ligated at the duodenal cap remained essentially constant following the administration of vitamin. The major portion of vitamin B_{12} administered by intravenous injection, in an amount approximately that found in plasma during oral absorption, results in a rapid elimination of the vitamin in the urine.

When normal rats were fed 0.005 µg of vitamin B_{12} labelled with 60Co the average amount excreted in the faeces was 66.-% of the dose. After the same dose of labelled vitamin B_{12} gastrectomised rats excreted an average of 93.8% of the dose. When given an extract of rat stomach along with the vitamin B_{12} the gastrectomised rats excreted an average of 69.5% of the dose. A dialysed filtrate of pig pyloric juice did not consistently reduce the amount of vitamin B_{12} excreted ([redacted]).

Radioactive vitamin B12, has been given orally to normal and gastrectomized rats and the absorption has been determined from estimation of faecal radioactivity ([redacted]). The relation between absorbed and ingested weights of the vitamin has been studied in normal animals. Over the range studied the percentage weight absorbed did not remain

constant; with doses of 5 µg or less there was no absorption at all. For a constant dose of 16 µg vitamin B12 normal rats showed a mean absorption of 42.9 ± 1.0%. In gastrectomized animals it was reduced to 5.5 ± 1.1%. Rat gastric juice given simultaneously with the vitamin produced no change in the absorption in normal rats, but raised that of the gastrectomized rats to 15.9 ± 1.2%. Though this increase was highly significant, it was not possible to increase the absorption to normal levels with rat gastric juice administered orally. Human gastric juice depressed the absorption in normal rats to 25.4 ± 2.5% and in gastrectomized rats to 1.5 ± 0.5%. A pig intrinsic-factor preparation did not affect the absorption when given to the gastrectomized animal.

No data are available on whether B_{12} absorption varies with B12 status, but fractional absorption decreases as the oral dose is increased. Total absorption increases with increasing intake. [redacted] measured fractional absorption of radiolabeled cyanocobalamin and reported that nearly 50% was retained at a 1 µg dose, 20% at a 5 µg dose, and just over 5% at a 25 µg dose. The second of two doses of B12 given 4 to 6 hours apart is absorbed as well as the first ([redacted]. When large doses of crystalline B12 are ingested, up to approximately 1% of the dose may be absorbed by mass action even in the absence of intrinsic factor ([redacted] & [redacted]).

B_{12} is continually secreted in the bile. In healthy individuals most of this B_{12} is reabsorbed and available for metabolic functions. [redacted] demonstrated that the secretion of B12 into the bile averaged 1.0 ± 0.44 nmol/day (1.4 µg/day) in eight cholecystectomized patients, and this represented 55 percent of total corrinoids. If approximately 50 percent of this B12 is assumed to be reabsorbed, the average loss of biliary B12 in the stool would be 0.5 nmol/day (0.7 µg/day). Research with baboons ([redacted] suggests that the form of B12 present in bile may be absorbed more readily than is cyanocobalamin, but the absorption of both forms was enhanced by intrinsic factor. [redacted]) reported data suggesting that bile enhances B12 absorption. However, in the absence of intrinsic factor, essentially all the B12 from the bile is excreted in the stool rather than recirculated. Thus, B12 deficiency develops more rapidly in individuals who have no intrinsic factor or who malabsorb B12 for other reasons than it does in those who become complete vegetarians and thus ingest no B12.

[redacted] estimated the bioavailability of dietary vitamin B12 in growing pigs. Two approaches, each using 2 quantities of dietary cyanocobalamin, were compared; the first was based on whole body retention for 8 d and the second was based on nycthemeral portal net flux of vitamin B12. In the first trial, 15 blocks of 3 pigs (31.7 ± 0.5 kg of BW) were formed according to their vitamin B12 status. Within each block, 1 pig (CONT) was killed and tissues were sampled for vitamin B12 determination. The remaining 2 piglets were fed 25 (B12-25) or 250 (B12-250) µg daily of cyanocobalamin for 8 d. Urine was sampled twice daily, and the pigs were killed and sampled as CONT pigs. The total content of vitamin B12 in the carcass, urine, and intestinal tract was affected by the dietary treatments ($P < 0.01$) but not in the liver ($P > 0.019$). The whole body retention of vitamin B(12) was greater ($P = 0.02$) in B12-250 than B12-25 pigs, but the corresponding bioavailability was estimated to be 5.3 and 38.2%, respectively. In trial 2, 11 pigs (35.1 ± 4.0 kg of BW and 75.4 ± 5.9 d of age) fed a diet unsupplemented with vitamin B12 from weaning at 28 d of age were surgically equipped with catheters in the portal vein and carotid artery and an ultrasonic flow probe around the portal vein. Each pig received 3 boluses of 0 (B12-0), 25, and 250 µg of dietary vitamin B12 according to a crossover design.

Postprandial nycthemeral arterial plasma concentrations of vitamin B12 reached minimum values ($P < 0.01$) between 15 and 18 h postmeal that were 29.6, 15.6, and 10.0% less than the premeal values for B12-0, B12-25, and B12-250 pigs, respectively (linear, $P < 0.01$). The cumulative net flux of vitamin B12 for 24 h corresponded to 2.4 and 5.1 µg for B12-25 and B12-250 treatments, respectively, and the corresponding bioavailability was estimated to be 9.7 and 2.0%, respectively. Although bioavailability estimates varied according to approaches, both showed the inverse relationship between dietary vitamin B12 and bioavailability of the vitamin. The dietary supplement of 25 µg was sufficient to maximize hepatic vitamin B12 retention and to attenuate the nycthemeral decrease of arterial plasma concentration of the vitamin.

The absorption of vitamin B12 from colon is questionable. [redacted]) investigated the absorption of B12 from the large intestine of rats. Labelled vitamin B12 (17 to 180 of µg 57Co- or 60Co-labeled vitamin B12 corresponding to 15,000 to 400,000 count/min) was injected into the colon ascendens or coecum of 104 rats (3 groups). The animals were killed at different time intervals (from 20 minutes to 29 days) and the tissue radioactivity was determined in a well-type scintillation counter. The total uptake, calculated for each animal, was expressed as percentage of the injected dose; large intestine (injection site) radioactivity was excluded. Thirty rats fed a stock diet containing non-labelled vitamin B12 (subdivided in 2 series) showed an average total uptake of 2.9% (range zero to 17.3) and of 2.0% of the injected dose (range 0.9 to 4.3), respectively. Animals fed vitamin B12-deficient diets showed a higher uptake of the labelled vitamin. In one series of 24 rats a total uptake of 8.3% of the injected dose (range 2.2 to 17.7) was obtained. Thirteen rats of a similar experiment showed an uptake of 5.0% of the injected dose (1.3 to 13.4) and 12 rats of 2 additional series showed an uptake of 6.8% (range 4.8 to 9.6) and 11.4% of the injected dose (range 4.0 to 17.3), respectively. The mechanism of the uptake of the radiovitamin was studied in the third group of 25 animals (series 7). In 23 of these rats the passage at the ileocoecal valve was occluded by ligation prior to injection. The average uptake was similar to that obtained in a parallel series. No evidence for leakage of the injected vitamin, either intraperitoneally or into the small intestine (by anti-peristaltic movement), could be found. It appears therefore that the labelled vitamin was absorbed from the large intestine, perhaps by nonspecific passive diffusion. Similarly, vitamin B12 synthesized in the large intestine by microbial flora could gain access to the tissues by such a mechanism. In humans, however, in neither the presence nor the absence of intrinsic factor are physiological doses of vitamin B12 absorbed from the colon [redacted] .

Distribution

Following IV infusion of 2.5, 5, 7.5 and 10 g of hydroxocobalamin at a constant rate of approximately 1 g/3 minutes, free cobalamin-(III) reached maximum concentrations generally at the end of the infusion. Fast complexation of hydroxocobalamin with plasma proteins is suggested by the findings of the study as Tmax observed for the free-cobalamins-(III) is very close to that observed with total cobalamins-(III). The volume of distribution at steady-state (Vss) for both free and total cobalamins-(III) is not dependent upon the administrated dose. Vss ranged from 280.7 up to 349.5 L for the free cobalamins-(III) and from 21.8 up to 25.6 L for total cobalamins-(III). This could be explained by the rapid distribution of free-cobalamins-(III) into tissues [redacted]

Metabolism

Ingested cobalamin is bound by gastric intrinsic factor, which facilitates cobalamin absorption in the distal small intestine. The subsequent plasma transport of cobalamin is mediated by transcobalamin II, which delivers cobalamin to various tissues, and by a granulocyte derived R-type cobalamin-binding protein, which delivers cobalamin exclusively to hepatocytes vie a mechanism that is enable of clearing asialoglycoproteins. Transcobalamin II-cobalamin and granulocyte R-type protein-cobalamin bind to cell surface receptors and are internalized by pinocytosis. Their protein moieties are then degraded after the pinocytotic vesicles fuse with lysosomes The liberated cobalamin is subsequently involved in several events which include: (a)return of cobalamin to the plasma, (b)intracellular retention of cobalamin and Its binding to an undefined intracellular cobalamin-binding protein (ICB), which has a molecular weight of greater than 100 kD and is immunologically distinct from transcobalamin II and granulocyte Rtype cobalamin-binding protein, and (c) a two-step reduction of the cobalt moiety of cobalamin and the subsequent conversion of cobalamin to either d'-deoxyadenosylcobalamin or to methylcobalamin.

After the release of cobalamin from the lysosome into the cytosol, the cobalt undergoes reduction (cob (III)alamin ? cob(I)alamin) followed by methylation using methionine synthase or by adenosylation in the mitochondrion. A number of genetic defects with variable deficiencies in these intracellular cobalamin processing steps comprise eight complementation groups (cblA-cblH), e.g. resulting in isolated methylmalonic aciduria or homocystinuria [redacted] .

Elimination

If the circulating B12 exceeds the B12 binding capacity of the blood, the excess is excreted in the urine. This typically occurs only after injection of B12. The highest losses of B12 ordinarily occur through the faeces. Sources of faecal B12 include unabsorbed B12 from food or bile, desquamated cells, gastric and intestinal secretions, and B12 synthesized by bacteria in the colon. Other losses occur through the skin and metabolic reactions. Faecal and urinary losses [redacted]) decrease when B12 stores decrease. Various studies have indicated losses of 0.1 to 0.2 percent of the B12 pool per day [redacted] regardless of the size of the store, with the 0.2 percent value generally applicable to those with pernicious anaemia.

Following IV injection of hydroxocobalamin, cobalamins (free and total) are slowly eliminated from plasma as the apparent plasma elimination half-live is approximately 30 h. The Cmax and AUC of both free and total cobalamins evolve proportionally to the dose over the studied range (2.5 up to 10 g). Cmax values ranged from 73.1 up to 197.2 µg eq/ml for free-cobalamins-(III) and from 287.6 up to 995.3 µg eq/ml for total cobalamins(III). AUC0-t values ranged from 188.4 up to 762.5 µg eq/ml·h for free cobalamins-(III) and 3566 up to 14271.5 µg eq/ml·h.

Estimated from AUC ratios, the systemic exposure to free cobalamins-(III) is approximately 5 % that of total cobalamins-(III). Kidney is a major route of elimination as up to 74 % of the administered dose is recovered in the urine. The total body clearance (CL) of free cobalamins-(III) ranged from 12.5 up to 13.2 L/h, which exceeds the normal glomerular filtration rate (approximately 4.8 up to 7.9 L/h). This high clearance may be due to the extensive binding of

free cobalamins to plasma proteins. Clearance of total cobalamins-(III) ranged from 0.566 to 0.645 L/h across all doses [redacted]

Pharmacokinetic Interactions

No formal pharmacokinetic interaction studies have been performed.

Absorption may be reduced by para-aminosalicylic acid, colchicine, biguanides, neomycin, cholestyramine, potassium chloride, methyldopa, and drugs decreasing gastric acid output (e.g. H2-blockers, proton pump inhibitors).

2.4.4 Toxicology

In humans vitamin B12 is usually nontoxic even in large doses; however, mild transient diarrhoea, peripheral vascular thrombosis, itching, transitory exanthema, urticaria, feeling of swelling of the entire body, anaphylaxis, and death have been reported. Although allergic reactions to vitamin B12 have generally been attributed to impurities in the preparation, a few patients have reacted positively to skin testing with purified cyanocobalamin.

2.4.4.1 Acute Toxicity

The oral LD50 of cyanocobalamin is >5 mg/kg in the mouse ([redacted]). In rats, the approximate LD50 of hydroxocobalamin ranged from 720 to 1200 mg/kg by intraperitoneal route. In dogs, the toxicity of hydroxocobalamin was studied by IV route after administration of a single dose (150, 300 and 1200 mg/kg). Clinical signs included red coloured urine, skin and mucous membranes and subcutaneous oedema around the head. It was considered unlikely that the subcutaneous oedema could be due to an immediate allergic reaction mediated by histamine release ([redacted]).

2.4.4.2 Repeat Dose Toxicity

In dogs, in addition to single doses (150, 300 and 1200 mg/kg), the toxicity of hydroxocobalamin was studied by IV route after administration of repeated doses (300, 600 and 1200 mg/kg/day for 3 days – 75, 150 and 300 mg/kg/day for 4 weeks). Platelet count was decreased in all these studies. Liver and kidneys were the main target organs. At the biochemistry level, liver enzymes (ALT, AST, ALP) were increased in all the studies but returned to baseline levels after withdrawal of the treatment. At the histopathological level, changes were attributed to an overload phenomenon. They occurred at ≥300 mg/kg and at ≥75 mg/kg in the single dose study and in repeat-dose studies, respectively, and were possibly associated with reactive and degenerative changes mainly in repeat-dose studies. Other kidney findings observed in the single dose study at 1200 mg/kg and in the 3-day study at ≥ 600 mg/kg were related to the redistribution of plasma water from the intravascular to the extravascular space occurring after hydroxocobalamin administration. This mechanism was also probably involved in the decrease in platelet count.

Single cell necroses, affecting mainly macrophages, were observed at all dose levels in the bone marrow. However, they did not impact either the cellularity of the bone marrow, or the bone marrow functioning. Moreover, histopathological examination in the 4-week toxicity study showed a trend towards recovery in terms of incidence and severity. Adverse effects were observed in other organs/tissues in 3-day and 4-week studies. In the 3-day toxicity study, heart alterations occurring at the top dose of 1200 mg/kg/day and spleen alterations observed at 600 and 1200 mg/kg/day were attributed to redistribution of plasma water. In the 4-week study, heart and spleen findings were also observed and were considered to result from a non specific inflammatory reaction and from the overload phenomenon, respectively. Taking into consideration the nature and reversibility of the effects reported in the single dose study, a single dose of 300 mg/kg is considered as well tolerated in dogs. With the exception of liver fibrosis reported at 300 mg/kg in the 4-week study, all the treatment-related findings observed in repeat-dose studies were either fully reversible or showed a trend to recovery after 8 weeks of treatment-free period. Liver fibrosis likely results from the inflammatory

reaction reported after 4-week treatment and as a sequel of the observed sinusoid oedema related to redistribution of plasma water and overload phenomenon.

In view of the nature of the adverse effects reported at ≤150 mg/kg/day, of their full or on-going reversibility after a 8-week recovery period, the dose of 150 mg/kg/day can be considered as a NOAEL.

Cyanocobalamin was administered intravenously to dogs for 2 weeks (40, 100 and 400 mg/kg/day). Kidney findings consistent with an overload phenomenon, and bone marrow finding similar to those reported with hydroxocobalamin were observed at the high dose level. Considering this NOAEL in dogs and cyanocobalamin levels in intoxicated victims treated with hydroxocobalamin, safety factors based on Cmax ranged form 1 to 3 [redacted] .

2.4.4.3 Genotoxicity

Hydroxocobalamin was non-genotoxic in a standard battery of in vitro (Ames tests, TK mouse lymphoma assays) and in vivo (rat micronucleus) tests [redacted] . The are no data on the mutagenic activity of cyanocobalamin.

2.4.4.4 Carcinogenicity

No carcinogenicity studies have been performed. Early studies have shown a procarcinogenic effect of vitamin B12. Vitamin B12 has been found to enhance markedly the carcinogenic effect of p-dimethylaminoazobenzene in rats receiving a methionine-deficient diet. However, a control group of rats receiving this diet without p-dimethylaminoazobenzene showed no hepatic tumours [redacted] .

Cobalamin is not considered to be a carcinogen by IARC.

2.4.4.5 Reproductive and Developmental Toxicity

During pregnancy cobalamin is essential, presumably because of its role in DNA synthesis and methionine synthesis; however, there are conflicting studies regarding an association between early pregnancy loss and cobalamin deficiency [redacted]).

Only limited embryo-foetal toxicity studies are available in rats and rabbits, and do not allow to draw any clear conclusion on the embryotoxic potential of cobalamin. However, it should be noted that no teratogenic effect was reported in both species with hydroxocobalamin [redacted] . No studies on fertility and on peri/postnatal development are available.

2.4.4.6 Local Tolerance

No local (injection site) intolerance problems have been reported in clinical practice.

2.4.4.7 Other Toxicity Studies

Hydroxocobalamin was tested for phototoxic potential in vitro in Balb/c 3T3 fibroblasts using the Neutral Red uptake assay. Concentrations of 0.316 to 1000µg/mL were used in the presence and absence of UV-A irradiation. There were no significant increases in Neutral Red uptake in the presence than in the absence of UV-A. Therefore, hydroxocobalamin is not considered to have a phototoxic potential. Although no phototoxicity studies have been performed with cyanocobalamin, the clinical experience suggests that the risk of phototoxicity in also unlikely [redacted]).

2.4.4.8 Ecotoxicity

This generic formulation of cyanocobalamin poses no additional environmental risk. As a vitamin, cyanocobalamin is unlikely to result in a significant risk to the environment.

2.4.4.9 Toxicology of Components of the Formulation

The dosage form excipients are approved and established agents in widespread use in the pharmaceutical manufacturing industry.

2.4.5 Integrated Overview and Conclusions

[redacted]

The above texts are redacted under sections 41 and 43 of the FOI Act

2.4.6 Literature References

Module	Number	Reference
4	1.	[redacted]
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The references are redacted under sections 41 and 43 of the FOI Act