CTD MODULE 2

2.5. CLINICAL OVERVIEW

CYANOCOBALAMIN 1 MG FILM-COATED TABLETS

Author: [redacted]

Redacted under section 40 of the FOI Act

Document status: final

Date: 11 September 2015

Number of pages: 36

TABLE OF CONTENTS

ABBREVIATIONS

AUC	Area Under the plasma concentration-time Curve
BDDCS	Biopharmaceutics Drug Disposition Classification System
BCS	Biopharmaceutics Classification System
CoA	Coenzyme A
CI	Confidence Interval
CV	Coefficient of Variation
EM(E)A	European Medicines Agency
FDA	Food and Drug Administration
HC	Homocysteine
HoloTC	Holotranscobalamin
IF	Intrinsic Factor
IM	Intramuscular(ly)
IV	Intravenous(ly)
Ke	elimination constant
MMA	Methylmalonic Acid
MMSE	Mini-Mental State Examination
OR	Odds Ratio
PO	Per Os
RCT	Randomised Controlled Trial
RDA	Recommended Dietary Allowance
SD	Standard Deviation
Tmax	Time to peak plasma concentration
t1/2	elimination half-life
THF	Tetrahydrofolate
UL	tolerable Upper intake Level

2.5.1. Product Development Rationale

Cyanocobalamin is the most common and widely produced form of the chemical compounds that have vitamin B12 activity. Cyanocobalamin usually does not occur in living organisms, but animals can convert commercially produced cyanocobalamin into active (cofactor) forms of the vitamin, such as 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 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. Cobalamin deficiency causes megaloblastic anaemia and neurocognitive abnormalities but effects on immune function and bone formation have also been described.

This 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. Websites of EMA and FDA were also searched for relevant data.

Cyanocobalamin is indicated for the treatment of haematological, neurological and other symptoms as a result of vitamin B12 deficiency. Malabsorption of vitamin B12, for example as a result of lack of intrinsic factor (pernicious anaemia), ventricular resection or small intestinal disease. Also indicated at aminosalicylic (PAS) 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.

2.5.2. Overview of Biopharmaceutics

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

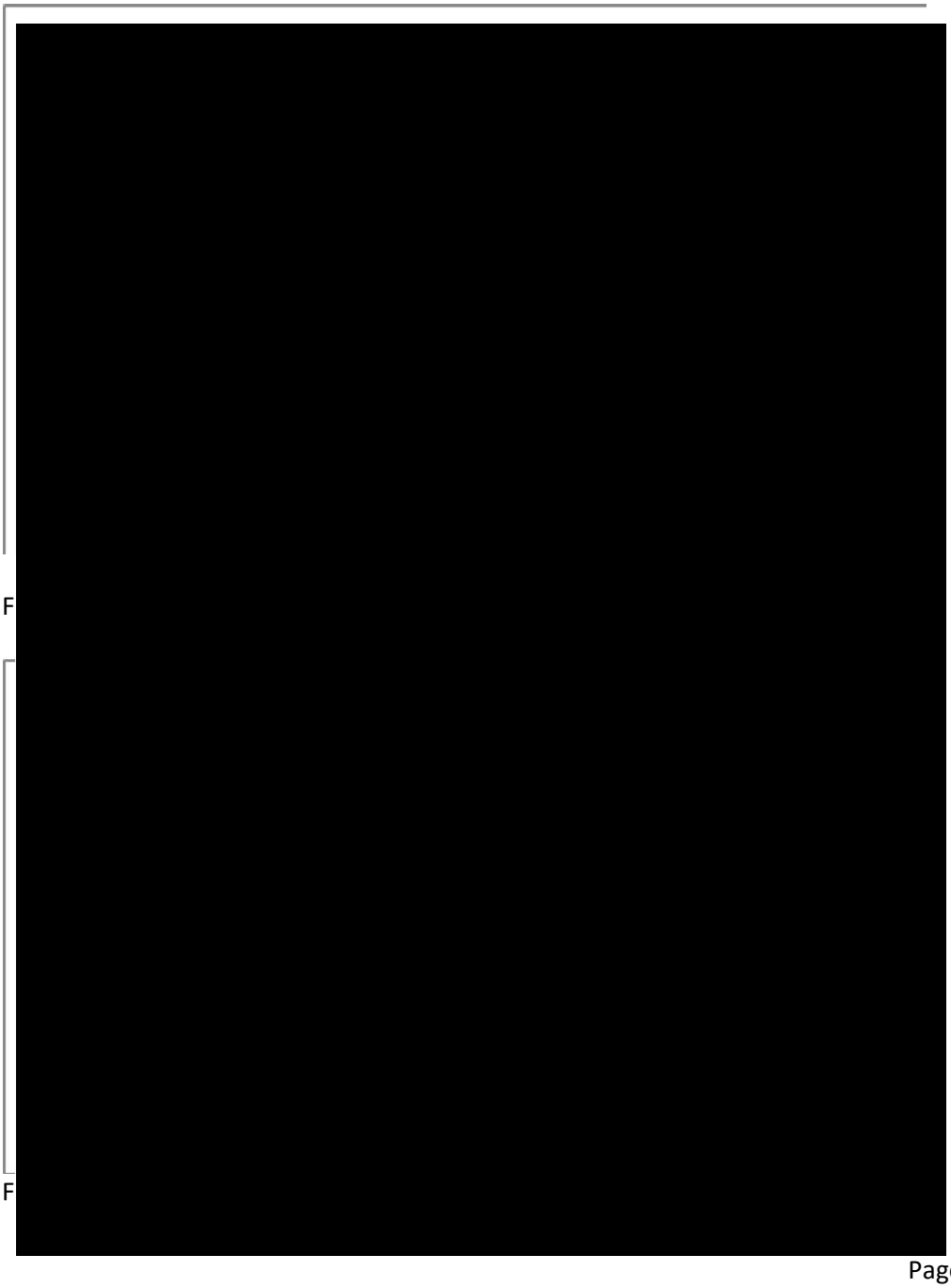
[redacted]

[redacted]

[redacted]

[redacted]

Redacted under sections 41 and 43 of the FOI Act



[redacted]

[redacted]

[redacted]

Redacted under sections 41 and 43 of the FOI Act

2.5.3. Overview of Clinical Pharmacology

2.5.3.1. Pharmacokinetics

Absorption

The whole body retention of radioactive cyanocobalamin. coenzyme B12, methylcobalamin, and hydroxocobalamin was measured by whole body monitoring after oral doses of 1, 5 and 25 µg. At each dose level there were significant differences between the values for whole body retention of the different cobalamins (Table 2) [redacted].

Table 2. Mean values and (in parentheses), standard deviations of percentages of doses retained by each group of patients at each dose level [redacted]

Oral dose	Number of subjects	Percentage retained from dose of: ^{58}Co cyanocobalamin	^{57}Co coenzyme B_{12}	^{58}Co methylcobalamin	^{57}Co hydroxocobalamin
1 µg	12	49.2(14.9)	33.7(11.1)	–	–
1 µg	10	–	–	44.4(10.0)	55.7(11.3)
5 µg	10	20.4(8.8)	12.9(10.7)	–	–
5 µg	10	–	–	18.8(7.1)	16.3(5.7)
25 µg	10	5.6(2.2)	7.9(3.6)	–	–
25 µg	11	–	–	6.1(2.3)	7.4(2.2)

[redacted] compared the pharmacokinetics of 2 oral formulations of cyanocobalamin -a marketed cyanocobalamin tablet (immediate-release B12 5 mg) and cyanocobalamin formulated with a proprietary carrier, sodium N-[8-(2-hydroxybenzoyl)amino]caprylate (SNAC)-to establish the feasibility of using an absorption enhancer with B12 to improve uptake of the vitamin. An open-label, randomized, single-dose, parallel-group study was conducted in healthy male subjects. Subjects were randomly assigned to 1 of 4 treatment groups: Treatment A subjects (n = 4) received 2 tablets of 5-mg cyanocobalamin formulated with 100-mg SNAC as part of a dose range-finding arm included to determine a dose to provide a measurable concentration of vitamin B12 at all time points when tested with the available vitamin B12 assay; treatment B subjects (n = 6) received 1 tablet of 5-mg cyanocobalamin formulated with 100-mg SNAC; treatment C subjects (n = 6) received 1 commercially available 5-mg cyanocobalamin tablet; and treatment D subjects (n = 4) received commercially available 1-mg cyanocobalamin IV. Treatment A was completed 3 weeks before treatments B, C, and D were studied. Human serum B12 was analyzed by chemiluminescence assay method. Validation procedures established that samples could be diluted up to 100 times without any effects on accuracy and precision. The pharmacokinetic properties of vitamin B12 were characterized by non-compartmental analysis. Vitamin B12 absolute bioavailability estimates were calculated between the oral (A, B, and C) and IV (D) treatments using non baseline-adjusted vitamin B12 concentrations as well as baseline-adjusted vitamin B12 concentrations, with or without body weight adjustments (Table 3). Twenty healthy male subjects, aged 20 to 45 years, participated in this study. Based on data from treatment A, a 5-mg cyanocobalamin dose was selected for use with treatments B and C. The oral cyanocobalamin formulation containing SNAC had greater mean absolute bioavailability than the commercial oral

Redacted under sections 41 and 43 of the FOI Act

formulation (5.09% vs. 2.16%, respectively), calculated on AUC0-last values uncorrected for baseline, weight, or body mass index. It also had a reduced Tmax compared with the commercial formulation (0.5 hours vs. 6.83 hours, respectively). The Ke was similar between treatments (0.028 1/h vs. 0.025 1/h). Comparable results were achieved using corrected values.

Table 3. Pharmacokinetic parameters of non–baseline-corrected serum cyanocobalamin following treatments A, B, C, and D ([redacted]).

Pharmacokinetic Parameters	Treatment A*		Treatment B†		Treatment C‡		Treatment D§	
	Mean (SD)	n	Mean (SD)	n	Mean (SD)	n	Mean (SD)	n
C_{max}, pg/mL	28,175 (13,681)	4	12,847 (6613)	6	1239 (450)	6	221,287 (80,248)	4
T_{max}, h	0.54 (0.32)	4	0.50 (0.21)	6	6.83 (3.19)	6	0.05 (0.03)	4
AUC_{0-last}, pg/mL/h	127,494 (65,790)	4	54,609 (16,405)	6	23,165 (8382)	6	214,738 (44,614)	4
$AUC_{0-\infty}$, pg/mL/h	‖		‖		‖		235,165 (43,854)	3
K_e, 1/h	0.03 (0.010)	3	0.025 (0.009)	5	0.028 (0.006)	3	0.048 (0.018)	3
$t_{1/2}$, h	25.31 (8.8)	3	30.06 (8.24)	5	25.95 (6.07)	3	15.53 (4.70)	3

SNAC = sodium N-[8-(2-hydroxybenzoyl)amino]caprylate.
*Treatment A: 2 × 5-mg cyanocobalamin/100-mg SNAC tablets.
†Treatment B: 1 × 5-mg cyanocobalamin/100-mg SNAC tablet.
‡Treatment C: 1 × 5-mg cyanocobalamin tablet.
§Treatment D: 1-mg cyanocobalamin IV (1 mg/mL solution).
‖Values missing or not reportable.

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]).

The absorption of vitamin B12 from colon is questionable. In humans, 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]

Redacted under sections 41 and 43 FOI Act

Metabolism

Ingested cobalamin is bound by gastric intrinsic factor (IF), 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]

Pharmacokinetics in Special Populations

Redacted under sections 41 and 43 of FOI Act

Malabsorption syndromes

The absorption of cobalamin is impaired in various causes of malabsorption: in autoimmune gastritis (pernicious anaemia), after total or partial gastrectomy, gastric bypass or other bariatric surgery, ileal resection or organ reconstruction surgery (ileal conduit diversion and ileocystoplasty), inflammatory bowel diseases, tropical sprue, Imerslud-Gräsbeck and other syndromes. A less profound vitamin B12 deficiency may occur in protein-bound vitamin B12 malabsorption, mild atrophic gastritis, strict vegetarian diet and in infants breast-fed by vitamin B12-deficient mothers ([redacted] .

First described [redacted] food-cobalamin malabsorption is a syndrome characterized by the inability of the body to release cobalamin from food or intestinal transport proteins, particularly in the presence of hypochlorhydria, where the absorption of 'unbound' cobalamin is normal ('maldigestion'). In our experience, this syndrome accounted for 60–70% of cases of cobalamin deficiency in elderly patients.14,15 This syndrome is characterized by cobalamin deficiency in the presence of sufficient food-cobalamin intake and a normal Schilling test ruling out malabsorption or pernicious anaemia. Thus in this syndrome, patients can absorb 'unbound' cobalamin through intrinsic factor or passive diffusion mechanisms [redacted]

Pharmacokinetics in the Elderly

[redacted] examined the intestinal absorption of cyano[^{57}Co]cobalamin from a non-protein-bound test dose given to 38 subjects from a population of elderly. The subjects were 76 years old and were apparently free from conditions known to affect cyanocobalamin absorption. Their gastric mucosal function was normal, as judged from determinations of serum gastrin and pepsinogen I. The urinary excretion of radioactivity during the first 24 h was 24 (SD 7)%, range 8.6 to 45.2%, corresponding to a health associated reference interval of 10 to 38%. The results indicate that cyanocobalamin absorption does not decline during normal aging. Duplicate studies were performed in another 20 subjects (70-81 years old) from the same population study; these subjects had a serum cobalamin concentration less than 130 pmol/L. The imprecision (CV) was 23%.

A modified protein-bound cobalamin absorption test was used to study dietary cobalamin absorption in healthy adults of different age groups and patients with isolated low serum concentrations of cobalamin ([redacted] . Dietary cobalamin absorption was significantly reduced in healthy adults aged 55-75 years compared with young adults, with a further reduction in those older than 75 years. No difference was detected between dietary cobalamin absorption in patients with isolated low serum cobalamin and controls of a similar age group. Cobalamin malabsorption was associated with elevated serum gastrin. The diagnostic value of this protein-bound cobalamin absorption test in the elderly was limited by the frequent finding of reduced absorption in healthy elderly people with normal serum cobalamin concentrations. The performance of such tests should be evaluated in different age groups before application in diagnosis.

The gold standard for cobalamin deficiency treatment is administration of cobalamin by intramuscular injection. The injection is painful and inconvenient, particularly for elderly persons. Cobalamin might also be administered intranasally. Previous studies do not provide insight into the pharmacokinetics of intranasal cobalamin administration in comparison with cobalamin injection. The aim of the study performed [redacted]) was to quantify

the pharmacokinetics of intranasally and intramuscularly administered cobalamin to determine if intranasal administration might be an alternative for intramuscular administration. Ten inpatients and outpatients of a geriatrics unit were recruited and randomly assigned to receive a single dose of 1000 µg cobalamin administered either by intranasal spray or intramuscular injection (5 per group). Inclusion criteria were written informed consent, age >65 years, and a cobalamin serum concentration <200 pmol/L. Total cobalamin serum concentrations were determined 10 times within 48 hours after administration. The differences in Cmax, Tmax, and AUC0-48 h per administration route were statistically compared using ANOVA. The average Cmax was 1 nmol/L after intranasal and 38.5 nmol/L after intramuscular administration. The average Tmax for intranasal and intramuscular administration was 42 minutes versus 342 minutes, respectively, and the AUC0-48 h was 1.3 µmol/L/min versus 45.4 µmol/L/min, respectively. These values also differed significantly (P <0.05). The estimated bioavailability of the intranasal administration was 2%. The pharmacokinetics of intranasal and intramuscular cobalamin administration in elderly, cobalamin-deficient patients differ significantly.

Pharmacokinetic Interactions

Absorption may be reduced by para-aminosalicylic acid (PAS), colchicine, biguanides, neomycin, cholestyramine, potassium chloride, methyldopa, and H2-blockers and proton pump inhibitors.

[redacted] evaluated the influence of PAS-treatment upon the Schilling-test and various other parameters in a series of 26 patients. No relationship between Schilling values and duration of treatment could be shown.

Vitamin B12 malabsorption has been found in 21 (30%) of 71 diabetic patients taking long-term metformin therapy in addition to dietary management ([redacted]). The patients with evidence of B12 malabsorption had significantly lower haemoglobin levels (and significantly higher serum folic acid levels) than those with normal B12 absorption. Steatorrhoea was found in only one patient. Stopping metformin therapy resulted in reversion of B12 absorption to normal in most patients examined. Four patients with B12 malabsorption were found to have pathologically low serum B12 levels. The authors conclude that all patients on long-term metformin therapy should have annual serum B12 estimations. The reduction of vitamin B12 may be induced by metformin in a dose dependent manner ([redacted]). Similar malabsorption has been observed with phenformin[redacted]

[redacted] performed a systematic review and meta-analysis of the published studies in order to see whether the atherogenic outcomes could be related to any serum biochemical abnormalities. Results showed that carbamazepine and sodium valproate consumption are associated with a significant elevation of the serum homocysteine levels. On the other hand, medication with carbamazepine is associated with a reduction of the level of folate in the serum and that of sodium valproate is associated with a reduction of serum level of vitamin B12.

[redacted] examined the possible association between use of prescription histamine H2 receptor antagonists (H2RA) or proton pump inhibitors (PPI) and vitamin B12 deficiency in older adults. This was a case-control study in a University-based geriatric primary care setting. Among patients aged 65 years or older with documented serum vitamin B12 studies between 1990 and 1997, 53 vitamin B12-deficient cases were compared with 212 controls for past or current use of prescription H2RA/PPI according to information in subjects'

medical records. Controlling for age, gender, multivitamin use, and Helicobacter pylori infection, chronic (≥ 12 months) current use of H2RA/PPI was associated with a significantly increased risk of vitamin B12 deficiency (OR 4.45; 95% CI 1.47-13.34). No association was found between past or short-term current use of H2RA/PPI and vitamin B12 deficiency. These findings support an association between chronic use of H2RA/PPI by older adults and development of vitamin B12 deficiency.

Patients treated with chloramphenicol may respond poorly to cobalamin therapy.

Serum levels of this medicine may be lowered by oral contraceptives. These interactions are unlikely to have clinical significance.

2.5.3.2. Pharmacodynamics

Mechanism of Action

Cobalamin participates in two enzymatic processes in mammalian cells.

In the methionine synthase reaction, homocysteine (HCys) is converted to methionine allowing for the ‘‘recycling’’ of 5-methyl-tetrahydrofolate (THF) to N5,10 methylene-THF which is needed for the de novo synthesis of thymidylic acid and ultimately, for DNA formation. Since conversion of N5,10-methylene-THF to N5-methyl-THF is irreversible, cobalamin deficiency ‘‘traps’’ folic acid as N5-methyl-THF. Concurrently, HCys accumulates while methionine decreases, leading to a decrease in S-adenosylmethionine which further limits N5,10-methylene-THF formation by decreasing the synthesis of formyl-THF (‘‘formate starvation’’). Decreased methionine and S-adenosylmethionine may limit many methylation reactions including those involving DNA and myelin basic protein.

In the methylmalonylCoA mutase reaction, methylmalonylCoA, derived from propionic acid synthesized by intestinal bacteria, is converted to succinylCoA, a precursor for fatty acid and heme synthesis. Thus, cobalamin deficiency results in methylmalonic acid (MMA) accumulation. Impaired thymidylate synthesis, detected by the deoxyuridine suppression test, is the most sensitive marker of cobalamin depletion, followed by elevation of MMA and HCys in peripheral blood. Similarly, decreased methylation of colonic DNA, increased uracil incorporation into DNA, and increased serum MMA and HCys levels antedate clinical changes.

Normal Vitamin B12 level

A strict vegetarian (vegan) diet contains very little cobalamin; less strict vegetarians (lacto-vegetarians, ovo-vegetarians and lacto-ovo-vegetarians) may have subclinical deficiency, as shown by increased blood methylmalonic acid (MMA) and homocysteine concentrations. Since there is clear evidence of abnormal cobalamin metabolism in vegetarians and hyper-homocysteinemia is a risk factor, especially for stroke and vascular dementia, vegetarians are advised to take cobalamin supplements lifelong. In developed countries, the diet is rich in meat and cobalamin-rich foods; thus, malabsorption is the most common cause of cobalamin deficiency. Infants born to vegetarian mothers are at risk of cobalamin deficiency and may present with megaloblastic anaemia, involuntary movements and skin pigmentation. Most people with insufficient cobalamin intake are, however, poverty-imposed vegetarians living in developing countries and represent a worldwide health problem. Some of the subjects with subclinical cobalamin deficiency may have normal serum cobalamin concentrations and may be classified as “normal asymptomatic subjects”, although the minimum concentrations of

serum vitamin B12 for optimal neuronal health are still unknown, especially in the later stages of life.

Based on studies of food cobalamin absorption in normal subjects and of parenteral cobalamin requirements in pernicious anaemia, the recommended daily allowance (RDA) of cobalamin was set at 2–3 µg/day (However, micronucleus formation in peripheral blood lymphocytes, an index of chromosome breakage and loss, is minimized at plasma cobalamin levels above 300 pmol/l (410 pg/ml), requiring a cobalamin intake of 7 µg/day ( ). Thus, the current RDA for cobalamin may be inadequate to ensure genomic stability.

It is not entirely clear what should be regarded as a clinically normal serum cobalamin level, although it has been proposed that a serum cobalamin of <148 pmol/l (200 ng/l) would have a sensitivity of diagnosing 97% of true cobalamin deficiency ( ). It is even less clear what levels of serum cobalamin represent 'subclinical' deficiency, i.e., a low serum cobalamin in the absence of clinical symptoms.

Measurement of serum cobalamin is commonly used to screen for cobalamin deficiency, but many patients with low cobalamin levels are not cobalamin deficient (i.e. ''false'' low values) while significant clinical impairment may occur despite normal cobalamin values (i.e. ''false'' high values). Cobalamin levels may reflect variations in the 2 major cobalamin transport proteins, transcobalamin (formerly transcobalamin II) and haptocorrin (formerly transcobalamin I). Transcobalamin-bound cobalamin represents less than 20% of circulating cobalamin and readily enters tissues via specific receptors. Haptocorrin-bound cobalamin represents more than 80% of circulating cobalamin but is metabolically inert ( ).

Asymptomatic women taking oral contraception or hormone replacement therapy (HRT) may have with mildly reduced serum cobalamin (110–148 pmol/l; 150–200 ng/l). They do not require further investigation but should be advised to review their dietary intake of cobalamin-rich foods, and cobalamin supplements may be considered ( ).

Pregnancy causes a lowering of serum cobalamin. In normal pregnancy, total serum cobalamin levels fall by 30% by the third trimester Serum cobalamin levels during pregnancy and are less reliable in determining underlying deficiency. During pregnancy, in the presence of strong suspicion of underlying deficiency, a short course of empirical hydroxocobalamin should be given, with further investigations post-partum. HoloTC may be more reliable than serum cobalamin in determining deficiency in pregnancy, and is recommended as the test of choice, if available (.

Vegetarians, particularly strict vegans, should be considered for monitoring of their cobalamin level according to clinical assessment. Dietary alterations or oral supplementation may be considered according to the clinical situation, particularly during pregnancy and breast-feeding).

Reduced serum cobalamin levels in infancy in the presence of clinical features should be treated promptly to prevent long term neurological sequelae. In the presence of clinical suspicion of underlying cobalamin deficiency, even in the presence of normal serum cobalamin levels, further biochemical tests, including MMA and total homocysteine, are recommended. No specific recommendation can be made regarding treatment because each case has to be judged individually. No specific recommendations can currently be made in

Redacted under sections 41 and 43 of the FOI Act

relation to breastfeeding-associated biochemical low cobalamin status in asymptomatic infants (.

There is no consensus on the cut-off points for vitamin B12 and its co-markers, such as folate, holotranscobalamin, methylmalonic acid and homocysteine. In order to establish the state of the art about cut-off points used to determine vitamin B12 deficiency in the last decades, the database was used for searching studies published in adults between December 1992 and May 2014 (69 articles), using search terms like 'vitamin B12', 'cobalamin', 'cut-off', 'deficiency' alone or in combinations ( ). Broad ranges of cut-off points for vitamin B12 and its biomarkers were identified: vitamin B12 ranged between 100 pmol/L and 350 pmol/L, holotranscobalamin 20-50 pmol/L, methylmalonic acid 0.210-0.470 µmol/L, homocysteine 10-21.6 µmol/L, serum folate 3.7-15.9 nmol/L and red blood cell 124-397 nmol/L. For the majority of studies, the potential influence of age, analytical methods, gender and fortified food consumption was not taken in account when choosing cut-off values. This could explain the discrepancies between studies on vitamin B12 and folate deficiency prevalences. The authors conclude that there is inconsistency in the literature regarding vitamin B12 cut-offs. It would be necessary to establish different reference cut-offs according to age, considering the analytical methods used.

Vitamin B12 Deficiency

Cobalamin (vitamin B12) deficiency may result from inadequate ingestion, malabsorption, increased excretion, increased requirements or defective transport to, or utilisation by, the target cells. 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 (Figure 4).

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 IF. About 50% of patients with pernicious anaemia have anti-IF antibodies.

studied the haematological manifestations or abnormalities in 201 patients (median age: 67 ± 6 years) with well-documented cobalamin deficiency (mean serum vitamin B12 levels 125 ± 47 pg/ml) extracted from an observational cohort study (1995–2003). Assessment included clinical features, blood count and morphological review. Haematological abnormalities were reported in at least two-third of the patients: anaemia (37%), leukopenia (13.9%), thrombopenia (9.9%), macrocytosis (54%) and hypersegmented neutrophils (32%). The mean haemoglobin level was 10.3 ± 0.4 g/dl and the mean erythrocyte cell volume 98.9 ± 25.6 fl. Approximately 10% of the patients have life-threatening haematological manifestations with documented symptomatic pancytopenia (5%), 'pseudo' thrombotic microangiopathy (2.5%), severe anaemia (defined as Hb levels <6 g/dl; 2.5%) and haemolytic anaemia (1.5%). Correction of the haematological abnormalities was achieved in at least two-thirds of the patients, equally well in patients treated with either intramuscular or oral crystalline cyanocobalamin.

Redacted under sections 41 and 43 of FOI Act

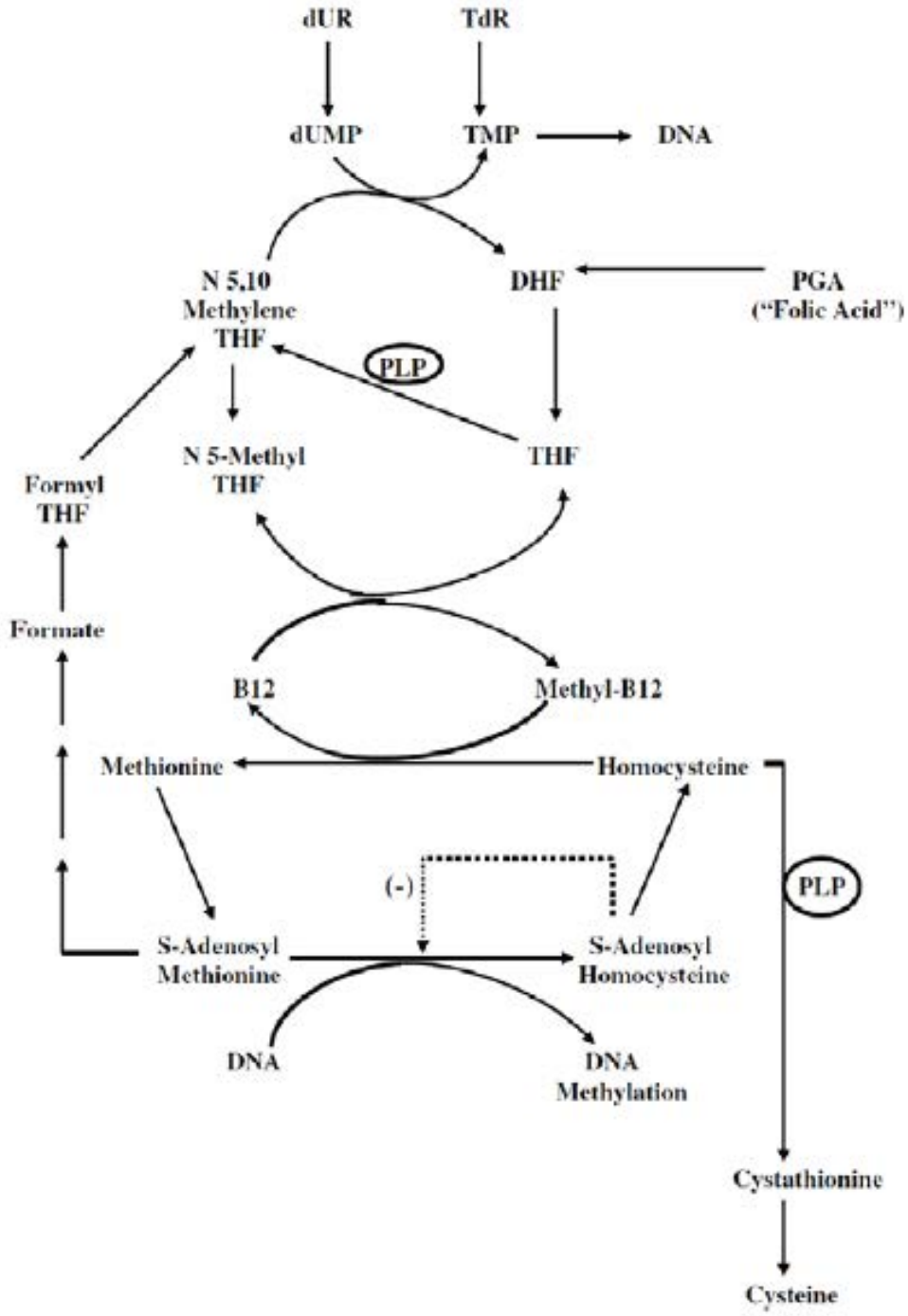


Figure 4. The methionine synthase pathway ([redacted]

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. While a role for both the methylmalonylCoA mutase and the methionine synthase pathways in the pathogenesis of neurocognitive dysfunction in cobalamin deficiency has been suggested, observations in experimental animals and in human subjects with inborn errors of cobalamin metabolism do not consistently support either hypothesis. According to more recent experiments, cobalamin-deficient (Cbl-D) 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) ([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.

Pharmacodynamic interactions

Some earlier case reports have observed accelerated neurological deterioration in patients with pernicious anaemia and severe vitamin B12 deficiency after treatment with folic acid. These observations in combination with the known metabolic interrelation of folate and vitamin B12 suggest that vitamin B12 status have been directly associated with cognitive performance.

A study examined the interactions between plasma concentrations of folate and vitamin B12 markers in relation to cognitive performance in Norwegian elderly who were unexposed to mandatory or voluntary folic acid fortification ([redacted] . Cognitive performance was assessed by six cognitive tests in 2203 individuals aged 72–74 years. A combined score was calculated using principal component analysis. The associations of folate concentrations, vitamin B12 markers (total vitamin B12, holoTC and MMA) and their interactions in relation to cognitive performance were evaluated by quantile regression and least-squares regression, adjusted for sex, education, apo-ε14 genotype, history of CVD/hypertension and creatinine. Cross-sectional analyses revealed an interaction (P = 0·009) between plasma concentrations of folate and vitamin B12 in relation to cognitive performance. Plasma vitamin B12 concentrations in the lowest quartile (>274 pmol/l) combined with plasma folate concentrations in the highest quartile (<185 nmol/l) were associated with a reduced risk of cognitive impairment compared with plasma concentrations in the middle quartiles of both vitamins (OR 0·22, 95% CI 0·05, 0·92). The interaction between folate and holoTC or MMA in relation to cognitive performance was not significant. In conclusion, this large study population unexposed to mandatory folic acid fortification showed that plasma folate, but not plasma vitamin B12, was associated with cognitive performance. Among the elderly participants with vitamin B12 concentrations in the lower range, the association between plasma folate and cognitive performance was strongest.

Cobalamin should not be used to treat megaloblastic anaemia of pregnancy because it is due to folate deficiency

2.5.4. Overview of Efficacy

Efficacy in Vitamin B12 Deficiency

Cobalamin (vitamin B12) deficiency, the most common cause of megaloblastic anaemia, is treated with intramuscular (IM) cobalamin. It has been suggested by some investigators that oral (PO) cobalamin treatment may be as effective in the treatment of this condition, with the advantages of ease of administration and lower cost.

[redacted]) investigated the efficacy of oral cobalamin therapy. They randomly assigned 38 newly diagnosed cobalamin deficient patients to receive cyanocobalamin as either 1 mg intramuscularly on days 1, 3, 7, 10, 14, 21, 30, 60and 90 or 2 mg orally on a daily basis for 120 days. Therapeutic effectiveness was evaluated by measuring haematologic and neurologic improvement and changes in serum levels of cobalamin (normal, 200 to 900 pg/mL) methylmalonic acid (normal, 73 to 271 nmol/L), and homocysteine (normal, 5.1 to 13.9 µmol/L). Five patients were subsequently found to have folate deficiency, which left 18 evaluable patients in the oral group and 15 in the parenteral group. Correction of haematologic and neurologic abnormalities was prompt and indistinguishable between the 2 groups. The mean pretreatment values for serum cobalamin, methylmalonic acid, and homocysteine were, respectively, 93 pg/mL, 3,850 nmol/L, and 37.2 µmol/L in the oral group and 95 pg/mL, 3,630 nmol/L, and 40.0 µmol/L in the parenteral therapy group. After 4 months of therapy, the respective mean values were 1,005 pg/mL, 169 nmol/L, and 10.6 µmol/L in the oral group and 325 pg/mL, 265 nmol/L, and 12.2 µmol/L in the parenteral group. The higher serum cobalamin and lower serum methylmalonic acid levels at 4 months post treatment in the oral group versus the parenteral group were significant, with P <0.0005 and P <0.05, respectively. In cobalamin deficiency, 2 mg of cyanocobalamin administered orally on a daily basis was as effective as 1 mg administered intramuscularly on a monthly basis and may be superior.

[redacted] assessed the effects and cost of PO versus IM cobalamin treatment in patients with megaloblastic anaemia due to cobalamin deficiency. This was a 90-day, prospective, randomized, open-label study conducted at the [redacted]). Patients aged ≥16 years with megaloblastic anaemia due to cobalamin deficiency were randomized to receive 1000-µg cobalamin PO once daily for 10 days (PO group) or 1000-µg cobalamin IM once daily for 10 days (IM group). After 10 days, both treatments were administered once a week for 4 weeks, and after that, once a month for life. Patients were assessed for the presence of reticulocytosis between treatment days 5 and 10 until it was detected. Therapeutic effectiveness was assessed by measuring haematologic parameters on days 0, 10, 30, and 90 and serum vitamin B12 concentration on days 0 and 90. The Mini–Mental State Examination was used before and after the B12 therapy for cognitive function assessment and 125-Hz diapozone was used for vibration threshold testing. Neurologic sensory assessment, including soft-touch and pinprick examinations, was used to identify neuropathy at baseline and study end. Tolerability was assessed using laboratory tests and patient interview. Cost was assessed using the cost of the study drug and of the injection. Sixty patients completed the study: 26 in the PO group (16 men, 10 women; mean [SD] age, 60 [15] years) and 34 in the IM group (17 men, 17 women; mean [SD] age, 64 [10] years). Reticulocytosis was observed in all patients. In the PO group, at days 30 and 90, all hematologic parameters changed significantly versus day 0 (mean haemoglobin levels

increased [both P < 0.001]; mean corpuscular volume decreased [both P < 0.001]; mean white blood cell count increased [day 30, P < 0.01; day 90, P < 0.001]; and mean platelet count increased [both P < 0.001]). The mean serum vitamin B12 concentration increased significantly from day 0 to 90 (P < 0.001). These hematologic parameters and the recovery patterns were similar between the 2 groups. Neurologic findings included sensitive peripheral neuropathy in 9 patients (15.0%), alteration of cognitive function (loss of memory, impaired concentration) in 7 patients (11.7%), and loss of sense of vibration in 5 patients (8.3%). Neurologic improvement was detected in 7 of 9 patients (77.8%) in the PO group and 9 of 12 patients (75.0%) in the IM group at day 30. In conclusions, in this study of patients with megaloblastic anaemia due to cobalamin deficiency, PO cobalamin treatment was as effective as IM cobalamin treatment. PO treatment also was better tolerated and less expensive compared with IM treatment. However, because of the small sample size and the short term of this study, further long-term studies are needed to determine the efficacy of PO cobalamin treatment.

[redacted]) conducted a randomized, parallel-group, double-blind, dosefinding trial to determine the lowest oral dose of cyanocobalamin required to normalize biochemical markers of vitamin B12 deficiency in older people with mild vitamin B12 deficiency, defined as a serum vitamin B12 level of 100 to 300 pmol/L (135-406 pg/mL) and a methylmalonic acid level of 0.26 µmol/L or greater. They assessed the effects of daily oral doses of 2.5, 100, 250, 500, and 1000 µg of cyanocobalamin administered for 16 weeks on biochemical markers of vitamin B12 deficiency in 120 people. The main outcome measure was the dose of oral cyanocobalamin that produced 80% to 90% of the estimated maximal reduction in the plasma methylmalonic acid concentration. Supplementation with cyanocobalamin in daily oral doses of 2.5, 100, 250, 500, and 1000 µg was associated with mean reductions in plasma methylmalonic acid concentrations of 16%, 16%, 23%, 33%, and 33%, respectively. Daily doses of 647 to 1032 µg of cyanocobalamin were associated with 80% to 90% of the estimated maximum reduction in the plasma methylmalonic acid concentration. In conclusion, the lowest dose of oral cyanocobalamin required to normalize mild vitamin B12 deficiency is more than 200 times greater than the RDA, which is approximately 3 µg daily.

[redacted]*)* compared the effectiveness of one-month oral vitamin B12 supplementation in patients with a subtle vitamin B12 deficiency to that of a placebo. This multicentre (13 general practices, two nursing homes, and one primary care centre in western Switzerland), parallel, randomised, controlled, closed-label, observer-blind trial included 50 patients with serum vitamin B12 levels between 125-200 pM/l who were randomized to receive either oral vitamin B12 (1000 µg daily, N = 26) or placebo (N = 24) for four weeks. The institution's pharmacist used simple randomisation to generate a table and allocate treatments. The primary outcome was the change in serum methylmalonic acid (MMA) levels after one month of treatment. Secondary outcomes were changes in total homocysteine and serum vitamin B12 levels. Blood samples were centralised for analysis and adherence to treatment was verified by an electronic device ([redacted] . Baseline characteristics and adherence to treatment were similar in both groups. After one month, one patient in the placebo group was lost to followup. Data were evaluated by intention-to-treat analysis. One month of vitamin B12 treatment (N = 26) lowered serum MMA levels by 0.13 µmol/l (95%CI 0.06-0.19) more than the change observed in the placebo group (N = 23). The number of patients needed to treat to detect a metabolic response in MMA after one month was 2.6 (95% CI 1.7-6.4). A significant change was observed for the B12 serum level, but not for the homocysteine level, haematocrit, or mean corpuscular volume. After three months without active treatment (at four months), significant differences in MMA levels were no longer detected. In conclusion, oral vitamin B12 treatment normalised the metabolic markers

of vitamin B12 deficiency. However, a one-month daily treatment with 1000 µg oral vitamin B12 was not sufficient to normalise the deficiency markers for four months, and treatment had no effect on haematological signs of B12 deficiency.

Although oral replacement with high doses of vitamin B12 is both effective and safe for the treatment of B12 deficiency, little is known about patients' views concerning the acceptability and effectiveness of oral B12. [redacted]) investigated patient perspectives on switching from injection to oral B12 therapy. This study involved a quantitative arm using questionnaires and a qualitative arm using semistructured interviews, both to assess patient views on injection and oral therapy. Patients were also offered a six-month trial of oral B12 therapy. One hundred and thirty-three patients who receive regular B12 injections were included from three family practice units (two hospital-based academic clinics and one community health centre clinic) in Toronto. Seventy-three percent (63/86) of respondents were willing to try oral B12. In a multivariate analysis, patient factors associated with a "willingness to switch" to oral B12 included being able to get to the clinic in less than 30 minutes (OR 9.3, 95% CI 2.2-40.0), and believing that frequent visits to the health care provider (OR 5.4, 95% CI 1.1-26.6) or the increased costs to the health care system (OR 16.7, 95% CI 1.5-184.2) were disadvantages of injection B12. Fifty-five patients attempted oral therapy and 52 patients returned the final questionnaire. Of those who tried oral therapy, 76% (39/51) were satisfied and 71% (39/55) wished to permanently switch. Factors associated with permanently switching to oral therapy included believing that the frequent visits to the health care provider (OR 35.4, 95% CI 2.9-432.7) and travel/parking costs (OR 8.7, 95% CI 1.2-65.3) were disadvantages of injection B12. Interview participants consistently cited convenience as an advantage of oral therapy. The authors conclude that switching patients from injection to oral B12 is both feasible and acceptable to patients. Oral B12 supplementation is well received largely due to increased convenience. Clinicians should offer oral B12 therapy to their patients who are currently receiving injections, and newly diagnosed B12-deficient patients who can tolerate and are compliant with oral medications should be offered oral supplementation.

Meta-analyses

[redacted]*)* performed a systematic review of randomized trials on the oral vitamin B12 versus intramuscular vitamin B12 for vitamin B12 deficiency. They searched databases for relevant RCTs. Outcomes included levels of serum vitamin B12, total serum homocysteine and methylmalonic acid, haemoglobin and signs and symptoms of vitamin B12 deficiency. Two RCTs comparing oral with intramuscular administration of vitamin B12 met the inclusion criteria. The trials recruited a total of 108 participants and followed up 93 of these from 90 days to 4 months. In one of the studies, mean serum vitamin B12 levels were significantly higher in the oral (643 ± 328 pg/ml; n = 18) compared with the intramuscular group (306 ± 118 pg/ml; n =15) at 2 months ($P < 0.001$) and 4 months (1005 ± 595 versus 325 ± 165 pg/ml; $P < 0.0005$) and both groups had neurological responses. In the other study, serum vitamin B12 levels increased significantly in those receiving oral vitamin B12 and intramuscular vitamin B12 ($P < 0.001$). The evidence derived from these limited studies suggests that 2000 µg doses of oral vitamin B12 daily and 1000 µg doses initially daily and thereafter weekly and then monthly may be as effective as intramuscular administration in obtaining short-term haematological and neurological responses in vitamin B12-deficient patients.

In a meta-analysis, [redacted]) assessed the effectiveness of oral vitamin B12 versus intramuscular vitamin B12 for vitamin B12 deficiency. Searches were undertaken of The [redacted] The bibliographies of all relevant papers identified using this strategy were searched. In addition, we contacted

authors of relevant identified studies and Vitamin B12 research and pharmaceutical companies to enquire about other published or unpublished studies and ongoing trials. Randomised controlled trials (RCTs) examining the use of oral or intramuscular vitamin B12 to treat vitamin B12 deficiency were selected. All abstracts or titles identified by the electronic searches were independently scrutinised by two reviewers. When a difference between reviewers arose, they obtained and reviewed a hard copy of the papers and made decisions by consensus. They obtained a copy of all preselected papers and two researchers independently extracted the data from these studies using piloted data extraction forms. The whole group checked whether inclusion and exclusion criteria were met, and disagreement was decided by consensus. The methodological quality of the included studies was independently assessed by two researchers and disagreements were brought back to the whole group and resolved by consensus. Two RCT's comparing oral with intramuscular administration of vitamin B12 met our inclusion criteria. The trials recruited a total of 108 participants and followed up 93 of these from 90 days to four months. High oral doses of B12 (1000 µg and 2000 µg) were as effective as intramuscular administration in achieving haematological and neurological responses. The evidence derived from these limited studies suggests that 2000 µg doses of oral vitamin B12 daily and 1000 µg doses initially daily and thereafter weekly and then monthly may be as effective as intramuscular administration in obtaining short term haematological and neurological responses in vitamin B12 deficient patients.

[redacted] designed a protocol to compare the effectiveness of orally and intramuscularly administered vitamin B12 in the treatment of patients ≥65 years of age with vitamin B12 deficiency. The proposed study involves a controlled, randomised, multicentre, parallel, non-inferiority clinical trial lasting one year, involving 23 primary healthcare centres in the Madrid region (Spain), and patients ≥65 years of age. The results of this study should help establish, taking quality of life into account, whether the oral administration of vitamin B12 is an effective alternative to its intramuscular administration. If this administration route is effective, it should provide a cheaper means of treating vitamin B12 deficiency while inducing fewer adverse effects. Having such an alternative would also allow patient preferences to be taken into consideration at the time of prescribing treatment. This trial has been registered with ClinicalTrials.gov, number [redacted] and under EUDRACT number [redacted]

An economic analysis aimed to estimate the cost savings of switching from intramuscular injections to high-dose oral supplements for patients aged 18 years and older with confirmed vitamin B12 deficiency ([redacted] . Population based administrative databases for Ontario were used to identify patients receiving vitamin B12 intramuscular injections in any fiscal year between 2006 and 2011. [redacted] database was used to identify patients who were prescribed vitamin B12 injections, and the Ontario Health Insurance Plan database was used to identify all physician claims for intramuscular injections as well as laboratory tests assessing vitamin B12 levels. The Registered Physicians Database was used to identify the type of physician; the analysis was restricted to family physicians and internists. Two cohorts of patients were identified. For cohort 1, the [redacted] database was used to identify patients who were prescribed vitamin B12 injections. Those covered under the [redacted] are 65 years of age or older and are economically deprived. A second cohort was created to capture those 18 to 64 years of age receiving injections. Cohort 2 consisted of patients (not in cohort 1) who received 6 or more intramuscular injections within 1 year and had a laboratory test 2 months before the intramuscular injection claim. Physician experts were consulted to estimate the resources and costs of converting patients to oral supplements. The Ministry of Health and Long-Term Care perspective was taken, and all costs are expressed in 2013

Canadian dollars. The budget impact analysis demonstrated costs of $2.8 million to the Ministry of Health and Long-Term Care in the first year of conversion; however, in subsequent years there are savings of $4.2 million per year. The cumulative 5-year budget impact demonstrates savings of $14.2 million to the health care system. This analysis represents the cost of conversion for those currently receiving intramuscular injections. There are no conversion costs for those who are prescribed oral supplements as an initial therapy, and so the savings could be even greater than reported. As well, an underlying assumption of this analysis is that patients will comply with oral supplementation. The authors conclude that over 5 years, there are savings of $14.2 million to the health care system from switching to vitamin B12 oral supplements.

Efficacy in Subnormal Serum Cobalamin Levels in Apparently Normal People

Vitamin B12 deficiency is common in older people and the prevalence increases with age. Vitamin B12 deficiency may present as macrocytic anaemia, subacute combined degeneration of the spinal cord, or as neuropathy, but is often asymptomatic in older people. The diagnosis and indications for treatment are clear for individuals with low plasma levels of vitamin B12 in the setting of megaloblastic anaemia and neuropathy, but the relevance of treatment of vitamin B12 deficiency in the absence of such clinical signs is uncertain.

[redacted] investigated the effect of supplementation on the cognitive function of older people with cobalamin deficiency by a randomized trial. Fifty Chinese subjects more than 60 years old with serum cobalamin level < 120 pmol/l were randomized into supplement and control groups. Fasting serum methylmalonic acid levels (MMA) were measured. A battery of neuropsychological tests was administered. The supplement group received intramuscular cyanocobalamin injections, while the control group received no intervention. They were followed up at around 4 months. 78% of the subjects had raised MMA, indicating metabolic cobalamin deficiency. Supplemented subjects improved in performance IQ, but the amount of improvement was not significantly more than that of control subjects. Moreover, the supplement group fared worse than the control group at follow-up in some motor function scores. Three out of seven demented subjects had improvement in Mini-Mental State Examination scores, but there was no consistent improvement in other neuropsychological scores. This study suggested that cobalamin deficiency did not invariably cause cognitive impairment in older people. There remain the possibilities that cobalamin deficiency causes cognitive impairment or exacerbates coexisting dementia in some older people.

[redacted] determined the effect of small doses of oral cyanocobalamin supplements in older patients with low or borderline serum vitamin B12 concentrations but no other evidence of pernicious anaemia. It was a randomized, double-blind, placebo-controlled study assessing the efficacy of oral cyanocobalamin 10 µg and 50 µg daily for 1 month. Thirty-one inpatients participated in the study, with serum vitamin B12 levels between 100 and 150 pmol/L, without pernicious anaemia, other malabsorption disorders, or progressive neurological or terminal illness. The mean age was 81.4 years. After informed consent, a medical and drug history was taken and the Mini-Mental State Examination (MMSE) completed. A dietitian made assessment of oral cobalamin intake. Blood was taken for serum vitamin B12, serum and red cell folate assay, full blood examination, fasting serum gastrin, parietal and IF antibodies, fasting serum homocysteine, and creatinine. Patients were then randomized to receive 10 µg oral cyanocobalamin, 50 µg oral cyanocobalamin, or placebo treatment for 1 month, after which the investigations and clinical examinations were repeated. Percentage change in the level of vitamin B12, homocysteine, folate, and red cell parameters and absolute changes in MMSE were calculated and compared between groups. The groups were compared on the

number of responders who improved their level of B12 by 20%. Chi-square calculations on changes in serum vitamin B12 concentration were also performed. Mean serum vitamin B12 ± standard deviation improved by 51.7 ± 47.1% in the 50-µg group, 40.2 ± 34.4% in the 10-µg group, and 11.7 ± 24.5% in the placebo group. The change in the 50-µg cyanocobalamin group was significantly greater than that in the placebo group (P = 0.044). The change in the 10-µg cyanocobalamin group was not significantly different from that in the placebo group (P = 0.186). Eight of 10 subjects in each treatment group were classified as responders, compared with two of 11 in the placebo group (P = 0.004). Homocysteine levels fell in patients receiving cyanocobalamin, but this fall failed to reach statistical significance. There were no significant changes in the other parameters measured. Cyanocobalamin supplementation of 50 µg but not 10 µg daily produced a significant increase in serum vitamin B12. This result has implications for the management of patients with subnormal or borderline serum vitamin B12 concentrations and for food fortification with vitamin B12.

Mildly cobalamin-deficient elderly were supplemented with 1000 µg cobalamin (group C, n = 34), 1000 g cobalamin with 400 µg folic acid (group CF, n = 31) or a placebo (n = 30) for 6 months ([redacted] . Participants provided one single blood sample 3, 5 or 7 months after cessation of supplementation to monitor early changes in plasma concentrations of cobalamin, holotranscobalamin (holoTC) and methylmalonic acid (MMA). At the end of supplementation (groups C+CF), one participant met our criteria for mild cobalamin deficiency, as did 13, 14 and 43% of the participants assessed at respectively 3, 5 and 7 months post-supplementation. Cobalamin and holoTC declined on average with 47 and 56% relative to concentrations at the end of supplementation for the group assessed at 7 months post-supplementation. Essentially similar declines were observed for those participants assessed at 3 and 5 months post-supplementation. Mean MMA concentrations increased by 15% (P = 0.07) in those participants assessed at 3 and 5 months post-supplementation, and increased by 50% (P = 0.002) in those participants assessed at 7 months post-supplementation. Considering MMA as a sensitive tissue marker for cobalamin status, oral supplementation may afford adequate cobalamin status for a period of up to 5 months after cessation in the majority of participants.

[redacted] compared the efficacy and safety profile of a new proprietary oral vitamin B12 formulation (oral B12)) with IM vitamin B12 (IM B12) in restoring normal serum B12 concentrations in patients with low cobalamin levels (<350 pg/mL). Patients were recruited from 5 centres and randomly assigned to receive oral B12 1000 µg, taken daily for 90 days, or IM B12 1000 µg, given on study days 1, 3, 7, 10, 14, 21, 30, 60, and 90. The patients were aged ≥60 years or aged ≥18 years and had gastrointestinal abnormalities or were on a restricted diet. The primary efficacy outcome compared the proportion of patients in each treatment arm in whom cobalamin levels were normalized (≥350 ng/mL) following 60 days of treatment. Secondary objectives included comparing the efficacy of the 2 formulations after 90 days of treatment, assessing time to normalization of B12 levels, and evaluating the changes in the levels of biomarkers MMA and homocysteine (HC). The effect on holotranscobalamin II (active B12) levels was assessed as an exploratory end point and correlated to serum cobalamin levels in both treatment groups. Blood samples were collected at baseline (day 1) and on days 15, 31, 61, and 91. Fifty patients were recruited. Forty-eight patients (96.0%) completed the study (22 patients [91.7%] in the oral B12 group and 26 patients [100%] in the IM B(12) group). All patients (100%) in both treatment groups and in both populations had a cobalamin level ≥350 pg/mL on day 61 and maintained it on day 91. The difference between the IM and oral treatment groups did not reach the planned level of statistical significance (P < 0.05) for mean percent change from baseline (PCFB) in serum cobalamin levels on day 61 and day 91. The difference between the IM and oral treatment groups did not reach the planned level of

statistical significance for mean PCFB in serum MMA levels on day 61. There was a statistical difference between the IM and oral treatment groups for mean PCFB in serum MMA levels on day 91 (P = 0.033), with lower values in the oral B12 group. The difference between the IM and oral treatment groups did not reach the planned level of statistical significance for mean PCFB in plasma HC levels on day 61 and day 91. All patients in each treatment group achieved normalization of serum cobalamin levels by day 15. All patients in both treatment groups and in both populations had plasma holotranscobalamin levels ≥40 pmol/L on day 61 and on day 91. No statistical analysis was planned or performed for safety end points, which were reported only descriptively. Most observed adverse effects were considered mild or moderate in intensity. All adverse effects that were considered severe in intensity were also considered by the investigator to be not related to the study drug. In this selected study population comprising individuals with low cobalamin levels but who otherwise were in good health, patients received oral B12 (1000 µg/d) or IM B12 (1000 µg in 9 injections over 3 months) for a total of 3 months. Both the oral and IM formulations were effective in restoring normal levels of serum cobalamin in all patients studied (100%). Both formulations used in this study were well tolerated at the dose studied.

Meta-analyses

[redacted] performed a systematic review of the effect of pyridoxine hydrochloride (hereinafter "vitamin B6"), cyanocobalamin or hydroxocobalamin (hereinafter "vitamin B12"), and folic acid supplementation on cognitive function. Literature search was conducted in [redacted] with supplemental articles from reviews and domain experts. They included English language randomized controlled trials of vitamins B6 and/or B12 and/or folic acid supplementation with cognitive function outcomes. Fourteen trials met their criteria; most were of low quality and limited applicability. Approximately 50 different cognitive function tests were assessed. Three trials of vitamin B6 and 6 of vitamin B12 found no effect overall in a variety of doses, routes of administration, and populations. One of 3 trials of folic acid found a benefit in cognitive function in people with cognitive impairment and low baseline serum folate levels. Six trials of combinations of the B vitamins all concluded that the interventions had no effect on cognitive function. Among 3 trials, those in the placebo arm had greater improvements in a small number of cognitive tests than participants receiving either folic acid or combination B-vitamin supplements. The evidence was limited by a sparsity of studies, small sample size, heterogeneity in outcomes, and a lack of studies that evaluated symptoms or clinical outcomes. The evidence does not yet provide adequate evidence of an effect of vitamin B6 or B12 or folic acid supplementation, alone or in combination, on cognitive function testing in people with either normal or impaired cognitive function.

[redacted]*)* systematically reviewed studies that investigated vitamin B12 intake and biomarkers of vitamin B12 status and estimated dose-response relations with the use of a meta-analysis. This systematic review included all RCTs, prospective cohort studies, nested case-control studies, and cross-sectional studies in healthy adult populations published through January 2010 that supplied or measured dietary vitamin B12 intake and measured vitamin B12 status as serum or plasma vitamin B12, methylmalonic acid (MMA), or holotranscobalamin. We calculated an intake-status regression coefficient ( ) for each individual study and calculated the overall pooled and SE ( ) by using random-effects meta-analysis on a double-log scale. The meta-analysis of observational studies showed a weaker slope of dose-response relations than the meta-analysis of RCTs. The pooled dose-response relation of all studies between vitamin B12 intake and status indicated that a doubling of the vitamin B12 intake increased vitamin B12 concentrations by 11% (95% CI: 9.4%, 12.5%). This

increase was larger for studies in elderly persons (13%) than in studies in adults (8%). The dose-response relation between vitamin B12 intake and MMA concentrations indicated a decrease in MMA of 7% (95% CI: -10%, -4%) for every doubling of the vitamin B12 intake. The assessment of risk of bias within individual studies and across studies indicated risk that was unlikely to seriously alter these results. The obtained dose-response estimate between vitamin B12 intake and status provides complementary evidence to underpin recommendations for a vitamin B12 intake of populations.

Dosing of Cobalamin

A review ( ) evaluated oral cobalamin (vitamin B12) therapy in adult and elderly patients, from the perspective of a haematologist. PubMed was systematically searched for English and French articles published from January 1990 to January 2007. Several prospective studies in well-determined population (n = 4), prospective randomized studies (n = 3) and a systematic review by the Cochrane group (n = 1) provide evidence that oral cobalamin therapy may adequately treat cobalamin deficiency, particularly haematological abnormalities or manifestations. These studies suggest that at least 1000 µg/day of oral cyanocobalamin are needed for pernicious anaemia and a mean daily dose of 250 µg for food-cobalamin malabsorption. This review confirms the previously reported efficacy of oral cobalamin treatment in adult and elderly patients. According to the Guideline of British Committee for Standards in Haematology (, current clinical practice within the UK is to treat cobalamin deficiency with hydroxocobalamin in the intramuscular form. Standard initial therapy for patients without neurological involvement is 1000 µg IM three times a week for 2 weeks. Patients presenting with neurological symptoms should receive 1000 µg IM on alternate days until there is no further improvement. However, the Guideline Writing Group (GWG) recommends a pragmatic approach in patients with neurological symptoms by reviewing the need for continuation of alternate day therapy after 3 weeks of treatment. Maintenance treatment for patients presenting without neurological deficit is with hydroxocobalamin 1000 µg IM every 3 months. Those with initial neurological deficit should receive hydroxocobalamin 1000 µg IM every 2 months. No further testing for cobalamin levels is required. Although there is little evidence that more frequent dosing is harmful, specific objective studies demonstrating clinical benefit are absent, and the GWG cannot make specific recommendations.

High dose oral cyanocobalamin (1000–2000 µg) is licensed for use in several countries outside the UK. The absorption of oral cobalamin has been documented in pernicious anaemia (. In one case daily doses of 5 µg. gave no response; in another, even 80 µg. a day for 24 days produced an increase in red blood cells no greater than would have been expected in 15 days from the injection of 2.5 µg. Initial treatment with oral cobalamin may not be appropriate in pernicious anaemia, but may be considered in maintenance or correction of suboptimal levels in asymptomatic patients (

Oral vitamin B12 at a daily dose of 2000 µg (20 µg absorbed on average) is equivalent to weekly injections of cyanocobalamin at a dose of 1000 µg (150 µg retained). Oral treatment has been successfully used in Sweden for almost 50 years. However, patients with life-threatening megaloblastic anaemia or severe myelopathy may benefit from rapid

replacement with daily injections, although in the past cure has been accomplished with much lower and less frequent doses ([redacted] .

2.5.5. Overview of Safety

No safety level (Tolerable Upper Intake Level [UL]) has been established for vitamin B12 ([redacted]). The absorption of vitamin B12 mediated by the glycoprotein, IF, is limited to 1.5–2.0 µg per meal because of the limited capacity of the receptors. In addition, between 1% and 3% of any particular oral administration of vitamin B12 is absorbed by passive diffusion. Thus, if 1000 µg vitamin B12 is taken orally, the amount absorbed would be 2.0 µg by active absorption plus up to about 30 µg by passive diffusion. Intake of 1000 µg vitamin B12 has never been reported to have any side-effects. Similar large amounts have been used in some preparations of nutritional supplements without apparent ill effects.

Cobalamin is generally well tolerated, though adverse effects include itching, exanthema, chills, fever, hot flushes, nausea, dizziness and, exceptionally, anaphylaxis ([redacted]). This may be due to hypersensitivity to cobalt or any of the other components of the medication. Acneiform eruptions have been reported rarely. Due to cross-sensitivity of hydroxocobalamin and cyanocobalamin, treatment of patients may be a challenge. Skin patch testing may help to choose an appropriate product. If absolutely necessary, treatment may be considered under hydrocortisone cover in a hospital setting where severe hypersensitivity can be managed.

Special Patient Groups

Children

Malnutrition is frequent in third-world children. A study measured the effect of daily supplementation of vitamin B12 and/or folic acid on development in young North Indian children ([redacted]). In a randomized, double blind trial, children aged six to 30 months, received supplement with placebo or vitamin B12 and/or folic acid for six months. Children were allocated in a 1:1:1:1 ratio in a factorial design and in blocks of 16. They measured development in 422 children by the Ages and Stages Questionnaire 3rd ed. at the end of the intervention. Compared to placebo, children who received both vitamin B12 and folic acid had 0.45 (95% CI 0.19, 0.73) and 0.28 (95% CI 0.02, 0.54) higher SD-units in the domains of gross motor and problem solving functioning, respectively. The effect was highest in susceptible subgroups consisting of stunted children, those with high plasma homocysteine (> 10 µmol/L) or in those who were younger than 24 at end study. With the exception of a significant improvement on gross motor scores by vitamin B12 alone, supplementation of either vitamin alone had no effect on any of the outcomes.

Elderly

Elderly persons are more likely to have low values for serum and erythrocyte folate, and for serum cobalamin ([redacted]). Many of those with low vitamin levels have biochemical abnormalities consistent with true deficiency, including increased formiminoglutamic acid excretion, abnormal marrow deoxyuridine suppression, and raised serum levels of methylmalonic acid and homocysteine. Therapy with the appropriate vitamin reverses the biochemical defect. Despite this, the clinical consequences for most elderly persons are remarkably few. True megaloblastic anaemia is rare, and the small number of therapeutic trials to date have not improved the levels of haemoglobin in the treated subjects, although the mean corpuscular volume has decreased significantly. There has been recent concern that these low blood vitamin levels might be important causes of nervous system damage, but

studies specifically of the elderly have not demonstrate overall improvements in neurological function following therapy. Vascular damage from high blood homocysteine levels secondary to cobalamin or folate deficiency remains a potential hazard. Dietary insufficiency, malabsorption of protein-bound vitamin B12 secondary to atrophic gastritis, and defective absorption of folyl polyglutamates seem the likeliest possible causes. Pernicious anaemia, although a common cause of severe megaloblasfic anaemia in the elderly, is an infrequent cause for the low cobalamin levels in population studies.

Although the benefits are uncertain, the balance of the evidence suggests that one should treat elderly persons with low values of cobalamin or folate. Crystalline vitamin B12 and folic acid are absorbed normally and are therefore suitable for replacement therapy, provided that pernicious anaemia is excluded.

Both oral and intramuscular formulations of cobalamin are well tolerated in the elderly [redacted]

Pregnancy and Breast Feeding

Absorption of orally administered vitamin B12 is significantly increased by pregnancy in the human and in the rat. As yet, pregnancy is. the only known method of increasing vitamin B12 absorption in the adult. In spite of increased vitamin B12 absorption the serum vitamin B12 level and the vitamin B12 content of the liver and kidney of pregnant rats actually show a decrease, presumably due to the demands of the foetus which seems to be the main beneficiary of the increased vitamin B12 absorption rate in pregnancy ([redacted] .

Pregnant women in resource-poor areas are at risk of multiple micronutrient deficiencies, and diets that are low in animal products place women at increased risk of vitamin B12 deficiency.

Pregnancy causes a lowering of serum cobalamin. In normal pregnancy, total serum cobalamin levels fall by 30% by the third trimester Serum cobalamin levels during pregnancy and are less reliable in determining underlying deficiency. During pregnancy, in the presence of strong suspicion of underlying deficiency, a short course of empirical hydroxocobalamin should be given, with further investigations post-partum. HoloTC may be more reliable than serum cobalamin in determining deficiency in pregnancy, and is recommended as the test of choice, if available ([redacted] .

The median value of milk vitamin B12 concentration was 1.2 µg/L whereas in 50% of malnourished mother the concentrations fell below the detection limit (50 pmol/L). However, supplementation of Guatemalan lactating women with vitamin B12 depletion (serum vitamin B12, 150–221 pmol/L) with 3–1000 mg/d of the vitamin for 2 months, even at the highest doses, resulted only in slight increase in concentration in breast milk (from 67 to only 180 pmol/L, <1% of any dose was transferred to milk, and no dose reduced infant serum or urinary methylmalonic acid. Thus, maternal supplementation with vitamin B12 in lactation may be too late to restore adequate milk concentrations and infant status ([redacted] Further studies of this important question are needed.

Overdosage

There are no reports on overdose. Overdosage is unlikely to require treatment.

2.5.6. Benefits and Risks Conclusions

[redacted]

[redacted]

[redacted]

[redacted].

Redacted under sections 41 and 43 of the FOI Act

2.5.7. Literature References

Module	Number	Reference
5	1.	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]

		[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]

[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]

		[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]

5	54.	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]

Redacted under sections 41 and 43 of FOI Act