CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

22-041

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW(S)

ADDENDUM
to
CLINICAL PHARMACOLOGY REVIEW

NDA: 22-041	Submission Date: 6/16/06
Submission Type; Code:	**3P**
Brand/Code Name:	Cyanokit™
Generic Name:	Hydroxocobalamin
Primary Reviewer:	David Lee, Ph.D.
Secondary Reviewer	Suresh Doddapaneni, Ph.D.
OCP Division:	Clinical Pharmacology II
OND Division:	Anesthesia, Analgesia, and Rheumatology Products
Sponsor:	EMD Pharmaceuticals, Inc.
Relevant IND(s):	-
Formulation; Strength(s):	2.5 g
Proposed Indication:	Treatment of known or suspected cyanide poisoning
Proposed Dosage Regimen:	• Single 5.0 g (maximum of 10.0 g) by IV infusion o 5.0 g, (2.5 g x 2 vials) administered by IV infusion over 15 minutes. o A second dose of 5.0 g may be administered by IV infusion, depending upon the severity of the poisoning and the clinical response,, over 15 minutes (for patients in extremis) to 2 hours based on patient condition.

1 Executive Summary

1.1 Recommendations

On Nov 27, 2006, the Office of Clinical Pharmacology / Division of Clinical Pharmacology Evaluation II (OCP/DCP-II) completed the Cyanokit NDA submitted on 6/16/06. At that time, DSI inspection of study H101 was still pending.

Although there were some issues identified from the inspection (DSI Inspection Review 12/12/06), overall the impact of these issues does not seem to significantly affect the overall understanding of the Clinical Pharmacology aspects of the product. From OCP perspective, the information submitted in the NDA is acceptable. No further clinical pharmacology issues are pending at this time.

1.2 Discussion

Of the issues identified in the inspection the following critical items are further discussed and the implications assessed on the overall understanding of the Clinical Pharmacology aspects of the product.

Analytical Site: [redacted]

b(4)

8. Lack of adequate documentation for the following aspects of the studies:

a. Processing of QCs with subject samples.

At least two analysts were involved in processing of subject samples in each analytical run. There was no documentation to confirm that each analyst who processed subject samples also processed QCs. During the inspection, the firm stated that their practice allows one analyst to exclusively process QCs and the other to process only subject samples. Processing of QCs with subject samples by each analyst is essential to assure that all samples are handled identically. This enables QCs to reflect the accuracy of the subject concentrations.

Reviewer discussion: *It is acknowledged and agreed with the DSI inspection team with respect to the proper handling of the samples, including QCs. It is likely that, in a given analysis day, the calibration curve samples and the subject samples were prepared by one analyst and that the QC samples prepared another. Looking at the calibration standard and QC outputs, the measured QC concentration values appear to be in range with each other. Thus, the impact of this issue may be minimal.*

9. No internal standard was used in the assays.

Specifically the total cobalamin assay in plasma does not include an internal standard to normalize the variability during sample processing. Due to the high run failure rate (20-30%) found in this audit for total cobalamins, the failure to utilize an internal standard is of concern.

Reviewer discussion: *It is acknowledged that using an internal standard will minimize the variability of the sample handling and address the concerns of accuracy and precision of the assay. The variability issue, and thus, use of no internal standard, may be addressed by looking at the overall 'trend' of the*

analytical assay performed over 6 months time period (October 2004 to April 2005).

There were a total 19 days to analyze the samples for the Study H101. Of the 19 days, 6 days were labeled as 'Reassay' (it is also acknowledged that the Applicant 'reassayed' without providing objective criteria (see #10 below)). Looking at the standard curve and QC values generated from 19 days, the precision and accuracy appear to be relatively small (see Section 2.6 Analytical Section, Clinical Pharmacology Review dated 11/27/06), indicating that the assay is 'stable' and thus the variability produced by the assay will have a minimal impact on the overall assessment.

10. No objective criteria for selecting pharmacokinetic (PK) repeats.

The sponsor identified the PK repeats without providing objective criteria. In addition, firm selected repeats for confirmation purposes without established criteria. Majority of these repeats for total cobalamins in urine and plasma matched the original values. However, 44% (12 of 27) of the repeats for free cobalamin differed from their original values by >30%, suggesting the lack of reproducibility and confidence in the free cobalamin assay.

Reviewer discussion: *There were more than 600 plasma samples analyzed from the pharmacokinetic portion of the study. First, the critical moiety in this study is the total cobalamin complexes; there is no information whether the cyanide binds to only 'free' cobalamin complexes. The observed 'free' cobalamin concentrations were less than that of the 'total' cobalamin concentrations. It is likely that cyanide will bind to all cobalamin complexes. Looking at the 'total' cobalamin complexes aspect, the inspection does not have any issues. Secondly, compared to the overall number of samples analyzed, the 12 samples in the 'repeated' assays seem too small to drastically impact the overall assay process. Thus, although there were some hints for lack of reproducibility and confidence in the 'free' cobalamin assay, it appears that 'free' cobalamin assay will have a minimal impact on the overall assay.*

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/s/

David Lee
12/15/2006 01:41:09 PM
BIOPHARMACEUTICS

Suresh Doddapaneni
12/15/2006 02:19:02 PM
BIOPHARMACEUTICS

CLINICAL PHARMACOLOGY REVIEW

NDA: 22-041	Submission Date: 6/16/06
Submission Type; Code:	**3P**
Brand/Code Name:	Cyanokit™
Generic Name:	Hydroxocobalamin
Primary Reviewer:	David Lee, Ph.D.
Secondary Reviewer	Suresh Doddapaneni, Ph.D.
OCP Division:	Clinical Pharmacology II
OND Division:	Anesthesia, Analgesia, and Rheumatology Products
Sponsor:	EMD Pharmaceuticals, Inc.
Relevant IND(s):	-
Formulation; Strength(s):	2.5 g
Proposed Indication:	Treatment of known or suspected cyanide poisoning
Proposed Dosage Regimen:	• Single 5.0 g (maximum of 10.0 g) by IV infusion o 5.0 g, (2.5 g x 2 vials) administered by IV infusion over 15 minutes. o A second dose of 5.0 g may be administered by IV infusion, depending upon the severity of the poisoning and the clinical response,, over 15 minutes (for patients in extremis) to 2 hours based on patient condition.

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1 Executive Summary

1.1 Recommendations

The Office of Clinical Pharmacology / Division of Clinical Pharmacology Evaluation II (OCP/DCP-II) has reviewed the Cyanokit NDA submitted on 6/16/06. At this time, results of DSI inspection of study H101 are still pending.

From OCP perspective, the information contained in the NDA is acceptable, provided that DSI inspection of study H101 does not uncover significant issues affecting the acceptability of the data.

1.2 Phase IV Commitments

Not applicable.

1.3 Summary of CPB Findings

EMD Pharmaceuticals, Inc. has submitted NDA 22-041, Cyanokit®, for treatment of known or suspected cyanide poisoning.

The NDA contains both non-clinical and clinical information of hydroxocobalamin (HOCo) in the treatment of known or suspected cyanide poisoning. Non-clinical studies include a controlled study of survival in cyanide-poisoned dogs. Clinical studies include studies from the literature (1 prospective study (Baud 1), and 3 retrospective studies

(Baud 2 and 3, and Fortin)) for efficacy and one single-dose pharmacokinetic (PK) study – EML 015722-H101. This review will mainly focus on the pharmacokinetic portion of H101 study.

> Note: Baud 1, Baud 2, and Fortin studies examined the safety and efficacy of HOCo in treating cyanide poisoning in victims of fire smoke inhalation. Baud 1 served as the basis for marketing approval of hydroxocobalamin for the treatment of acute cyanide poisoning in France. Another retrospective study (Baud 3) examined the safety and efficacy of hydroxocobalamin in treating cyanide poisoning in victims exposed to very high amounts of cyanide from sources other than fire smoke.

Due to the ethical and practical difficulties in obtaining controlled clinical data of HOCo for the indication of acute cyanide poisoning, a non-clinical study was conducted under Good Laboratory Practice (GLP) conditions in dogs, to support efficacy under 21 CFR 314 subpart I, to provide evidence of efficacy when human efficacy studies are not ethical or feasible. This non-clinical study was agreed to by the Agency at a meeting with EMD Pharmaceuticals, Inc. on April 29, 2003.

Additionally, the clinical development program to evaluate the safety of HOCo included an additional controlled clinical study in healthy subjects, Study EML 015722-H101. This study was also agreed to by the Agency at the meeting with the Applicant on April 29, 2003, and was further evaluated by the Agency via a special protocol assessment prior to commencement of the study. Pharmacokinetic (PK) evaluations were also included for a subset of subjects in this study. There were several communications with the Applicant regarding this study (April 8, 2004, April 8, 2005, and May 23, 2005)

Study H101 results

After 2.5, 5, 7.5 and 10.0 g of HOCo administration, the Cmax and AUC values of both free and total cobalamins-(III) generally increased in proportion to dose, over the range of HOCo doses studied. A deviation from dose-proportionality was found for Cmax of free cobalamins-(III) in the 2.5 g dose group; this may have been due to the time required to achieve equilibrium between free and protein-bound cobalamins-(III), relative to the short infusion time in the 2.5 g dose group (7.5 minutes). Hydroxocobalamin reacts with plasma constituents to form various cobalamin-(III) complexes. The exact structure of these metabolites of hydroxocobalamin has not been investigated. In cyanide-poisoned individuals, hydroxocobalamin binds cyanide to form cyanocobalamin.

Cmax values ranged from 73.1 to 197.2 mcg eq/mL for free cobalamins-(III), and from 287.6 to 995.3 mcg eq/mL for total cobalamins-(III). AUCo-t values ranged from 188.4 to 762.5 mcg.eq.h/mL for free cobalamins-(III) and from 3566.0 to 14,271.5 mcg eq. h/mL for total cobalamins-(III). The average ratio of free to total cobalamins-(III), as estimated by the ratio of the respective mean AUCo-t and AUCo-inf amounted to approximately 5% in all dose groups.

The volume of distribution at steady state (Vss) for both free and total cobalamins-(III) showed no apparent relationship to dose. The Vss ranged from 280.7 to 349.5 L for free cobalamins-(III), and from 21.8 to 25.6 L for total cobalamins-(III). The comparatively high values for Vss of free cobalamins-(III) are due to the high protein binding of HOCo as it reacts in the blood with plasma constituents to form cobalamins-(III) complexes and the rapid distribution of free cobalamins-(III) into tissues. Reports from the literature indicate that "hydroxocobalamin" is able to cross the blood-brain barrier and be transported into cells.

The half-life (tI12) of free cobalamins-(III), and of total cobalamins-(III), was similar across dose groups and ranged from 25.9 to 30.3 hours for free cobalamins-(III), and from 29.6 to 32.8 hours for total cobalamins-(III).

The total body clearance (CL) of free cobalamins-(III) across all dose groups ranged from 12.5 to 13.2 L/h, which exceeds the normal glomerular filtration rate (approx. 4.8 to 7.9 L/h). This is suggestive of additional elimination of free cobalamins-(III), e.g. binding to proteins. Clearance of total cobalamins-(III) ranged from 0.566 to 0.645 L/h across all dose groups.

The percentage of total cobalamins-(III) excreted in urine from time zero to infinity (Aeo-inf) ranged from 58.2 to 73.8 %. These results suggest that hydroxocobalamin is eliminated largely via the renal route of excretion. There was somewhat less urinary recovery of total cobalamins-(III) at the 10.0 g dose than at lower doses. It should be noted that total cobalamins-(III) are not completely stable in native human urine particularly at 37°C, i.e., at the conditions in the bladder. This may represent the major reason for incomplete urinary recovery.

Special Populations

No PK or pharmacodynamic (PD) studies in special subject populations (e.g., subjects with renal or hepatic impairment, or in pediatric or elderly subjects) were performed.

In Study EML 015722-H10l, male and female subjects revealed no major differences in the plasma PK parameters of free and total cobalamins-(III), except for Vss and CL, which tended to be slightly higher in male than in female subjects. However, these differences were negligible when Vss and CL were normalized to body weight.

Drug Interactions

No specific PK studies were performed to study the interaction of hydroxocobalamin with other drugs, as part of the clinical development program. However, one clinical study report in the literature described the PK of hydroxocobalamin in the presence and absence of sodium thiosulfate. Peak hydroxocobalamin levels were slightly lower in subjects in the presence of sodium thiosulfate. The reason for the lower peak hydroxocobalamin levels is unknown but could be due, at least in part, to binding of

hydroxocobalamin by sodium thiosulfate. Several publications have reported binding of hydroxocobalamin to thiosulfate to form thiosulfatocobalamin.

Cyanocobalamin

No measurements of cyanocobalamin were performed by the sponsor in the clinical studies described in this submission.

> Note: It is noted that there are several literature reports that quantified cyanocobalamin after cyanide exposure, which the assays reported in the literature are vastly different from the assay methodology utilized by the Applicant; the discussion in this section is solely for the purpose of global understanding of cyanocobalamin detected in vivo after cyanide exposure. The reported studies speculated that there is an association between blood cyanide levels and cyanocobalamin formation. The concentrations of cyanocobalamin in humans, following high cyanide exposure and subsequent administration of 5.0 g of hydroxocobalamin, were in the range of 212.4 ± 30.9 mcmol/L (282 ± 41 mc eq/mL; Houeto et al. (1996)) and 227 ± 95 mcmol/L (302 ± 126 mc eq/mL, n=12; Houeto et al. (1995)). Maximum cyanocobalamin concentrations in cyanide-exposed patients treated with 5.0 g hydroxocobalamin measured by Astier and Baud (1995) were also within that range (318 ± 35 mc eq/mL, n=5 highest exposed patients). Houeto et al. (1995) measured a plasma concentration of 544 mcmol/L (723 mcg eq/mL) cyanocobalamin. Comparing cyanocobalamin levels with the dog efficacy study results, the reported cyanocobalamin concentrations are well below the 718 mcmol/L observed in dogs in the 14-day toxicity study T8380 after the maximum feasible dose of 400 mg/kg/d cyanocobalamin (see below paragraph). This dose of 400 mg/kg/d was considered to be the NOAEL in dogs; higher dose levels could not be tested due to limited solubility in water, which results in excessive administration volume at higher doses. Cyanocobalamin is excreted more rapidly than hydroxocobalamin. The tl/2 of cyanocobalamin in humans was reported to be 13.6 hours (±1.2 hours) by Houeto et al. (1996) and 9.3 hours (±3.2 hours) by Astier and Baud, (1995).

Dog cyanide efficacy study

The primary non-clinical efficacy study of hydroxocobalamin was conducted in dogs. The doses to be tested in the dogs were based on the stoichiometric binding information of HOCo to cyanide (1:1 binding), a lethal dose of cyanide in a 70 kg human (approx. 2.7 mmol), and from the literature data which the 'efficacious' dose started at 5.0 g. Additionally, the Applicant conducted dog intravenous toxicokinetic study (Study T8374) to supplement the dog efficacy study, which the results showed that dog's mean Cmax for total cobalamins-(III), following the 150 mg/kg dose, falls between healthy subjects' intravenous administration doses of 5.0 and 10.0 g. The no adverse effects level (NOAEL) was determined to be 300 mg/kg (calculation of safety margins is based on a human dose of 5.0 and 10.0 g).

Comparison of Pharmacokinetic Parameters Between Humans and Dogs:

Dose (mg/kg)	Gender	Dog Toxicity Study (IV) T8374		Human Safety Study (IV) EML 016722-H101	
		C_{max} [1]	AUC [2]	C_{max} [1]	AUC [2]
Free Cobalamins-(III) [6]					
75	M+F	-	-	113 ±21 [3]	395 ±37 [3]
150	M	410 ±54	308 ±32	197 ±40 [4]	814 ±153 [4]
150	F	429 ±31	304 ±22		
300 [5]	M	511 ±61	608 ±68	-	-
300 [5]	F	676 ±218	785 ±327	-	-
1200	M	1480 ±160	3520 ±1520	-	-
1200	F	1650 ±320	2730 ±130	-	-
Total Cobalamins-(III) [6]					
75	M+F	-	-	579 ±113 [3]	9420 ±2990 [3]
150	M	773 ±66	2350 ±180	995 ±149 [4]	15700 ±2570 [4]
150	F	922 ±73	2160 ±120		
300 [5]	M	1260 ±180	4110 ±490	-	-
300 [5]	F	1540 ±390	4850 ±1120	-	-
1200	M	3370 ±460	18800 ±5300	-	-
1200	F	3320 ±370	14500 ±400	-	-

1 (µg eq/mL)
2 (µg eq/mL•h)
3 5.0 g dose
4 10.0 g dose
5 NOAEL in T15026 and T8374
6 all means rounded to 3 significant digits, SD with same precision as the mean

The non-clinical efficacy study in dogs with cyanide (infusion of 0.4 mg/kg/min potassium cyanide) and HOCo (75 and 150 mg/kg doses) all dogs treated with high-dose HOCo survived while 79% (n=15) of dogs treated with the low-dose HOCo survived. However, only 17% (n=3) dogs survived from the vehicle treated group. This study showed that 75 and 150 mg/kg doses were highly effective in treating cyanide poisoning in dogs. See below for the PK parameters from this study.

Table 1 **Summary of important PK parameters from the dog efficacy study (m+f)**

Analyte	PK parameter	0 mg/kg OHCo group (n = 17)	75 mg/kg OHCo group (n = 19)	150 mg/kg OHCo group (n = 18)
CN	C_{max}	128±19.0	120±34.4	114±28.3
	$C_{EOI\ Cyanokit/saline}$	72.8±20.4	41.1±8.71	29.2±6.28
	$AUC_{0.1-2h}$	115±13.5	47.2±11.1	30.6±7.60
CNCo	C_{max}	-	78.1±10.5	99.3±15.4
	AUC_{0-2h}	-	45.5±6.72	52.5±7.39
Co_{tot}	C_{max}	a)	474±205	1190±599
	AUC_{0-2h}	a)	816±102	1820±219

a) not evaluated, since bioanalytical data indicated no exposure to cobalamins

- not investigated

Survival in Cyanide-Intoxicated Dogs:

Parameter	Treatment		
		Hydroxocobalamin (mg/kg)	
	Vehicle N=17 n (%)	75 mg/kg N=19 n(%)	150 mg/kg N=18 n(%)
Survival at Hour 4	7 (41.2)	18 (94.7)	18 (100.0)
Survival at Day 14	3 (17.6)	15 (78.9)	18 (100.0)

Therefore, considering the HOCo exposure from the Study T8374 and efficacy results from Study N106342, and considering the clinical use of hydroxocobalamin as a potentially life-saving drug, the safety margins based on the free cobalamins-(III), Cmax, and exposure (AUC) appear to be appropriate to recommend hydroxocobalamin for the treatment of patients with known or suspected cyanide poisoning at an initial dose of 5.0 g, which can be repeated once depending upon patient response (see 2.4.5).

DSI Inspection:

With respect to inspection of the Study H101 site, the DSI inspection is still pending. The results from the inspection will be provided in the subsequent review of this NDA as an addendum.

Overall, the information contained in this Submission for Cyanokit is acceptable, pending the favorable inspection from DSI. Since there is no human efficacy information from a prospectively designed study due to the ethical consideration, the Applicant is highly encouraged to gather information from the post-marketing of Cyanokit on the efficacy of HOCo and measurements of cyanocobalamin and HOCo in cyanide poisoning subjects.

2 QBR

2.1 General Attributes of the Drug

2.1.1 What is Cyanokit?

Cyanokit® for injection is an antidotal treatment for cyanide poisoning. The active ingredient in the Cyanokit is hydroxocobalamin (HOCo), the hydroxylated active form of vitamin B12. It is a highly hygroscopic, odorless, dark red, crystalline powder that is freely soluble in water, which binds cyanide directly.

The dosage form of Cyanokit is composed of 1 vial of lyophilized powder for IV infusion containing 2.5 g lyophilized HOCo. Two vials are contained in a single marked package.

Composition of a Cyanokit 2.5 g vial

Components	Quantity for 1 vial	Function	Quality Standards
Hydroxocobalamin	[redacted]	Drug substance	In-house monograph
[redacted]			

b(4)

2.1.2 What is cyanide poisoning? What is the proposed mechanism of action and therapeutic indication of hydroxocobalamin?

Cyanide affects all body tissues, since it is readily diffused into cells. Following absorption, cyanide binds ferric iron in mitochondrial cytochrome oxidase to form a reversible complex. Cyanide inhibits electron transport chains, oxidative phosphorylation, and reduces the cellular redox potential. This leads to cytotoxic anoxia, which shifts the cell into anaerobic metabolism, resulting in lactic acid production and eventual cell death.

Cyanide can be produced by smoke from household and industrial fires. Hydrogen cyanide and its salts (e.g., sodium, potassium, mercury, silver, and gold) are extensively used in industry. As well, cyanide and cyanogenic compounds are available for intentional misuse in suicide attempts and acts of terror.

Signs of cyanide poisoning include altered mental status, hypertension followed by hypotension (systolic blood pressure <90 mmHg), seizures or coma, mydriasis, tachypnea followed by bradypnea (respiratory rate below 10/min), cardiovascular collapse, vomiting, and/or lactate level ≥8 mmol/L. Blood concentrations of cyanide are usually unknown at the time when a treatment decision must be made.

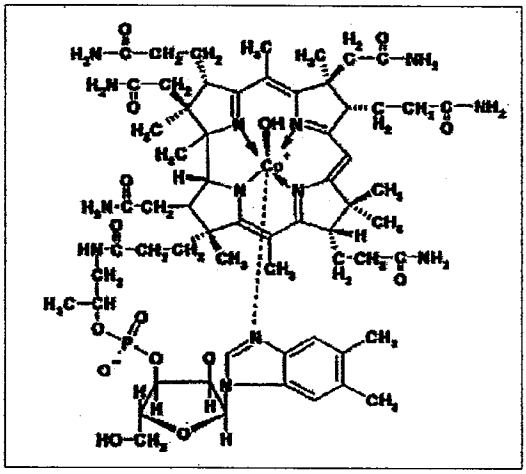
When HOCo is administered in vivo, plasma constituents 'initially' binds to HOCo to form cobalamin-(III) 'complexes.' Plasma constituents replace HO- portion of the HOCo

ligand (see below for HOCo structure), forming cobalamin-(III) complexes. There are two species: a) 'high-molecular' complex – large 'proteins' binding to HO-site, such as histidine- and possibly thiol-groups, and b) 'low-molecular' complex – small individual molecule binding to HO-site, such as thiols, histidine, glycine, thocyanate, etc.

After the initial binding to form both high- and low-complexes, these cobalamin-(III) complexes bind to cyanide to form 'cyanocobalamin-complexes.' Each HOCo molecule can bind one cyanide ion by substituting the hydroxo-ligand linked to the trivalent cobalt ion.

In vivo HOCo behavior had subsequent impact on the assay development. The Applicant stated that they developed an assay to distinguish both of above mentioned species. Thus, with respect to sample analysis, the Applicant used the term 'total' cobalamin-(III) complexes for the measurement of both of high- and low-complexes. The 'free' cobalamin-(III) complexes were defined as 'non-protein-bound' cobalamin-(III) complexes, that is, 'low-molecular' complexes. According to the literature, cyanocobalamin is cleared from the body via the kidneys with half-life of approx. 6 days (400 days in the liver).

Hydroxocobalamin :



2.1.3 How is this drug developed?

The first study describing the use of HOCo as an antidote for cyanide poisoning was reported in 1952. Since the 1970s, HOCo has been used to treat humans with acute cyanide poisoning, particularly in France. Cyanokit development started in 1994 and was approved by the French Authority in 1996 as an antidotal treatment for acute cyanide poisoning. The product also has a marketing authorization in Hong Kong. In other countries, Cyanokit has been distributed either with special permission in order to import or with an import certificate due to its emergency drug status.

By the end of the year 2005, the Applicant stated that approximately —— kits have been sold worldwide (e.g., ——kits ordered by the French government in 2002 and 2004, and ——kits ordered by the Italian government in 2004). However, the Applicant stated that the extent of patient exposure is unknown. b(4)

The one antidote currently available in the United States is the Cyanide Antidote Kit from Taylor Pharmaceuticals. However, Cyanide Antidote Kit is not currently available to the public since it is not marketed anymore.

Hydroxocobalamin is available in the United States in low doses as an intramuscular injection for the treatment of Vitamin B12 deficiency and pernicious anemia. It is available as a solution of 1000 mcg/mL; a dose of 100 to 1000 mcg once monthly is commonly used in clinical practice, which is considerably less than the initial adult dose of 5.0 g of HOCo used for the treatment of known or suspected cyanide poisoning.

2.1.4 What are the proposed dosage and route of administration?

The initial dose of Cyanokit® for adults is 5.0 g, (2 vials) administered by IV infusion over 15 minutes. Depending upon the severity of the poisoning and the clinical response, a second dose of 5.0 g may be administered by IV infusion up to a total dose of 10.0 g. The rate of infusion for the second dose ranges from 15 minutes (for patients in extremis) to 2 hours based on patient condition. The 5.0 g adult dose was proposed from the dog studies (toxicokinetic and cyanide- administered-hydroxocobalamin-antidote dog efficacy studies). See Section 2.2.1 for further discussion.

2.2 General Clinical Pharmacology

2.2.1 What are the design features of the pivotal clinical trials?

Since it is not ethical (i.e., dosing healthy subjects with cyanide) and due to the practical difficulties in obtaining controlled clinical data of HOCo for the indication of acute cyanide poisoning, a non-clinical efficacy study (Study no.: N106342) was conducted under Good Laboratory Practice (GLP) conditions in dogs, to support efficacy under 21 CFR 314 subpart I (this is to provide evidence of efficacy when human efficacy studies are not ethical or feasible). Additionally, the Applicant conducted dog toxicokinetic study (Study T8374) to supplement the dog efficacy study. The doses to be tested in the dogs were based on the stoichiometric binding information of HOCo to cyanide (1:1 binding), a lethal dose of cyanide in a 70 kg human (approx. 2.7 mmol), and from the literature data which the 'efficacious' dose started at 5.0 g. From a clinical perspective, the Baud studies were submitted to demonstrate the efficacy of HOCo in subjects with cyanide poisoning.

Non clinical studies

Study T8374:

Study T8374 was a single dose intravenous study, which hydroxocobalamin (0, 150, 300 or 1200 mg/kg, iv., 10 mL/min of 25 mg/mL) was administered to beagle dogs (n=4/sex/dose; 14-14.5 months of age; males: 7.6-10 kg; females 6.4-8.2 kg). Two dogs of each sex/dose combination were observed for 2 week following treatment (recovery dogs). Blood samples were analyzed for free- and total- cobalamin-(III). The results showed that dog's mean Cmax for total cobalamins-(III), following intravenous administration of the 150 mg/kg dose, falls between healthy subjects' intravenous administration doses of 5.0 and 10.0 g.

Comparison of Pharmacokinetic Parameters Between Humans and Dogs:

Dose (mg/kg)	Gender	Dog Toxicity Study (IV) T8374		Human Safety Study (IV) EML 015722-H101	
		C_{max}[1]	AUC[2]	C_{max}[1]	AUC[2]
Free Cobalamins-(III)[6]					
75	M+F	-	-	113 ±21[3]	395 ±37[3]
150	M	410 ±54	308 ±32	197 ±40[4]	814 ±153[4]
150	F	429 ±31	304 ±22		
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300[5]	F	676 ±218	785 ±327	-	-
1200	M	1480 ±160	3520 ±1520	-	-
1200	F	1650 ±320	2730 ±130	-	-
Total Cobalamins-(III)[6]					
75	M+F	-	-	579 ±113[3]	9420 ±2990[3]
150	M	773 ±66	2350 ±180	995 ±149[4]	15700 ±2570[4]
150	F	922 ±73	2160 ±120		
300[5]	M	1260 ±180	4110 ±490	-	-
300[5]	F	1540 ±390	4850 ±1120	-	-
1200	M	3370 ±460	18600 ±5300	-	-
1200	F	3320 ±370	14500 ±400	-	-

1 (µg eq/mL)
2 (µg eq/mL×h)
3 5.0 g dose
4 10.0 g dose
5 NOAEL in T15086 and T8374
6 all means rounded to 3 significant digits, SD with same precision as the mean

Study N106342:

Study N106342 determined the efficacy of intravenous administered HOCo when dogs were injected with cyanide. Cyanide poisoning was induced by intravenous infusion of 0.4 mg/kg/min potassium cyanide. Potassium cyanide was infused until apnea was seen and thereafter for additional 3 minutes. This produced severe cardiovascular and respiratory deterioration that could lead to death without therapy. All animals were mechanically ventilated with 100% oxygen at a rate of 10 to 20 breaths/minute and a tidal volume of ~15 mL/kg (parameters were set to approximate the baseline minute volume values of the individual dog). The dogs received either vehicle (n=17), low-dose HOCo (75 mg/kg i.v., n=19) or high-dose HOCo (150 mg/kg i.v., n=18) over 7.5 min. The animals were removed from the ventilator after a 15-minute period from end of apnea and allowed to breathe medical grade air.

Animals surviving the 2-hour monitoring period after the vehicle- or HOCo infusion were allowed to recover from anesthesia, had all catheters and monitoring equipment removed, then returned to their home cages and maintained for 14 days. The primary endpoint of the study was 14 days survival. Secondary endpoints were given by cardiovascular, ECG, and respiratory measurements, clinical chemistry and hematology, clinical observations, and histopathology.

Exposure to KCN was comparable between dose groups based on plasma mean (SD) Cmax values (vehicle = 128 ± 19 nmol/mL, low-dose = 120 ± 34 nmol/mL, high-dose = 114 ± 28 nmol/mL). These exposures resulted in similar cardiovascular deterioration and time to apnea in all groups.

At the end of HOCo infusion, cyanide concentration decreased to 30-40 nmol/mL, while in the non-treated placebo group cyanide concentrations remained at approximately 70 nmol/mL. Additionally, the mean cyanide AUC0-2h in HOCo treated dogs was reduced by 59% (in the low-dose group) and 73% (in the high-dose group) as compared to the respective mean AUC in the vehicle group. In parallel to the initial decrease in cyanide, formation of considerable amounts of cyanocobalamin was observed in the HOCo treated group. The mean Cmax of cyanocobalamin was 27% higher in dogs receiving the high-dose Cyanokit compared to the dogs receiving the low dose. Within the first four hours after KCN poisoning, approximately 60% (n= 1 0) of the vehicle dogs died, whereas only 5% (n=l) of the dogs treated with the low-dose Cyanokit died. No dog treated with high-dose Cyanokit died within 4 hours of KCN poisoning. At Day 14 all dogs treated with the high-dose HOCo survived and 15 (79%) dogs treated with the low-dose HOCo survived. However, only 3 (17%) dogs survived from the vehicle treated group. This study showed that 75 and 150 mg/kg doses were highly effective in treating cyanide poisoning in dogs.

Pharmacokinetic parameters from the efficacy dog study:

Table 1 **Summary of important PK parameters from the dog efficacy study (m+f)**

Analyte	PK parameter	0 mg/kg OHCo group (n = 17)	75 mg/kg OHCo group (n = 19)	150 mg/kg OHCo group (n = 18)
CN	C_{max}	128±19.0	120±34.4	114±28.3
	$C_{EOI\ Cyanokit/saline}$	72.8±20.4	41.1±8.71	29.2±6.28
	$AUC_{0.1\text{-}2h}$	115±13.5	47.2±11.1	30.6±7.60
CNCo	C_{max}	-	78.1±10.5	99.3±15.4
	$AUC_{0\text{-}2h}$	-	45.5±6.72	52.5±7.39
CO_{tot}	C_{max}	a)	474±205	1190±599
	$AUC_{0\text{-}2h}$	a)	816±102	1820±219

a) not evaluated, since bioanalytical data indicated no exposure to cobalamins
- not investigated

Survival in Cyanide-Intoxicated Dogs:

Parameter	Treatment		
		Hydroxocobalamin (mg/kg)	
	Vehicle N=17 n (%)	75 mg/kg N=19 n(%)	150 mg/kg N=18 n(%)
Survival at Hour 4	7 (41.2)	18 (94.7)	18 (100.0)
Survival at Day 14	3 (17.6)	15 (78.9)	18 (100.0)

Therefore, considering the HOCo exposure from the Study T8374 and efficacy results from Study N106342, the Applicant proposed the starting dose of 5.0 g for the treatment of known or suspected cyanide poisoning in humans. For additional information the reader is referred to Dr. Lawrence Leshin's Pharm/Tox Review.

Clinical literature information:

Baud 1 was a prospective study which included 69 smoke inhalation victims, 36 women and 33 men, who received HOCo (initial dose of 5.0 g up to a maximum total dose of 15.0 g; the median HOCo dose was 5.0 g (range: 4.0 to 15.0 g) and the median infusion time was 30 minutes.) either at the site of a fire, upon admission to hospital, or at both sites between 1987 and 1994. The median age of the patients was 44 years (range: 20 to 94 years). Fifteen of the 69 patients (22%) were ~65 years of age and among these, 10 were ~75 years of age. Fifty (50) of 69 patients (72%) survived following HOCo treatment. In patients with blood cyanide (BCN) levels considered toxic, 28 of 42 patients (66.6%) survived, including 11 of 19 patients (58%) with BCN levels considered as potentially lethaL. The median baseline BCN concentration was 52 mcmol/L (range: 0 to 250 mcmol/L). Forty-two (42) patients had baseline BCN concentrations ≥39 mcmol/L and were considered to have cyanide poisoning. Hydroxocobalamin was administered without awaiting laboratory confirmation of cyanide poisoning. Forty-one

(41) of the 50 patients who survived were discharged without sequelae, 9 patients had neuropsychiatric sequelae; 19 patients died, of which 13 were due to decerebration. In patients with a marked reduction in BP or those in initial cardiac arrest, the combination of symptomatic treatment and the specific antidote hydroxocobalamin was associated with hemodynamic recovery.

Baud 2 was a retrospective study which included 61 smoke inhalation patients, 31 females and 30 males, who received HOCo (the average total dose of HOCo administered at the fire scene plus the hospital (intensive care unit-ICU) was 7.1 ± 3.5 g with a range of 2.5 to 15.0 g) either at the scene of a fire, upon admission to the hospital, or at both sites between 1988 and 2004. The average age of patients in the study was 54.3 ± 18.3 years and ranged from 19.7 years to 91.9 years. 53 patients had soot deposits and, in this subgroup, the majority (67.9%) had soot in their lower airways. Fifty-one of the 61 patients (83.6%) had neurological symptoms. In the group of 51 patients with neurological symptoms, 34 (66.7%) had an initial loss of consciousness, 35 (68.6%) were in a coma, and 15 (29.4%) were diagnosed as having psycho-organic syndrome. Twenty-four patients (39.3%) died either in the ICU or other hospital departments and 34 patients (55.7%) survived and were discharged. For the remaining 3 patients (4.9%), the hospital outcome was missing. Death occurred, on average, 6.1 ± 7.0 days after the event. The causes of death were decerebration (5 patients), septic shock (2 patients), and other reasons (16 patients). Information regarding the cause of death was not available for 1 patient. Fifteen of the 17 patients who experienced cardiac arrest at the fire scene died whereas only 9 of the 44 patients who did not experience cardiac arrest at the fire scene died. Patients who were in cardiac arrest at the fire scene had a restoration of circulation by the time they were admitted to the hospital. Blood concentrations of cyanide, carbon monoxide, and lactate decreased within the first 24 hours following hospital admission.

Baud 3 was a retrospective study which included 14 patients, who were hospitalized between 1988 and 2003, with a history of acute cyanide poisoning, 13 by ingestion, and 1 by inhalation. Twelve of 14 cases were attempts at suicide. The 2 female patients and the 12 male patients had a mean age of 36.3 ± 13.2 years (14.8 to 64.0). Twelve had pretreatment blood sampling for blood cyanide. Initial blood cyanide ranged from 12.7 mcmol/L (0.3 mg/L) to 260 mcmol/L (6.8 mg/L) with a mean 159.0 ± 66.3 mcmol/L. In 11 of 12 cases, blood cyanide concentrations exceeded lethal concentrations (100 mcmol/L), and in 3 of these, were twice the lethal concentration. Mean initial systolic blood pressure (SBP) was 93.2 ± 53.9 mmHg (range 0 to 200). Two patients were found in cardiac arrest, 4 were found in shock. Five patients had a Glasgow Coma Score (GCS) of 3 at arrival of emergency medical services. The average total dose of HOCo administered at the scene plus the Intensive Care Unit (ICU) was 9.2 ± 4.3 g with a range of 5 to 20 g. Other antidotes (sodium thiosulfate and/or dicobalt edetate, or dimercaprol and dimethylsuccinic acid) were given in addition to HOCo in 6 patients.

Fortin was a retrospective study which included 101 smoke inhalation patients, 48 females and 53 males, who received Cyanokit (HOCo) that was given at the scene of the fire by the Paris Fire Brigade emergency medical team between 1995 and 2003. The median age of patients in the study was 48.5 years with a range from 2 to 88 years. The

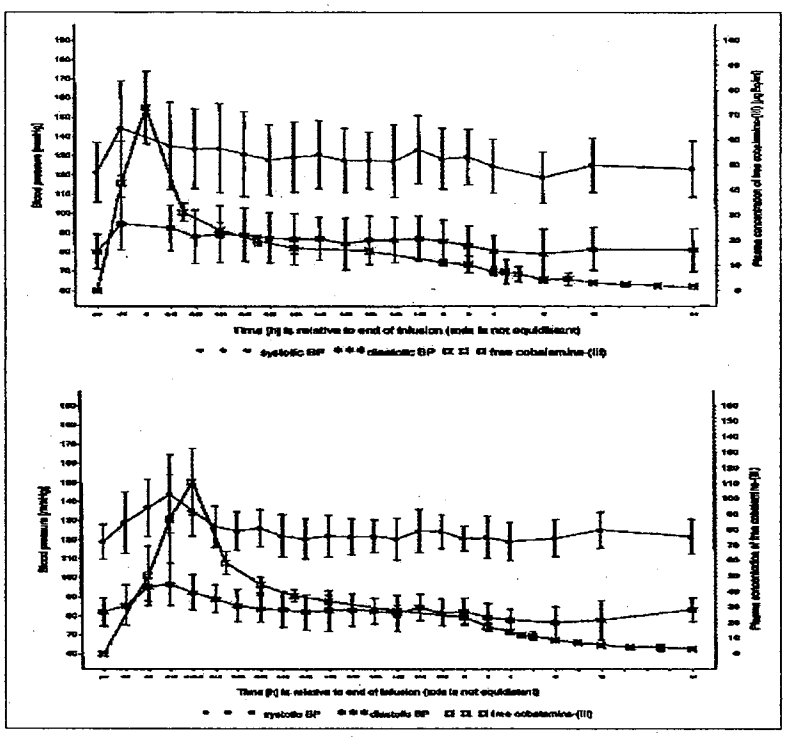
majority (n=90) of patients were adults (~19 years); 8 patients were children (c:19 years). Smoke inhalation was concomitant with burns in 53 patients, with multiple trauma in 2 patients and with a suicide attempt with drugs in 3 patients. Smoke inhalation was confirmed by the presence of soot in 72 patients (71.3%). The median dose of Cyanokit was 5.0 g (range: 1.0 to 10.0 g) in the total population, 1.5 g (range: 1.0 to 3.5 g) in children and 5.0 g (range: 2.0 to 10.0 g) in the adult population; two patients received 10.0 g. Of the 101 patients treated with Cyanokit at the fire scene, 84 survived and were admitted to the hospital and 17 died at the scene of the fire due to cardiac arrest. Death occurred at the Intensive Care Unit (ICU) in 25 of 84 hospitalized patients. Survival was documented in 30 patients. Hospital outcome was unknown in the remaining 29 patients due to the lack of hospital discharge summaries, which were not available for this study. Of the 38 patients with initial cardiac arrest, 21 patients (55.3%) had a return of spontaneous circulation on site and were admitted to the hospital; 17 of these 21 patients died within 1 to 8 days. Of the 12 patients who were hemodynamically unstable (o<systolic blood pressure (SBP ~90 mmHg), 9 patients (75%) recovered in approximately 30 minutes after the start of Cyanokit administration.

In all, the Applicant stated that HOCo was administered without significant problem in both pre- and hospital settings in patients with suspected cyanide poisoning associated with smoke inhalation. For additional information the reader is referred to Dr. Arthur Simone's Medical Officer's Review.

2.2.2 **What are the characteristics of the exposure-response relationships (dose-response, concentration-response) for safety? If relevant, indicate the time to the onset and offset of the pharmacological response or clinical endpoint.**

The results from correlations between plasma concentration and safety parameters indicated that there were no noteworthy changes when HOCo was administered:

Mean (SD) Plasma Concentration of Free Cobalamins-(III) and Mean (SD) Blood Pressure Versus Time in the 2.5- (top) and 5.0-g OH-Co Dose Group - PK Population



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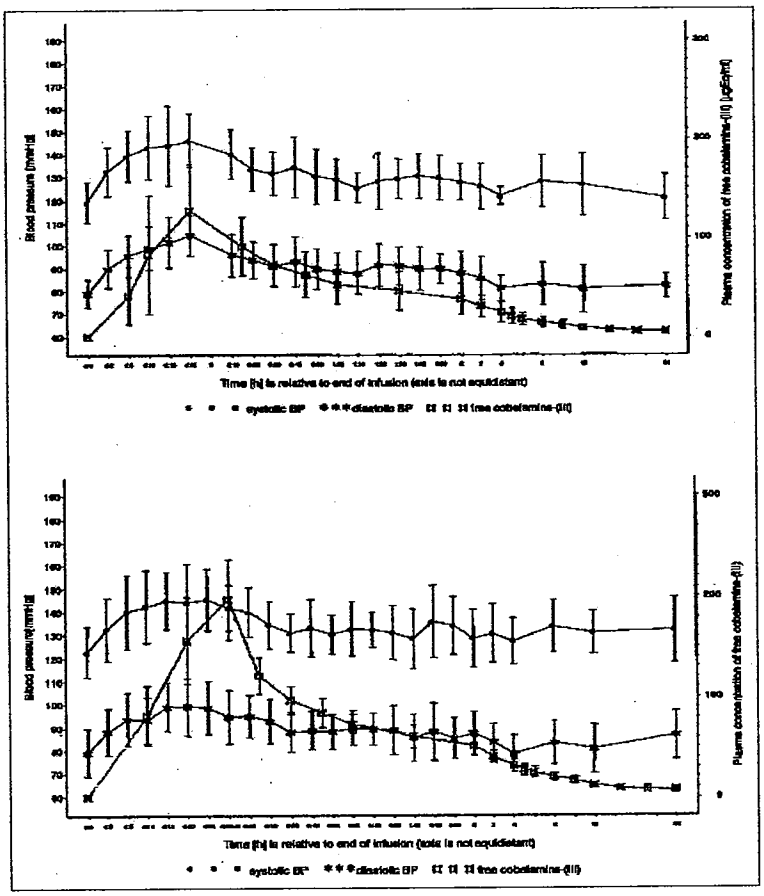
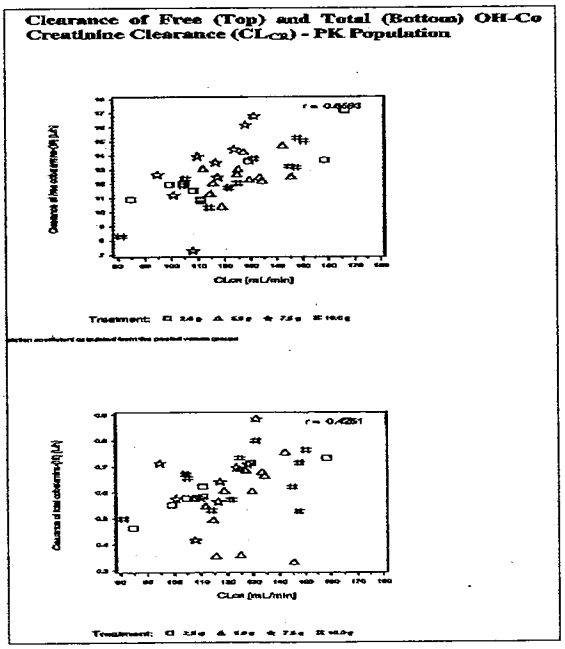


As expected a correlation between CLCR and HOCo was observed:



Overview on the Correlation Coefficients, r, of Laboratory Versus Pharmacokinetic Parameters

Correlation parameters		Time after end of infusion (n=41)					
Laboratory parameter	Plasma concentration of cobalamins-(III)	Day 1 2 h	Day 1 4 h	Day 1 12 h	Day 2	Day 3	Day 4
PT [%]**	Free	−0.67	−0.57	—	—	—	—
	Total	−0.63	−0.58	—	—	—	—
APTT [s]*	Free	0.63	0.46	—	0.35	—	—
	Total	0.57	0.43	0.31	0.45	0.41	0.34
Lymphocytes [10^9/L]	Free	—	—	−0.41	−0.43	—	0.31
	Total	—	—	—	−0.43	—	—
Lymphocytes [%]	Free	—	—	−0.44	−0.44	—	—
	Total	—	−0.32	−0.36	−0.42	—	—
Hemoglobin [G/L]*	Free	—	—	—	—	—	—
	Total	−0.31	—	—	—	—	—
LDH [IU/L]	Free	—	0.34	0.32	0.37	0.31	—
	Total	—	0.38	0.31	0.41	0.31	—
Total bilirubin [µmol/L]*	Free	0.92	0.91	0.89	0.89	0.83	0.68
	Total	0.93	0.89	0.82	0.77	0.71	0.65
Creatinine [µmol/L]*	Free	—	—	—	—	—	—
	Total	—	—	—	—	—	—
Triglycerides [mmol/L]*	Free	—	—	—	—	—	—
	Total	0.36	—	—	—	—	—
Cholesterol [mmol/L]*	Free	—	—	—	—	—	—
	Total	—	—	—	—	—	—
GPT(Cl) (ALT) [IU/L]**	Free	—	—	—	—	—	—
	Total	—	—	—	—	—	—
GPT(K) (ALT) [IU/L]* and**	Free	−0.53	−0.43	—	—	—	—
	Total	−0.51	−0.39	—	—	—	—
GOT (AST) [IU/L]	Free	—	—	—	0.41	0.39	—
	Total	—	—	0.32	0.48	0.43	0.34
Creatine kinase [IU/L]**	Free	—	—	—	—	—	—
	Total	—	—	—	—	—	—
Amylase [IU/L]**	Free	—	—	—	—	—	—
	Total	—	—	—	—	—	—
Uric acid [mmol/L]**	Free	0.31	—	—	—	—	—
	Total	—	—	—	—	—	—
Bicarbonate [mmol/L]***	Free	—	—	—	—	—	—
	Total	—	—	—	—	—	—

r-values >0.7 ("high correlation") are bolded, values −0.3 < r < 0.3 ("no apparent correlation") are indicated as —

*Parameter interfered positively with cobalamins-(III) during measurement

**Parameter interfered negatively with cobalamins-(III) during measurement

***Parameter interfered to <10% with cobalamins-(III) during measurement

2.2.3 Does this drug prolong the QT or QTc interval?

No formal QT study was conducted. However, twelve-lead ECG data from the Study H101 did not indicate any noteworthy changes.

2.2.4 What are the single dose PK parameters in healthy volunteers?

After 2.5, 5, 7.5 and 10.0 g of HOCo administration, the Cmax and AUC values of both free and total cobalamins-(III) generally increased in proportion to dose, over the range of

HOCo doses studied. A deviation from dose-proportionality was found for Cmax of free cobalamins-(III) in the 2.5 g dose group; this may have been due to the time required to achieve equilibrium between free and protein-bound cobalamins-(III), relative to the short infusion time in the 2.5 g dose group (7.5 minutes).

The following tables show mean PK parameters for free and total cobalamin-(III):

a. Pharmacokinetic Parameters of **Free** Cobalamins-(III) Following i.v. Administration of 2.5 to 10.0 g HOCo

Dose		C_{max} (µg eq/mL)	t_{max} (h)	AUC_{0-t} (µg eq/mL*h)	$AUC_{0-\infty}$ (µg eq/mL*h)	$t_{1/2}$ (h)	V_{ss} (L)	CL (L/h)
2.5 g (N=9)	Mean	73.1	0.142	188.4	201.3	30.3	325.6	12.5
	SD	14.5	0.041	25.5	28.1	2.4	41.0	2.1
	Min	46.8	0.125	136.1	143.6	27.5	285.5	10.8
	Max	90.8	0.250	212.9	227.6	35.0	405.2	17.2
	CV%	19.9	29.3	13.5	13.9	7.8	12.6	16.4
5 g (N=12)	Mean	112.7	0.242	366.0	394.6	30.2	349.5	12.6
	SD	20.8	0.029	37.2	36.9	6.7	99.1	1.2
	Min	68.4	0.183	311.2	335.2	23.8	243.4	10.4
	Max	150.4	0.283	446.3	473.8	49.5	629.4	14.7
	CV%	18.4	12.0	10.2	9.3	22.1	28.4	9.2
7.5 g (N=9)	Mean	128.6	0.371	562.3	593.4	27.5	333.1	13.2
	SD	46.9	0.047	167.7	171.5	2.5	91.5	2.8
	Min	78.9	0.261	411.4	440.6	25.4	152.3	7.3
	Max	235.6	0.416	973.7	1013.2	31.5	455.8	16.8
	CV%	36.4	12.8	29.8	28.9	9.0	27.5	21.4
10 g (N=11)	Mean	197.2	0.506	762.5*	813.8	25.9	280.7	12.5
	SD	40.3	0.013	141.9	153.3	2.7	62.0	2.0
	Min	125.3	0.500	612.4	647.5	22.6	203.3	8.3
	Max	249.6	0.532	1114.4	1190.7	30.0	406.6	15.2
	CV%	20.4	2.6	18.6	18.8	10.4	22.1	18.1

b. Pharmacokinetic Parameters of **Total** Cobalamins-(III) Following i.v. Administration of 2.5 to 10.0 g HOCo

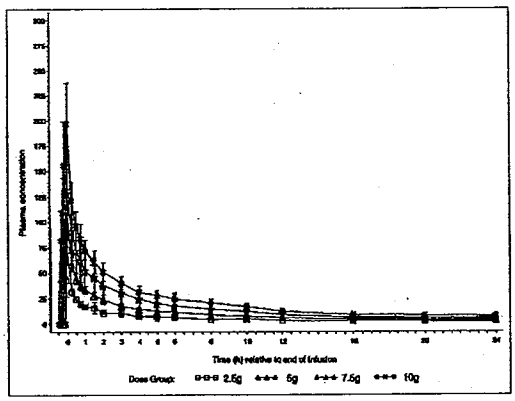
Dose		C_{max} (µg eq/mL)	t_{max} (h)	AUC_{0-t} (µg eq/mL*h)	$AUC_{0-\infty}$ (µg eq/mL*h)	$t_{1/2}$ (h)	V_{ss} (L)	CL (L/h)
2.5 g (N=9)	Mean	287.6	0.225	3566.0	4018.4	32.8	25.6	0.633
	SD	32.5	0.119	636.9	728.3	2.9	4.8	0.122
	Min	253.5	0.125	2475.2	2820.3	29.6	19.9	0.464
	Max	363.2	0.375	4705.5	5322.2	38.5	35.6	0.875
	CV%	11.3	53.0	17.9	18.1	8.9	18.6	19.3
5.0 g (N=12)	Mean	579.0	0.324	8453.7	9422.9	31.0	21.8	0.566
	SD	112.6	0.110	2639.8	2991.6	2.8	5.0	0.148
	Min	441.9	0.250	6091.2	6525.1	25.6	13.1	0.333
	Max	778.8	0.500	13007.4	14826.0	35.0	28.4	0.757
	CV%	19.4	34.1	31.2	31.7	8.9	22.8	26.1
7.5 g (N=9)	Mean	740.3	0.525	10815.0	11963.6	30.5	24.3	0.644
	SD	182.7	0.118	2479.2	2640.2	4.6	5.9	0.131
	Min	541.6	0.367	7396.6	8363.3	26.8	13.9	0.418
	Max	1180.3	0.649	16439.3	17715.7	41.1	34.9	0.885
	CV%	24.7	22.5	22.9	22.1	15.2	24.3	20.3
10 g (N=11)	Mean	995.3	0.551	14271.5	15681.1	29.8	23.0	0.645
	SD	149.1	0.099	2166.5	2571.5	4.7	2.7	0.103
	Min	770.7	0.500	11863.3	12339.5	21.6	18.1	0.498
	Max	1240.6	0.750	18324.1	19824.6	39.2	26.9	0.800
	CV%	15.0	18.0	15.2	16.4	15.8	11.7	16.0

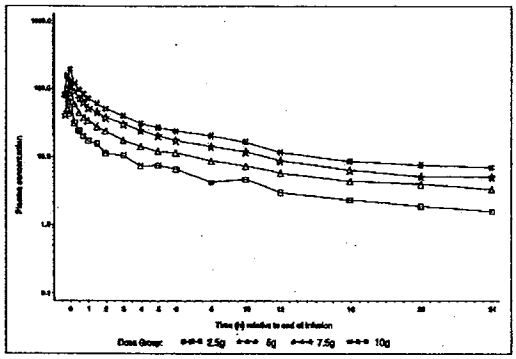
c. Pharmacokinetic Parameters of **Total** Cobalamins-(III) in **Urine** After Administration of 2.5 to 10.0 g OH-Co

Dose		$Ae_{0\text{-}72}$ (g eq)	$Ae_{0\text{-}72}$ (%)	CL_R (L/h)	$Ae_{0-\infty}$ (g eq)	$Ae_{0-\infty}$ (%)
2.5 g (N=9)	Mean	1.46	59.0	0.459	1.80	72.7
	SD	0.29	11.7	0.122	0.38	15.3
	Min	0.92	37.3	0.260	1.09	44.4
	Max	1.97	79.7	0.683	2.45	99.3
	CV%	19.8	19.8	26.6	21.0	21.0
5 g (N=12)	Mean	3.01	60.9	0.414	3.64	73.8
	SD	0.54	11.0	0.122	0.67	13.6
	Min	2.14	43.3	0.210	2.58	52.4
	Max	3.92	79.4	0.585	4.75	96.2
	CV%	18.1	18.1	29.5	18.5	18.5
7.5 g (N=9)	Mean	4.26	57.5	0.443	5.11	69.1
	SD	0.42	5.7	0.092	0.54	7.3
	Min	3.67	49.5	0.299	4.35	58.7
	Max	4.81	64.9	0.567	5.79	78.2
	CV%	9.9	9.9	20.7	10.5	10.5
10 g (N=11)	Mean	4.84	49.0	0.372	5.74	58.2
	SD	1.35	13.6	0.118	1.65	16.7
	Min	2.01	20.4	0.159	2.33	23.6
	Max	6.65	67.3	0.538	7.90	80.0
	CV%	27.8	27.8	31.6	28.8	28.8

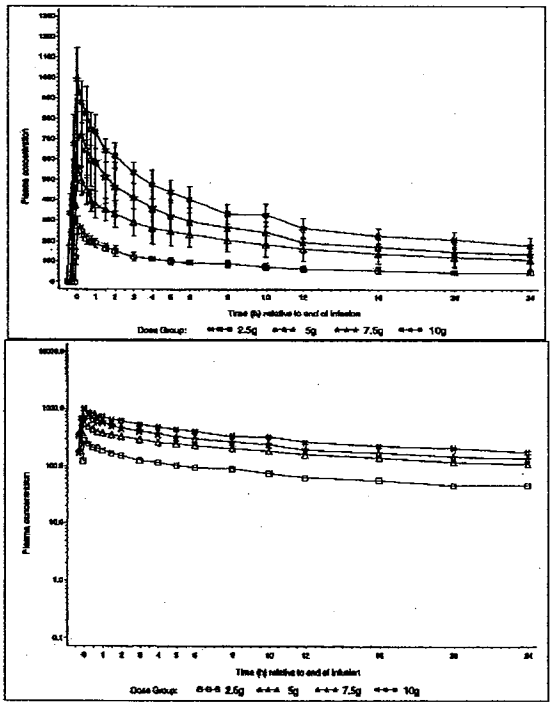
The following figures represent free and total cobalamin-(III) observed in all dosing groups:

a. Free Cobalamins-(III) Mean (SD) Plasma Concentrations Over Time of Each Dose Group on a Linear (Top) and Semi-Logarithmic Scale (Bottom)



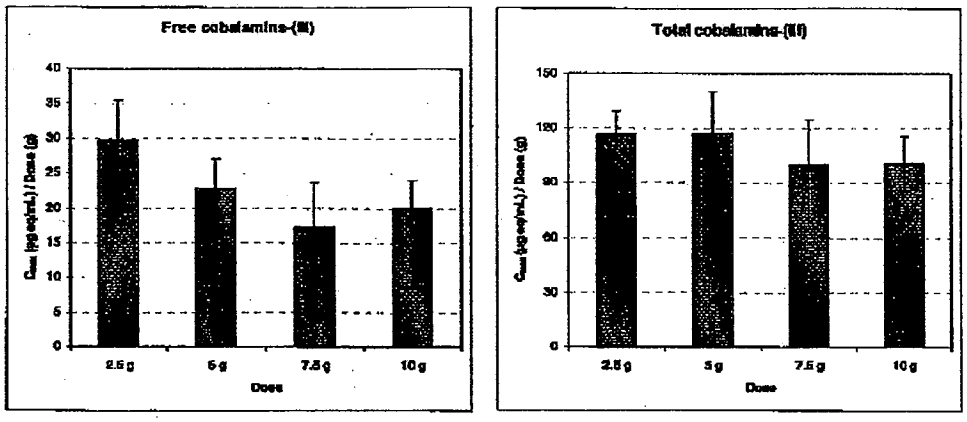


b. Total Cobalamins-(III) Mean (SD) Plasma Concentrations Over Time of Each Dose Group on a Linear (Top) and Semi-Logarithmic Scale (Bottom)

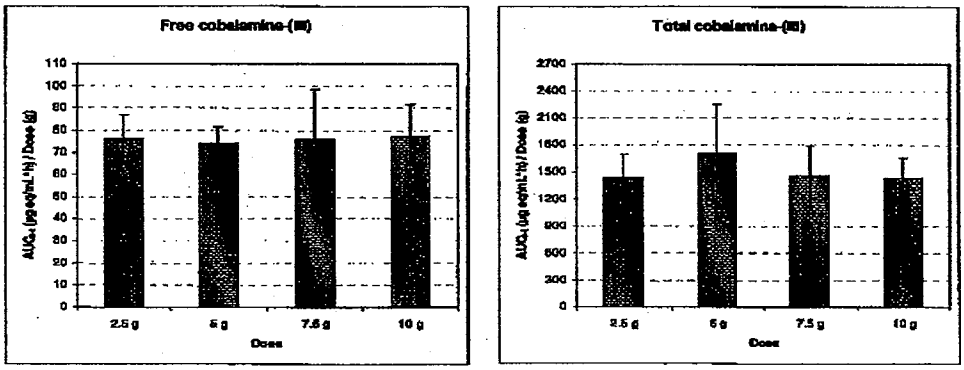


The following figures capture the cobalamin-(III) exposure comparison across all dosing groups.

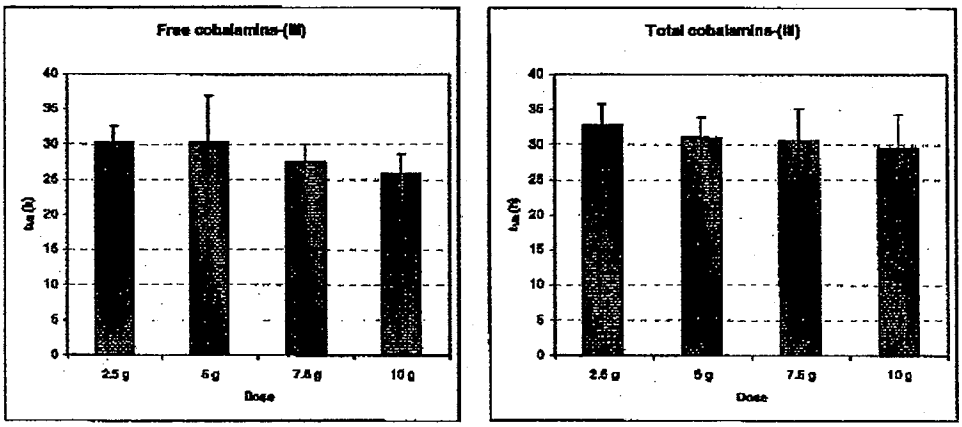
a. Mean (SD) Dose-Normalized Cmax of Free and Total Cobalamins-(III) in Plasma



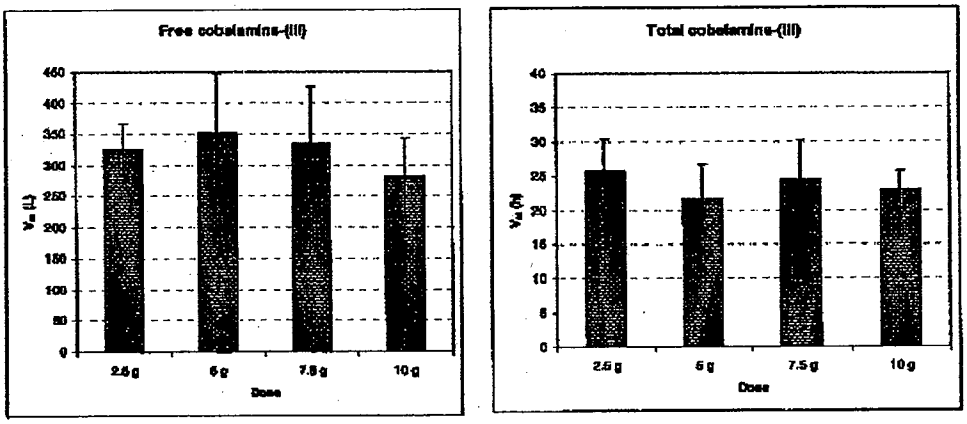
b. Mean (SD) Dose-Normalized AUC0-t of Free and Total Cobalamins-(III) in Plasma



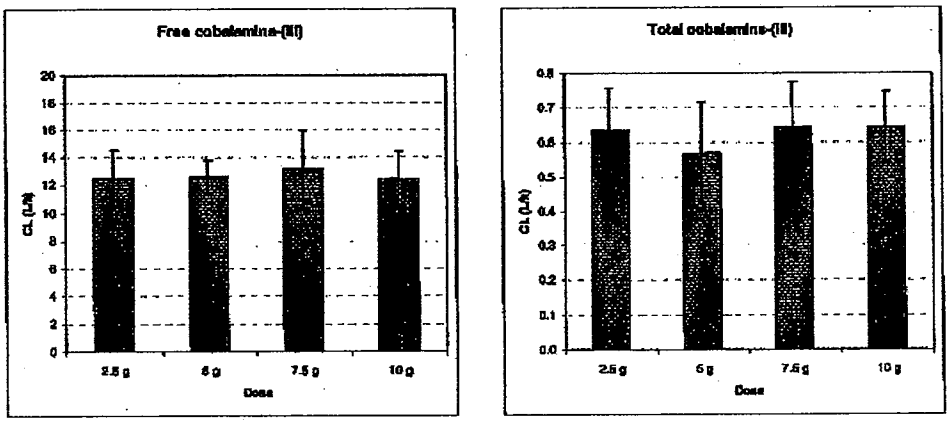
c. Mean (SD) t1/2 of Free and Total Cobalamins-(III) in Plasma



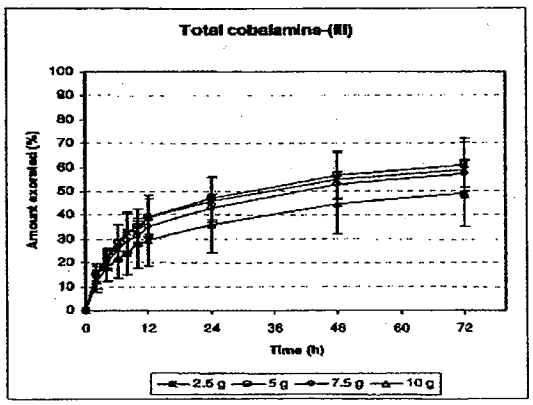
d. Mean (SD) Vss of Free and Total Cobalamins-(III) in Plasma



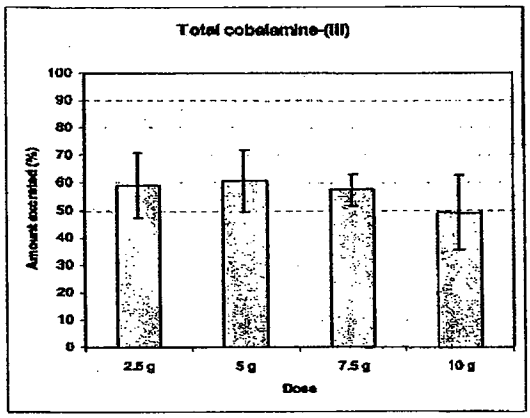
e. Mean (SD) CL of Free and Total Cobalamins-(III) in Plasma



f. Mean (SD) Cumulative Percentage Amount of Total Cobalamins-(III) Excreted in Urine Versus Time - PK Population



g. Mean (SD) Ae (% of Dose) of Total Cobalamins-(III) Versus Dose - PK Population



Special Populations
No PK or pharmacodynamic (PD) studies in special subject populations (e.g., subjects with renal or hepatic impairment, or in pediatric or elderly subjects) were performed.

In Study EML 015722-H10l, male and female subjects revealed no major differences in the plasma PK parameters of free and total cobalamins-(III), except for Vss and CL, which tended to be slightly higher in male than in female subjects. However, these differences were negligible when Vss and CL were normalized to body weight.

Drug Interactions
No specific PK studies were performed to study the interaction of hydroxocobalamin with other drugs, as part of the clinical development program.

Cyanocobalamin
No measurements of cyanocobalamin were performed by the sponsor in the clinical studies described in this submission.

2.2.5 **What are the characteristics of drug distribution?**

The volume of distribution at steady state (Vss) for both free and total cobalamins-(III) showed no apparent relationship to dose. The Vss ranged from 280.7 to 349.5 L for free cobalamins-(III), and from 21.8 to 25.6 L for total cobalamins-(III). The comparatively high values for Vss of free cobalamins-(III) are due to the high protein binding of HOCo as it reacts in the blood with plasma constituents to form cobalamins-(III) complexes and the rapid distribution of free cobalamins-(III) into tissues. Reports from the literature

indicate that "hydroxocobalamin" is able to cross the blood-brain barrier and be transported into cells.

2.2.6 What are the characteristics of drug metabolism? [Include- high vs low extraction ratio drug; metabolic scheme; enzymes responsible for metabolism; fractional clearance of drug]

Hydroxocobalamin does not undergo metabolism.

2.2.6.1 What are the characteristics of drug excretion?

Hydroxocobalamin is mainly excreted in urine.

2.3 Intrinsic Factors

2.3.1 What is the status of pediatric studies and/or any pediatric plan for study?

The Applicant did not address the pediatric issues. The appropriate dosage in the pediatric population should be determined for the safe and effective use of this product. If the drug product is approved now, proper usage or information of this product in pediatric population should be obtained as a post marketing commitment..

2.3.2 Special populations Gender

No PK or pharmacodynamic (PD) studies in special subject populations (e.g., subjects with renal or hepatic impairment, or in pediatric or elderly subjects) were performed.

In Study EML 015722-H10l, male and female subjects revealed no major differences in the plasma PK parameters of free and total cobalamins-(III), except for Vss and CL, which tended to be slightly higher in male than in female subjects. However, these differences were negligible when Vss and CL were normalized to body weight.

2.3.3 Renal impairment

No studies were conducted in renally impaired subjects. However, since HOCo is mainly excreted in urine, the subjects with impaired renal function should be closely monitored, although the HOCo is administered as a single dose. The recommended dosing regimen is 5.0 g of HOCo initially, followed by another 5.0 g, as needed, for the maximum of 10.0 g.

2.4 Extrinsic Factors

2.4.1 Drug-Drug Interactions

No specific PK studies were performed to study the interaction of hydroxocobalamin with other drugs, as part of the clinical development program. Since HOCo does not undergo metabolism and excretes as is, the drug-drug interaction is not likely to occur.

2.5 General Biopharmaceutics – not applicable

2.6 Analytical Section

2.6.1 What is the range of the standard curve? How does it relate to the requirements for clinical studies? What curve fitting techniques are used? What are the lower and upper limits of quantification (LLOQ/ ULOQ)? What is the accuracy, precision and selectivity at these limits? What is the sample stability under the conditions used in the study? (long-term, freeze-thaw, sample-handling, sample transport, autosampler) What is the QC sample plan?

Overall, the Applicant's approach and results from assay development and analytical runs are acceptable. Cobalamin-(III) complexes were measured by an HPLC-UV system.

Total Cobalamins-(III) in plasma

The lower limit of quantification (LLOQ) for the determination of Total Cobalamins-(II) was set to 0.1 mcg eq/mL. The concentration of the calibration curves was in the range from 0.1 mcg eq/mL up to 500 mcg eq/mL. According to the analysis of the calibration samples the assay was linear with determination coefficients (r2) of 0.99386 or higher. Summary statistics of the calibration samples show that the accuracy (expressed as bias) was between -7.21 % to 4.38% and the precision ranged from 1.87% to 5.95%. The inter-batch accuracy (expressed as bias) was between -2.88% to 3.50% for Cyanocobalamin QC samples, -8.47% to -3.07% for the spiked Hydroxocobalamin QC samples (including diluted QC samples) and the inter-batch precision given as RSD% was between 4.33% to 9.42% for Cyanocobalamin QC samples, 1.26% to 10.98% for the spiked Hydroxocobalamin QC samples.

Calibration standards:

		Calibration standard (µg eq/mL)									
Injection date	Analysis	0.100	0.200	0.500	2.000	5.000	20.000	50.000	200.000	400.000	500.000
21-Oct-04	Assay 1	0.109	0.194	0.478	2.020	4.835	20.184	49.570	204.374	404.608	496.204
		0.097	0.189	0.458	2.089	4.976	20.528	50.474	208.584	411.417	493.803
22-Oct-04	Assay 2	0.095	0.199	0.466	1.992	4.710	19.147	50.886	213.106	434.254	527.205
		0.112	0.189	0.435	1.960	4.628	18.730	51.403	211.344	429.497	524.991
25-Oct-04	Assay 3	0.106	0.190	0.466	2.001	4.870	19.999	49.866	209.001	413.506	505.527
		0.101	0.194	0.467	2.063	4.764	20.380	49.102	211.510	412.622	506.494
26-Oct-04	Reassay 1	0.099	0.190	0.455	1.952	4.706	20.255	49.807	208.781	412.552	485.589
		0.102	0.215	0.482	1.936	4.812	20.641	49.676	212.335	417.765	504.239
02-Nov-04	Reassay 2	0.096	0.207	0.426	-	4.630	20.575	50.982	216.174	414.615	476.342
		0.110	0.186	0.463	1.927	4.961	20.807	50.611	216.039	420.240	477.624
10-Nov-04	Reassay 3	0.099	0.213	0.473	2.013	4.834	20.623	49.335	205.587	414.648	481.918
		0.096	0.211	0.504	1.934	4.762	20.756	48.730	204.304	411.661	485.643
03-Dec-04	Assay 4	0.110	0.190	0.455	1.967	4.962	20.859	51.555	208.128	409.677	508.533
		0.103	0.173	0.445	2.020	4.995	20.683	51.262	205.331	406.137	504.541
03-Dec-04	Assay 5	0.103	0.182	0.502	2.021	4.859	20.189	51.360	203.032	411.267	497.648
		0.105	0.183	0.523	1.930	4.739	20.073	51.179	201.706	416.909	499.626
06-Dec-04	Assay 6	0.106	0.201	0.456	2.023	4.855	20.786	50.729	203.312	422.628	503.844
		0.097	0.197	0.469	1.969	4.850	20.369	49.574	201.069	413.102	497.700
07-Dec-04	Assay 7	0.103	0.185	0.470	2.041	5.074	20.421	49.866	208.550	406.142	508.086
		0.104	0.202	0.447	1.924	4.907	20.378	50.069	203.491	413.546	508.275
08-Dec-04	Reassay 4	0.101	0.188	0.484	2.032	4.837	20.076	50.503	200.755	420.261	500.155
		0.105	0.193	0.491	1.978	4.785	20.406	50.084	203.243	422.519	500.099
09-Dec-04	Reassay 5	0.110	0.207	0.475	1.936	4.616	20.292	50.436	203.564	421.535	494.484
		0.094	0.181	0.506	1.939	5.014	20.597	50.610	201.172	427.387	497.063
06-Jan-05	Reassay 6	0.110	0.189	0.460	1.896	4.673	20.201	48.772	-	394.344	531.913
		0.096	0.193	0.453	1.886	5.496	19.680	47.916	228.206	430.784	527.743
17-Feb-05	Assay 8	0.103	0.197	0.454	1.875	4.926	19.585	54.141	202.265	428.428	489.249
		0.103	0.191	0.479	1.995	4.909	19.631	53.478	200.531	426.836	488.725
18-Feb-05	Assay 9	0.105	0.175	0.443	1.861	4.896	20.222	51.987	206.365	425.524	508.233
		0.105	0.206	0.434	1.861	5.067	20.340	52.590	208.318	425.096	514.178
22-Feb-06	Assay 10	0.107	0.213	0.453	1.877	4.920	20.636	52.482	208.480	421.621	462.504
		0.092	0.201	0.494	1.884	5.024	20.157	53.114	205.545	424.591	457.629
23-Feb-05	Assay 11	0.095	0.195	0.465	1.835	4.893	19.882	52.148	202.739	418.786	525.722
		0.112	0.185	0.494	1.868	4.893	19.869	51.635	203.739	423.822	519.965
17-Mar-05	Assay 12	0.108	0.207	0.452	1.930	4.946	20.536	51.725	228.226	404.786	505.551
		0.101	0.184	0.428	1.851	4.731	20.072	50.543	224.231	405.402	503.197

		Calibration standard (µg eq/mL)									
Injection date	Analysis	0.100	0.200	0.500	2.000	5.000	20.000	50.000	200.000	400.000	500.000
22-Mar-05	Assay 13	0.110	0.191	0.442	1.933	5.283	20.469	52.327	208.523	414.302	503.997
		0.100	0.190	0.446	1.912	5.128	19.903	50.803	206.532	410.045	503.604
23-Mar-05	Assay 14	0.104	0.217	0.443	1.802	5.079	21.026	52.264	211.101	418.649	493.609
		0.093	0.215	0.451	1.872	4.933	20.452	50.720	203.316	413.477	484.568
24-Mar-05	Assay 15	0.117	0.196	0.440	1.927	4.988	20.114	52.124	208.440	419.959	493.726
		0.095	0.176	0.435	1.936	5.032	20.548	51.975	211.800	426.648	490.905
25-Mar-05	Assay 16	0.101	0.209	0.444	1.889	5.024	20.403	51.851	207.852	420.584	485.640
		0.101	0.199	0.453	1.863	5.032	20.443	52.514	208.040	417.414	481.619
29-Mar-05	Assay 17	0.108	0.183	0.453	1.952	5.053	20.270	52.446	217.233	424.275	472.881
		0.105	0.184	0.473	1.950	4.780	20.043	52.493	215.551	416.038	474.847
14-Apr-05	Assay 18	0.105	0.184	-	1.816	4.672	19.983	51.205	204.015	414.168	536.931
		0.105	0.183	0.476	1.578	4.961	20.246	51.068	-	412.341	536.437
20-Apr-05	Assay 19	0.105	0.179	0.489	1.815	4.855	19.660	51.647	202.985	422.081	538.359
		0.099	0.212	0.472	1.749	4.828	20.254	50.265	204.363	421.890	540.949

n	50	50	49	49	50	50	50	48	50	50
Mean	0.103	0.194	0.464	1.933	4.910	20.248	51.035	208.186	417.519	501.725
SD	0.005	0.012	0.022	0.073	0.157	0.424	1.295	6.532	7.820	19.231
RSD	5.30%	5.85%	4.76%	3.78%	3.21%	2.09%	2.54%	3.14%	1.87%	3.83%
Bias	3.02%	-2.84%	-7.21%	-3.35%	-1.80%	1.24%	2.07%	4.09%	4.38%	0.35%
Min. value	0.092	0.173	0.426	1.749	4.616	18.730	47.916	200.531	394.344	457.629
Max. value	0.117	0.217	0.523	2.089	5.496	21.026	54.141	228.226	434.254	540.949

Cyanocobalamin quality control data:

		QC samples (µg eq/mL)			
Injection date	Analysis	0.200	20.000	400.000	500.000
21-Oct-04	Assay 1	0.177	19.918	-	500.672
		0.189	19.449	-	505.603
22-Oct-04	Assay 2	0.155	20.683	-	528.489
		0.185	20.496	-	538.490
25-Oct-04	Assay 3	0.180	18.618	-	483.609
		0.173	13.639	-	371.967
26-Oct-04	Reassay 1	0.206	20.429	-	519.326
		0.196	20.705	-	506.802
02-Nov-04	Reassay 2	0.209	20.336	356.606	522.661
		0.205	20.279	353.323	506.763
10-Nov-04	Reassay 3	0.185	19.908	403.420	-
		0.182	19.611	401.734	-
02-Dec-04	Assay 4	0.212	20.337	421.775	-
		0.186	20.683	410.623	-
03-Dec-04	Assay 5	0.208	20.291	408.951	-
		0.183	20.095	417.090	-
06-Dec-04	Assay 6	0.202	20.388	421.010	-
		0.177	20.117	424.785	-
07-Dec-04	Assay 7	0.182	20.365	407.748	-
		0.216	20.490	426.070	-
08-Dec-04	Reassay 4	0.189	19.802	413.207	-
		0.211	19.574	411.603	-
09-Dec-04	Reassay 5	0.216	21.392	417.353	-
		0.165	20.582	426.823	-
06-Jan-05	Reassay 8	0.218	19.353	399.359	-
		0.189	20.097	396.706	-
17-Feb-05	Assay 8	0.192	19.690	406.586	-
		0.183	19.716	400.103	-
18-Feb-05	Assay 9	0.198	21.162	430.934	-
		0.222	20.961	440.979	-
22-Feb-05	Assay 10	0.152	21.109	434.700	-
		0.184	21.056	429.160	-
23-Feb-05	Assay 11	0.187	20.457	426.961	-
		0.215	20.172	421.395	-
17-Mar-05	Assay 12	0.186	20.114	395.739	-
		0.199	20.265	400.695	-

		QC samples (µg eq/mL)			
Injection date	Analysis	0.200	20.000	400.000	500.000
22-Mar-05	Assay 13	0.179	20.538	410.466	-
		0.186	20.210	412.173	-
23-Mar-05	Assay 14	0.192	22.689	424.152	-
		0.220	22.384	431.580	-
24-Mar-05	Assay 15	0.194	20.700	418.038	-
		0.188	20.505	414.191	-
25-Mar-05	Assay 16	0.201	21.016	419.216	-
		0.219	20.913	415.367	-
29-Mar-05	Assay 17	0.189	20.056	410.707	-
		0.191	20.075	406.227	-
14-Apr-05	Assay 18	0.201	22.328	444.900	-
		0.198	22.268	440.143	-
20-Apr-05	Assay 19	0.217	20.637	415.781	-
		0.220	20.551	423.666	-

n	50	50	42	10
Mean	0.194	20.347	413.982	498.458
SD	0.016	1.236	17.946	46.942
RSD	8.46%	6.09%	4.33%	9.42%
Bias	-2.88%	1.74%	3.50%	-0.31%
Min. value	0.152	13.639	353.323	371.967
Max. value	0.222	22.689	444.900	536.490

Hydroxocobalamin quality control data:

		QC samples (µg eq/mL)				
Injection date	Analysis	0.200	20.000	400.000	500.000	2000.000
21-Oct-04	Assay 1	0.190	19.781	-	482.583223	-
		0.195	19.068	-	477.713823	-
22-Oct-04	Assay 2	0.182	18.953	-	477.431136	-
		0.196	18.604	-	477.896725	-
25-Oct-04	Assay 3	0.182	17.780	-	467.555503	-
		0.206	18.940	-	475.834456	-
26-Oct-04	Reassay 1	0.196	18.453	-	462.836102	-
		0.188	18.937	-	471.69932	-
02-Nov-04	Reassay 2	0.178	17.537	404.205	470.042	-
		0.178	17.460	407.518	477.895	-
10-Nov-04	Reassay 3	0.204	17.808	400.500	-	1933.704
		0.195	18.075	402.895	-	1945.829
02-Dec-04	Assay 4	0.187	18.352	340.074	-	-
		0.205	18.423	339.229	-	-
03-Dec-04	Assay 5	0.186	17.755	356.346	-	-
		0.166	17.703	411.975	-	-
06-Dec-04	Assay 6	0.198	20.496	415.273	-	-
		0.196	20.536	422.169	-	-
07-Dec-04	Assay 7	0.157	17.016	348.653	-	-
		0.228	17.027	341.140	-	-
08-Dec-04	Reassay 4	0.176	19.300	365.579	-	1906.172
		0.175	19.342	376.491	-	1892.946
09-Dec-04	Reassay 5	0.227	17.974	362.545	-	1810.837
		0.150	18.193	357.363	-	1828.493
06-Jan-05	Reassay 6	0.185	17.808	353.197	-	1701.258
		0.188	17.618	350.682	-	1702.868
17-Feb-05	Assay 8	0.191	19.482	345.616	-	1726.005
		0.176	17.483	342.452	-	1673.439
18-Feb-05	Assay 9	0.176	17.466	363.068	-	1784.529
		0.217	18.706	363.251	-	1811.369
22-Feb-05	Assay 10	0.219	20.086	368.815	-	1810.002
		0.201	18.781	363.994	-	1810.103
23-Feb-05	Assay 11	0.177	17.842	354.084	-	1816.659
		0.186	17.667	353.132	-	1757.893
17-Mar-05	Assay 12	0.178	17.696	340.336	-	1763.726
		0.194	17.772	337.421	-	1737.481

		QC samples (µg eq/mL)				
Injection date	Analysis	0.200	20.000	400.000	500.000	2000.000
22-Mar-05	Assay 13	0.194	17.621	344.963	-	1769.560
		0.170	17.691	353.916	-	1766.570
23-Mar-05	Assay 14	0.209	22.334	450.258	-	2289.065
		0.229	21.781	451.064	-	2341.941
24-Mar-05	Assay 15	0.220	23.289	384.648	-	1939.640
		0.213	22.359	383.393	-	1993.369
25-Mar-05	Assay 16	0.214	19.135	377.276	-	1858.635
		0.217	18.547	376.933	-	1847.777
29-Mar-05	Assay 17	0.178	18.052	363.603	-	1697.479
		0.206	17.975	363.337	-	1751.917
14-Apr-05	Assay 18	0.200	19.347	371.023	-	1847.510
		0.201	19.161	379.747	-	1858.614
20-Apr-05	Assay 19	0.199	17.955	342.907	-	2060.720
		0.195	17.749	345.415	-	1114.182

n	50	50	42	10	32
Mean	0.194	18.703	370.631	474.149	1830.665
SD	0.017	1.396	29.411	5.962	201.079
RSD	8.82%	7.47%	7.94%	1.26%	10.98%
Bias	-3.07%	-6.49%	-7.34%	-5.17%	-8.47%
Min. value	0.150	17.018	337.421	462.836	1114.182
Max. value	0.229	23.289	451.084	482.583	2341.941

Free Cobalamins-(III) in plasma ultrafitrate

The lower limit of quantification (LLOQ) for the determination of Free Cobalamins-(II) was set to 0.1 mcg eq/mL. The concentration of the calibration curves was in the range from 0.1 mcg eq/mL up to 500 mcg eq/mL. According to the analysis of the calibration samples the assay was linear with determination coefficients (r2) of 0.99285 or higher. Summary statistics of the calibration samples show that the accuracy (expressed as bias) was between -6.95% to 4.85% and the precision ranged from 2.05% to 5.44%. The inter-batch accuracy (expressed as bias) was between -1.39% to 4.38% for Cyanocobalamin QC samples, 1.01 % to 2.54% for spiked Hydroxocobalamin QC samples and the inter-batch precision given as RSD% was between 4.06% to 7.37% for Cyanocobalamin QC samples, 4.05% to 8.29% for spiked Hydroxocobalamin QC samples.

Calibration standards:

	Calibration standards (µg eq/mL)									
Injection date	0.100	0.200	0.500	2.000	5.000	20.000	50.000	200.000	400.000	500.000
21-Oct-04	0.093	0.191	0.461	1.994	4.718	20.601	49.565	206.456	410.271	506.308
	0.115	0.196	0.482	2.019	4.819	20.806	49.911	209.910	412.073	506.935
22-Oct-04	0.100	0.197	0.473	1.939	4.810	20.125	49.325	206.263	408.028	501.071
	0.102	0.203	0.460	1.977	4.894	20.415	50.168	209.479	416.137	507.816
26-Oct-04	0.105	0.189	0.483	1.931	4.825	20.382	50.022	205.701	417.789	509.348
	0.096	0.207	0.481	1.978	4.806	20.244	49.809	206.468	420.305	498.483
27-Oct-04	0.102	0.196	0.463	1.901	4.776	20.462	50.242	212.022	423.569	512.640
	0.101	0.207	0.441	1.922	4.744	20.367	50.020	209.990	420.009	514.724
29-Oct-04	0.106	0.206	0.458	1.915	4.862	20.612	49.791	216.746	420.923	498.319
	0.096	0.191	0.464	1.910	4.776	20.206	49.693	211.255	421.416	503.861
02-Nov-04	0.068	0.205	0.485	1.965	4.748	20.175	50.512	210.461	430.767	475.507
	0.102	0.202	0.484	1.929	4.789	19.996	49.442	212.134	428.397	461.445
02-Dec-04	0.095	0.209	0.433	1.965	4.753	20.606	49.964	206.679	415.202	500.285
	0.107	0.195	0.493	1.939	4.823	20.804	50.114	206.312	415.607	499.758
03-Dec-04	0.106	0.211	0.465	1.913	4.696	20.225	49.894	206.049	436.404	508.574
	0.100	0.174	0.485	1.845	4.663	20.426	49.765	208.269	437.258	509.556
06-Dec-04	0.100	0.207	0.476	1.967	4.991	21.213	48.049	207.495	421.809	476.774
	0.102	0.195	0.458	1.991	4.884	20.413	50.127	206.438	424.875	475.788
07-Dec-04	0.101	0.188	0.460	1.924	4.723	20.806	50.516	210.030	429.737	516.359
	0.110	0.185	0.438	1.915	4.725	20.799	50.577	210.070	429.568	517.203
07-Jan-05	0.105	0.194	0.445	1.922	4.750	20.636	50.579	-	415.131	558.472
	0.102	0.200	0.437	1.922	4.706	20.495	50.407	-	414.210	560.401
14-Feb-05	0.105	0.203	0.473	1.924	5.018	20.223	53.765	209.722	417.574	454.062
	0.095	0.208	0.458	1.847	5.018	20.311	53.630	209.627	420.291	455.198
15-Feb-05	0.096	0.200	0.477	1.842	4.930	20.156	53.246	211.106	423.116	474.758
	0.107	0.200	0.471	1.790	4.946	20.334	53.246	210.811	422.954	478.572
17-Feb-05	0.106	0.180	0.450	1.804	-	20.201	54.272	202.783	418.161	520.648
	0.108	0.180	0.466	1.864	4.983	20.188	54.244	206.274	415.431	518.330
22-Feb-05	0.100	0.195	0.487	1.881	5.147	20.340	52.697	208.344	421.252	468.387
	0.104	0.199	0.472	1.874	5.166	20.220	52.857	205.585	417.476	464.963
17-Mar-05	0.096	0.194	0.452	2.012	5.071	21.258	48.454	199.639	401.598	557.993
	0.111	0.192	0.466	1.768	5.092	20.334	54.465	199.518	398.190	504.096
16-Mar-05	0.106	0.184	0.445	1.904	5.121	20.202	51.918	210.395	435.685	519.316
	0.105	0.180	0.472	1.888	4.594	20.245	51.850	211.310	-	521.289
24-Mar-05	0.106	0.179	0.504	1.867	4.752	17.837	54.333	213.456	405.369	557.948
	0.106	0.175	0.465	1.911	4.555	20.697	51.649	206.711	416.818	541.656

	Calibration standards (µg eq/mL)									
Injection date	0.100	0.200	0.500	2.000	5.000	20.000	50.000	200.000	400.000	500.000
25-Mar-05	0.107	0.193	0.462	1.838	4.776	21.923	52.484	205.531	411.809	538.077
	0.104	0.181	0.431	-	4.311	19.819	50.132	224.770	423.532	503.032
13-Apr-05	0.098	0.180	0.435	1.808	4.986	20.136	51.708	207.858	-	-
	0.112	0.188	0.462	1.896	5.133	20.797	52.899	210.411	419.049	529.094

n	40	40	40	39	39	40	40	38	36	39
Mean	0.103	0.194	0.465	1.908	4.843	20.401	51.159	208.817	419.369	506.181
SD	0.005	0.010	0.018	0.062	0.190	0.585	1.762	4.463	8.602	27.521
RSD	4.93%	5.30%	3.95%	3.24%	3.72%	2.77%	3.44%	2.14%	2.05%	5.44%
Bias	3.16%	-3.00%	-5.95%	-4.58%	-3.15%	2.01%	2.32%	4.41%	4.85%	1.24%
Min. value	0.093	0.174	0.431	1.759	4.311	17.837	48.049	196.639	399.190	454.082
Max. value	0.115	0.211	0.504	2.012	5.166	21.923	54.465	224.770	437.258	560.401

Cyanocobalamin quality control data:

		QC samples (µg eq/mL)			
Injection date	Analysis	0.200	20.000	400.000	500.000
21-Oct-04	Assay 1	0.200	20.369	-	517.793
		0.203	20.357	-	519.070
22-Oct-04	Assay 2	0.190	19.063	-	497.936
		0.212	19.054	-	499.199
25-Oct-04	Assay 3	0.207	20.079	-	516.166
		0.183	20.006	-	506.946
27-Oct-04	Reassay 1	0.193	20.907	-	529.662
		0.201	21.006	-	532.049
29-Oct-04	Reassay 2	0.204	20.388	-	556.901
		0.189	20.387	-	474.152
02-Nov-04	Reasssy 3	0.203	20.385	-	529.694
		0.206	19.884	-	528.489
02-Dec-04	Assay 4	0.210	21.650	428.640	-
		0.192	21.602	431.049	-
03-Dec-04	Assay 5	0.191	21.331	418.116	-
		0.198	21.374	418.413	-
06-Dec-04	Assay 6	0.202	20.853	421.244	-
		0.183	20.573	421.881	-
07-Dec-04	Assay 7	0.191	21.360	431.140	-
		0.201	21.350	429.798	-
07-Jan-05	Reassay 4	0.216	20.893	418.795	-
		0.205	20.819	418.319	-
14-Feb-05	Assay 8	0.189	20.408	396.458	-
		0.205	20.336	403.576	-
15-Feb-05	Assay 9	0.182	18.564	381.189	-
		0.179	18.624	382.576	-
17-Feb-05	Assay 10	0.189	18.733	366.912	-
		0.178	18.949	380.423	-
22-Feb-05	Reassay 5	0.171	18.202	364.650	-
		0.205	18.407	366.415	-
17-Mar-05	Assay 11	0.187	21.757	431.936	-
		0.196	21.265	407.497	-
18-Mar-05	Assay 12	0.180	18.883	424.672	-
		0.197	18.991	415.606	-
24-Mar-05	Assay 13	0.211	22.707	449.142	-
		0.206	21.601	476.951	-

		QC samples (µg eq/mL)			
Injection date	Analysis	0.200	20.000	400.000	500.000
25-Mar-05	Assay 14	0.220	24.664	498.887	-
		0.224	22.295	445.193	-
13-Apr-05	Assay 15	0.193	21.626	433.315	-
		0.192	22.182	433.936	-

n	40	40	28	12
Mean	0.197	20.555	417.523	517.540
SD	0.012	1.358	30.764	21.032
RSD	6.07%	6.60%	7.37%	4.06%
Bias	-1.38%	2.78%	4.38%	3.51%
Min. value	0.171	18.202	364.650	474.152
Max. value	0.224	24.664	498.887	556.901

Hydroxocobalamin quality control data:

		QC samples (µg eq/mL)				
Injection date	Analysis	6.200	20.000	400.000	500.000	2000.000
21-Oct-04	Assay 1	0.207	19.710	-	491.246	1936.557
		0.207	19.744	-	490.211	1936.006
22-Oct-04	Assay 2	0.201	19.262	-	469.095	1980.130
		0.172	18.057	-	481.625	1967.081
25-Oct-04	Assay 3	0.212	19.334	-	487.370	-
		0.184	19.879	-	489.521	-
27-Oct-04	Reassay 1	0.201	20.568	-	504.972	2025.465
		0.198	20.196	-	517.782	1792.455
29-Oct-04	Reassay 2	0.208	19.836	-	538.678	2018.424
		0.194	19.947	-	542.020	1995.880
02-Nov-04	Reassay 3	0.238	19.556	-	516.906	-
		0.185	19.649	-	511.406	-
02-Dec-04	Assay 4	0.204	20.858	407.097	-	2076.936
		0.216	21.334	406.781	-	2066.556
03-Dec-04	Assay 5	0.217	21.407	422.101	-	2073.412
		0.205	21.776	422.641	-	2059.865
06-Dec-04	Assay 6	0.199	21.171	412.195	-	2135.020
		0.193	21.167	414.460	-	2088.158
07-Dec-04	Assay 7	0.200	21.526	422.717	-	2161.297
		0.199	21.465	426.228	-	2158.999
07-Jan-05	Reassay 4	0.292	20.877	415.582	-	-
		0.267	20.956	414.314	-	-
14-Feb-05	Assay 8	0.223	20.909	396.877	-	-
		0.220	21.756	420.379	-	-
15-Feb-05	Assay 9	0.206	18.682	368.684	-	-
		0.196	18.456	368.639	-	-
17-Feb-05	Assay 10	0.192	18.881	373.854	-	-
		0.186	19.245	378.214	-	-
22-Feb-05	Reassay 5	0.183	18.192	358.112	-	-
		0.180	18.426	358.674	-	-
17-Mar-05	Assay 11	0.214	20.054	379.228	-	-
		0.190	20.489	399.755	-	-
18-Mar-05	Assay 12	0.212	20.050	360.977	-	-
		0.191	18.157	369.523	-	-
24-Mar-05	Assay 13	0.218	22.986	452.509	-	-
		0.253	22.676	456.260	-	-
25-Mar-05	Assay 14	0.240	22.313	458.347	-	-
		0.227	21.125	423.554	-	-
13-Apr-05	Assay 15	0.208	20.501	408.429	-	-
		0.210	20.598	410.569	-	-

n	39	40	28	12	16
Mean	0.205	20.314	405.247	505.070	2029.470
SD	0.017	1.219	27.442	20.468	95.321
RSD	8.29%	6.00%	6.77%	4.05%	4.70%
Bias	2.54%	1.57%	1.31%	1.01%	1.47%
Min. value	0.172	18.157	358.112	481.625	1792.455
Max. value	0.253	22.986	458.347	542.020	2161.297

Total Cobalamins-(III) in urine

The lower limit of quantification (LLOQ) for the determination of Total Cobalamins-(II) was set to 5 mcg eq/mL. The concentration of the calibration curves was in the range from 5 mcg eq/mL up to 1000 mcg eq/mL. According to the analysis of the calibration samples the assay was linear with determination coefficients (r2) of 0.98710 or higher. Summary statistics of the calibration samples show that the accuracy (expressed as bias)

was between -5.60% to 4.03% and the precision ranged from 2.86% to 7.05%. The inter-batch accuracy (expressed as bias) was between -7.69% to 4.40% for Cyanocobalamin QC samples, -6.85% to -3.54% for spiked Hydroxocobalamin QC samples (including diluted QC samples) and the inter-batch precision given as RSD% was between 5.43% to 8.27% for Cyanocobalamin QC samples, 2.75% to 8.58% for spiked Hydroxocobalamin QC samples.

Calibration standards:

	Calibration standards (ug eq/mL)								
Injection date	5.000	10.000	20.000	50.000	100.000	200.000	400.000	500.000	1000.000
02-Nov-04	5.062	9.794	20.182	49.639	100.044	203.295	406.179	461.408	-
	5.008	9.900	19.946	50.077	-	202.900	403.861	455.820	1124.758
03-Nov-04	4.989	9.882	20.156	48.476	101.322	202.131	395.391	448.625	1123.282
	4.913	10.331	20.823	50.145	98.987	202.504	402.142	452.647	1030.501
20-Dec-04	4.879	10.765	18.884	46.344	106.291	218.073	365.280	444.309	1069.865
	4.797	11.155	19.086	46.220	107.947	221.140	367.357	445.021	1005.427
21-Dec-04	4.848	11.098	19.881	45.444	107.069	226.705	341.640	441.092	-
	4.677	11.122	19.448	45.857	106.085	224.486	363.320	444.645	1089.132
17-Jan-05	5.230	10.308	18.842	46.514	107.531	183.664	375.183	555.472	-
	4.535	11.305	19.205	46.717	107.548	183.471	376.754	555.230	1029.040
07-Feb-05	4.841	10.463	20.646	51.434	103.392	209.384	412.164	458.859	1014.920
	4.866	10.175	20.394	51.741	103.413	205.923	410.985	445.559	878.576
08-Feb-05	4.669	10.290	19.969	51.667	103.674	204.475	411.233	469.543	1018.921
	5.232	9.968	19.277	52.775	103.176	205.996	406.934	463.076	888.471
09-Feb-05	4.866	10.128	19.504	51.357	100.233	197.741	397.590	468.846	1029.574
	5.111	10.067	19.525	52.091	101.384	193.100	409.727	468.297	1070.251
05-Apr-05	4.994	10.155	19.334	50.333	98.237	201.692	400.038	497.875	1024.479
	4.910	10.354	20.235	50.826	98.218	201.813	398.173	495.140	1018.417
07-Apr-06	4.921	10.879	19.670	49.342	102.816	202.767	407.504	479.459	950.973
	4.901	9.905	20.012	49.605	102.178	199.039	411.997	488.264	-

n	20	20	20	20	19	20	20	20	16
Mean	4.915	10.403	19.750	49.330	103.029	204.215	396.172	472.019	1022.912
SD	0.172	0.481	0.566	2.353	3.727	11.404	17.396	33.269	70.194
RSD	3.50%	4.62%	2.86%	4.77%	3.62%	5.58%	4.39%	7.05%	6.86%
Bias	-1.71%	4.03%	-1.25%	-1.34%	3.03%	2.11%	-0.96%	-5.60%	2.29%
Min. value	4.535	9.794	18.842	45.444	98.218	183.471	341.640	441.092	878.576
Max. value	5.232	11.305	20.823	52.775	106.085	226.705	412.164	556.472	1124.758

-: Concentration level not used for calibration curve

Cyanocobalamin quality control data:

		QC samples (µg eq/mL)		
Injection date	Analysis	15.000	100.000	600.000
02-Nov-04	Assay 1	13.911	93.139	622.034
		13.799	93.114	607.692
03-Nov-04	Assay 2	14.763	93.957	609.793
		14.315	91.963	793.081
20-Dec-04	Assay 3	16.153	109.506	720.697
		16.492	106.136	695.977
21-Dec-04	Assay 4	16.143	106.596	849.301
		15.785	107.717	756.795
17-Jan-05	Reassay 1	16.259	106.426	674.093
		16.819	106.470	757.020
07-Feb-05	Assay 5	15.602	106.219	710.057
		16.865	107.389	755.535
08-Feb-05	Assay 6	15.359	103.370	637.812
		15.083	103.664	726.528
09-Feb-05	Reassay 2	16.113	102.817	726.108
		15.544	103.696	644.448
05-Apr-05	Assay 7	15.822	103.196	707.653
		15.766	101.926	656.664
07-Apr-05	Assay 8	16.175	107.305	720.901
		16.416	107.678	797.781

n	20	20	20
Mean	15.660	103.225	736.509
SD	0.869	5.610	81.078
RSD	5.57%	5.43%	8.27%
Bias	4.40%	3.22%	-7.68%
Min. value	13.799	91.963	637.812
Max. value	16.865	109.506	849.301

Hydroxocobalamin quality control data:

		QC samples (µg eq/mL)			
Injection date	Analysis	15.000	100.000	600.000	2000.000
02-Nov-04	Assay 1	14.973	101.214	762.852	-
		15.350	99.938	770.748	-
03-Nov-04	Assay 2	15.412	98.962	763.430	-
		14.891	94.301	759.337	-
20-Dec-04	Assay 3	14.109	92.942	752.443	1939.895
		14.032	92.624	753.337	1945.335
21-Dec-04	Assay 4	13.757	91.307	744.025	1890.601
		13.692	91.646	742.900	1882.392
17-Jan-05	Reassay 1	13.427	92.297	745.556	1733.415
		14.161	91.737	742.202	1776.384
07-Feb-05	Assay 5	15.921	96.380	808.164	1684.723
		15.809	96.071	793.644	1749.891
08-Feb-05	Assay 6	12.847	88.509	726.301	1704.502
		12.898	89.724	737.585	1674.947
09-Feb-05	Reassay 2	15.284	93.960	765.051	1825.891
		14.763	94.689	756.603	1815.869
05-Apr-05	Assay 7	14.996	93.636	757.421	1931.899
		13.887	97.229	771.651	1866.146
07-Apr-05	Assay 8	14.615	97.142	784.139	2180.886
		14.459	96.640	793.435	2225.371

n	20	20	20	16
Mean	14.469	94.757	761.096	1863.008
SD	0.888	3.550	20.966	158.903
RSD	6.14%	3.75%	2.75%	8.58%
Bias	-3.54%	-5.24%	-4.66%	-6.85%
Min. value	12.847	88.509	726.301	1674.947
Max. value	15.921	101.214	809.164	2225.371

10 Page(s) Withheld

_____ Trade Secret / Confidential (b4)

✓ Draft Labeling (b4)

_____ Draft Labeling (b5)

_____ Deliberative Process (b5)

Withheld Track Number: Clin Pharm/Bio- 1

b(4)

4.2 Individual Study Review

Study EML 015722 - H101 : A double-blind, randomized, placebo-controlled, single-ascending-dose study, with a 4-week follow-up, of the safety, tolerabilty and pharmacokinetics of 4 intravenous doses (2.5 g, 5 g, 7.5 g and 10 g) of hydroxocobalamin in healthy subjects

Study phase - Clinical Phase I
Drug dosage - 2.5 g, 5 g, 7.5 g or 10 g per subject as single intravenous infusion
Treatment duration - Single dose
Study period - 24 September 2004 - 11 May 2005 b(4)
Coordinating/rincipal investigator - Georg Golor, PD, MD and PhD, PAREXEL International GmbH, Berlin
Sponsor - Merck Santé s.a.s., 37 rue Saint-Romain, F-69379 Lyon Cedex 08, France

Objectives:
Primary: To determine the safety and tolerabilty of four single intravenous doses of hydroxocobalamin (intravenous infusion: 2.5 g for 7.5 min, 5 g for 15 min, 7.5 g for 22.5 min and 10 g for 30 min) compared with placebo.

Secondary: To determine the pharmacokinetics (PK) of free and total cobalamins-(II) in plasma and total cobalamins-(II) in urine in a subgroup of 12 subjects (9 on OH-Co and 3 on placebo*) each for the 2.5-g and the 7.5-g dose groups and 16 subjects (12 on OH-Co and 4 on placebo*) each for the 5-g and 10-g dose groups.

Methodology: Single-center, double-blind, randomized, placebo-controlled, singleascending dose study, with a 4-week follow-up, of the safety, tolerabilty and PK of 4 intravenous doses (2.5, 5, 7.5 and 10 g) of HOCo in 200 planned healthy volunteers (12 each in the dose groups 2.5 g and 7.5 g and 88 each in the dose groups 5 g and 10 g; 150 subjects on drug and 50 subjects on placebo were planned to be included).

The start of the second dose group commenced after completion of treatment in the first dose group and after review of the interim safety data of the first dose group. The same procedure was applied for each following dose group. In case of doubtful results regarding safety parameters at any dose level, the corresponding PK data were required for further safety evaluation.

Number of subjects		Planned	Analyzed		
		Per Protocol	Active Drug	Placebo	Total
Doses Per protocol	2.5 g	12	9	3	12
	5.0 g	88	66	22	88
	7.5 g	12	9	3	12
	10.0 g	88	18	6	240
Intention-to-treat		200	102	34	136
Safety		200	102	34	136
Pharmacokinetics		56	41	0	41

Diagnosis and main criteria for inclusion: Healthy male and female subjects, aged 18 to 60 years; healthy as defined by medical history, physical and biological examinations performed before inclusion and wiling to give written informed consent.

Test product: OH-Co lyophilizate 2.5 g and solution (sodium chloride 0.9 %) used as solvent for single intravenous (i. v.) use. Batch No.: EC 463 (manufacturing batch 3034A)

Duration of treatment: One single i.v. infusion of 2.5 g = 7.5 min, 5 g = 15 min, 7.5 g = 22.5 min or 10 g = 30 min or placebo. If infusion rates were not tolerated well, slower infusion rates of 15, 30, 45 and 60 min, respectively, could have been used. The PK sampling times during infusion were to be adapted accordingly.

Criteria for evaluation: Safety and tolerability: Physical examination, vital signs (blood pressure, pulse rate, respiratory rate, body temperature and pulse oximetry), 12-lead ECG recordings / telemetry, biochemistry, hematology, blood coagulation parameters and urinalysis. Arterial and capilary blood gases (PK subjects: 5-g and 10-g dose groups only), spirometry, neurology tests (non-PK subjects only): Mini-Mental State, gross neurological examination (finger-nose, knee-heel test, nystagmus), sensory and motor assessment and reflex testing, recording of adverse events and local tolerabilty.

Pharmacokinetics: Cmax, Tmax, AUC, AUC, AUMC, t1/2, MRT, Vss, Ae, CL, CLR and Ae of free and total cobalamins-(II) (plasma) and total cobalamins-(II) (urine).

Statistical methods: All parameters were analyzed in a descriptive way. No confirmatory statistical tests were performed.

Effcacy results: No efficacy results were obtained from this study.

Results:

1. Demographics

		2.5 g OH-Co	5 g OH-Co	7.5 g OH-Co	10 g OH-Co	Total
		Verum (n=9)	Verum (n=12)	Verum (n=9)	Verum (n=11)	(n=41)
Age (years)	mean	38.7	35.3	32.8	33.5	35.3
	SD	10.5	9.1	9.7	11.2	10.0
	Range	26 – 57	24 – 46	21 – 45	21 – 56	21 – 57
Weight (kg)	mean	72.99	73.76	68.41	70.42	71.52
	SD	11.72	12.74	9.00	10.92	11.07
	Range	52.6–94.3	55.5–94.5	55.6–84.8	52.5–87.5	52.5–94.5
Height (cm)	mean	172.3	173.2	171.0	174.2	172.8
	SD	9.9	7.2	7.9	9.2	8.3
	Range	156–190	161–184	160–182	161–190	156–190
Males/females (n)		5/4	6/6	4/5	6/5	21/20
Ethnic origin						
Caucasian	(n)	9	12	9	11	41
Black	(n)	-	-	-	-	-
Asian	(n)	-	-	-	-	-
Other	(n)	-	-	-	-	-

2. Safety results:

- All subjects of the 2.5-g (n=9), 5.0-g (n=66) and 7.5-g (n=9) of the safety population and 17 of 18 subjects at 10 g received the planned amount of OH-Co. Subject 4018 received approximately 3.9 g (156 mL) of 10 g (400 mL infusion solution), because the infusion was discontinued due to AEs.

  Note: Several laboratory parameters were shown to be influenced by HOCo. The interference was investigated in a separate in-vitro study. The following table shows an overview of the results obtained for selected biochemistry parameters.

Interference/	Analyzer used (for VitroslB 950 analyzer interferences are listed for measured parameters only)	
Trend	Cobas™ Integra	VitroslB 950
< 10%	Albumin, alkaline phosphatase, bicarbonate (2nd reagent), calcium, chloride, CRP, GGT, glucose, potassium, sodium, total protein, urea	
Positive Interference	Cholesterol, creatinine, LDH (at low LDH), total bilirubin, triglycerides	
Negative Interference	Amylase, bicarbonate (1st reagent), creatine kinase, GOT (AST), GPT (AL T), uric acid	GPT (AL T), phosphate
Time dependency	Amylase, uric acid	GPT (AL T)
No reliable results	CKMB	

CRP = C-reactive protein; GGT = Gamma-glutamyl transferase / transpeptidase; LDH = Lactic dehydrogenase;
GOT (AST) = Glutamate oxaloacetate transaminase (aspartate aminotransferase);
GPT (AL T) = Glutamate pyruvate transaminase (alanine aminotransferase);
CKMB = MB isoenzyme of creatine kinase (CK)

In addition to biochemistry parameters, hemoglobin, mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were also positively influenced by the presence of HOCo. Other hematology parameters (erythrocyte, leukocyte and platelet counts or mean corpuscular volume (MCV)) only showed negligible interference. The coagulation parameter prothrombin time (PT) showed negative and activated partial thromboplastin time (aPTT) positive interference with HOCo. Interferences at HOCo levels of 0.4 g/L or higher were also observed for urinary parameters. Such interferences were less significant for pH, glucose, ketones, bilirubin, urobilinogen, but more significant for protein, erythrocytes, leukocytes and nitrite. Regarding urine sediment no interference was expected but in all dose groups an increase of oxal~te crystals in urine was observed.

Thus, no valid discrimination between in-vitro interference, if present, and a potential drug effect was possible for the affected laboratory parameters, in particular during the time of high OH-Co concentrations on Day 1.

- A total of 36 subjects exhibited deviations in hematology (21 deviations) or biochemistry (33 deviations) parameters that were judged as being clinically relevant by the Investigator. All hematology and biochemistry parameters were followed until normal or clinically not relevant values were reached at follow-up. Among hematology parameters, clinically significant abnormalities comprised decreases in lymphocytes percentages to 0:10% and in hemoglobin concentrations to below the reference range. The decreases in lymphocytes percentages to 0:0% may be considered drug-related, as they mainly occurred 2 - 4 hours after infusion of 2.5 - 10.0 g OH-Co on Day 1, but not after placebo infusion.

  Moreover, the incidence of decreases in lymphocytes to 0:0% was higher in the 7.5- and 10.0-g (33.3% and 16.7%, respectively) OH-Co groups than in the lower active treatment groups (11.1 % and 9.1 % for 2.5 and 5-g dose group, respectively). Nevertheless, the absolute lymphocytes counts were within the normal laboratory range. Absolute neutrophils counts and percentage showed mean increases within the reference range, but values above or below normal were confined to single subjects and were of no clinical significance. Smaller increases from baseline, on average, were also observed for total WBC (leukocytes) counts 4 - 12 hours post-dose, most pronounced after infusion of 7.5-g OH-Co, which may support the assumption of drug-related changes in WBC counts and differentials by OH-Co infusion.

- Clinically relevant abnormalities in biochemistry comprised elevated CRP (10 cases, following verum only), CK (5 cases following verum, 1 case following placebo),

glucose (1 case following verum, 2 cases following placebo), amylase (1 case following verum) , total bilirubin (1 case following placebo) and triglycerides (1 case following verum) and a decrease in inorganic phosphate (1 case following placebo). Abnormal changes in CK, glucose, total bilrubin and inorganic phosphate could be explained by reasons other than OH-Co infusion, but clear explanations were not found for the isolated abnormal increases in amylase activity after 5.0 g OH-Co and in triglyceride concentrations after 10.0 g OH-Co.

- Abnormal increases in CRP occurred at various time points between 12 hours post-dose on Day 1 and Day 28 after infusion of 2.5 to 10.0 g OH-Co. A possible relationship to study drug infusion was only assumed by the Investigator for the abnormal CRP increases in single subjects treated with 7.5 g and 10.0 g OH-Co. Mean increases from 12 hours post-dose until follow-up compared to baseline occurred after 5.0, 7.5 and 10 g OH-Co and were highest on Day 3 (48 h) at the 7.5-g OH-Co dose leveL. In four subjects, raised neutrophils percentages to above normal and decreases in lymphocytes proportions to below normal at 4 and/or 12 hours post-dose preceded the increase in CRP levels. Such combination of changes in inflammation markers after infusion of 5.0, 7.5 and 10.0 g OH-Co was not observed in any placebo subject, and may point to drug-mediated effects.

- Vital signs: Blood pressure (BP) clearly indicated drug-related mean increases from baseline amounting up to maxima of 22.9 - 27.0 mmg for systolic blood pressure (SBP), 14.3 - 25.4 mmg for diastolic blood pressure (DBP) and 17.1 - 25.8 mmg for mean arerial BP (MAP). These mean increases were somewhat higher in the dose range of 7.5 - 10.0 g compared to 2.5 - 5.0 g OH-Co, but not proportionally elevated with dose. Typically, BP started to increase within 2 - 5 mi after start of OH-Co infusion, reached a maximum around the end of infusion and returned to near baseline values within 4 - 8 hours post-dose. A smaller maximum mean increase in BP was observed in the small number of corresponding placebo groups by up to 6.7 mmHg (SBP, 7.5-g placebo group) that may be associated to the increasing volume and duration of infusion. Dose-related increases were also observed for the derived blood pressure parameter DeltaMAPmax and AUC (DeltaMAP, Inf), whereas the drug-related effects in AUC (DeltaMAP, 4h) and AUC (DeltaMAP, 24h) appeared to be masked by the circadian rhythm of BP. Subgroup evaluation showed that female subjects on active treatment were, on average, more affected by the increase in blood pressure than male subjects as shown by their MAP values. However, the observed differences in mean MAP between females and males may be more related to weight rather than gender differences.

- Coinciding with the increase in BP, mean pulse rate decreased from baseline by -8.2 bpm after 2.5-g up to -14.2 bpm in the 1O.0-g OH-Co group. This apparently drug-related effect led to a doserelated reduction in the pulse rate parameter DeltaPULSEmin. In general, individual decreases in DeltaPULSE correlated with increases in DeltaMAPmax and individual increases in DeltaMAP coincided with decreases in DeltaPULSEmin at the respective time points. The following table

summarizes the maximum mean (::SD) changes from baseline after OH-Co infusion for selected vital signs parameters:

Dose level:	2.5 g		5.0 g		7.5 g		10.0 g	
Treatment:	OH-Co (n=9)	Placebo (n=3) *	OH-Co (n=66)	Placebo (n=22) *	OH-Co (n=9)	Placebo (n=3) *	OH-Co (n=18)	Placebo (n=6)*
Systolic blood pressure [mmHg]	22.9 ±18.7	2.7 ±7.6	22.6 ±16.8	0.2 ±6.3	27.0 ±10.0	6.7 ±8.1	25.7 ±13.2	4.0 ±8.0
	5 min during infusion		10 min during infusion		20 min during infusion		25 min during infusion	
Diastolic blood pressure [mmHg]	14.3 ±8.3	−3.0 ±14.2	17.7 ±9.8	1.6 ±4.1	25.4 ±4.7	3.0 ±6.2	22.6 ±10.1	3.8 ±3.9
	5 min during infusion		15 min during infusion		20 min during infusion		20 min during infusion	
Pulse rate [bpm]	−8.2 ±10.8	−0.3 ±8.5	−11.5 ±6.0	1.2 ±4.7	−12.6 ±11.0	3.7 ±12.5	−14.2 ±6.4	3.0 ±7.4
	5 min during infusion		10 min during infusion		20 min during infusion		10 min during infusion	
Body temperature [°C]	0.29 ±0.29	0.07 ±0.21	−0.22 ±0.38	−0.15 ±0.46	−0.42 ±0.33	−0.13±0.15	−0.25 ±0.31	0.08 ±0.39
	3 h after infusion		Day 15		10 min after infusion		10 min after infusion	
Mean arterial blood pressure (MAP) [mmHg]	17.1 ±11.3	−1.0 ±12.5	18.1 ±11.3	−0.5 ±9.7	25.8 ±5.5	4.3 ±6.8	23.9 ±10.9	5.2 ±9.8
	5 min during infusion		10 min during infusion		20 min during infusion		20 min during infusion	
DeltaMAPmax [mmHg]	18.6 ±10.1	7.7 ±4.7	22.8 ±9.4	7.3 ±4.8	26.5 ±5.9	11.3 ±4.0	27.5 ±8.1	13.0 ±5.8
	From start up to 24 hours after end of infusion							
AUC (DeltaMAP, Inf) [mmHg·h]	1.29 ±0.65	−0.14 ±0.81	3.81 ±1.92	0.05 ±0.78	7.36 ±2.11	1.53 ±2.31	9.51 ±3.67	2.05 ±1.02
AUC (DeltaMAP, 4 h) [mmHg·h]**	25.5 ±25.8	−4.5 ±2.2	22.1 ±20.5	-8.6 ±12.7	43.3 ±20.8	10.4 ±25.5	37.4 ±21.5	12.9 ±16.7
AUC (DeltaMAP, 24 h) [mmHg·h]**	38.5 ±202.3	−70.6 ±2.6	67.3 ±111.8	−57.0 ±111.7	108.6 ±96.6	18.7 ±86.8	128.0 ±141.9	94.4 ±153.4

*For maximum mean change in the placebo groups, the values at time point of maximum mean change in the corresponding active group are given.
**Time interval: end of infusion up to 4 or 24 h, respectively.

- The frequency of vital signs AEs appeared to be higher in the 7.5- and 10.0-g dose groups (55.6% and 27.8%, respectively), compared to the 2.5- and 5.0-g groups (22.2% and 18.2%, respectively). Overall, female subjects were more frequently affected by vital signs AEs than male subjects (36.0% versus 11.5%). Subjects with vital signs AEs showed similar mean increases in MAP of around 30 mmg irrespective of the dose administered, but subjects without vital signs AE showed increases in MAP comparable to the 2.5- or 5-g dose groups (13.3 or 16.5 mrg) and to the 7.5- or 10-g dose groups (21.8 or 20.8 mrg), respectively.

- A small mean decrease in body temperature not exceeding -0.42 °C occurred shortly after end of infusion in the two higher dose groups; only marginal changes were found in the two lower OH-Co dose groups and after placebo. There was no effect after infusion of 2.5 to 10.0 g OH-Co on respiratory rate and pulse oximetry.

- Adverse Events: A total of 458 AEs occurred during the study, 435 in 102 subjects during or after infusion of OHCo and 23 AEs in 15 subjects during or after infusion of placebo. No serious AE occurred and none of the subjects died during the study. All AEs were followed up until they were resolved. A total of 19 subjects received corrective treatment. In one subject of the 10-g dose group, the infusion was prematurely discontinued after 11.9 min due to an allergic reaction. The number of AEs and subjects with AEs by dose group is given in the following table.

	2.5 g OH-Co		5 g OH-Co		7.5 g OH-Co		10 g OH-Co		Total	
Number of	Verum (n=9)	Placebo (n=3)	Verum (n=66)	Placebo (n=22)	Verum (n=9)	Placebo (n=3)	Verum (n=16)	Placebo (n=6)	Verum (n=102)	Placebo (n=34)
AEs/ subjects with AES	23/9	0/0	234/66	11/9	60/9	5/2	118/18	7/4	435/102	23/15

The main AEs, considered by the Investigator to be possibly study-drug related, comprised the events chromaturia, erythema (skin redness), pustular rash, headache, increased diastolic blood pressure, decreased lymphocyte count and erythema at the injection site. In addition, two allergic reactions (5 and lO-g dose group) were observed. All cases of skin reddening and chromaturia were graded by the Investigator as severe (throughout the report, the term "intense" wil be used for severe skin reddening and chromaturia, since the coloration of skin and urine does not interfere with usual daily activities).

The number of subjects with drug-related adverse events by leading system organ class and preferred term (safety population, n = number of subjects) is given in the following table. All AEs presented occurrng in at least five subjects in at least one dose group

	2.5 g OH-Co		5 g OH-Co		7.5 g OH-Co		10 g OH-Co		Total
	Verum (n=9)	Placebo (n=3)	Verum (n=66)	Placebo (n=22)	Verum (n=9)	Placebo (n=3)	Verum (n=16)	Placebo (n=6)	(n=136)
Any adverse event	9	—	66	2	9	—	18	—	104
Blood pressure diastolic increased	—	—	6	—	5	—	5	—	16
Blood pressure increased	2	—	6	—	—	—	—	—	8
Chromaturia	9	—	66	—	9	—	18	—	102
Erythema (skin redness / redness all over the body)	—	—	61/1	—	9/0	—	18*/0	—	89
Headache	1	—	4	1	5	—	6	—	17
Infusion and injection site erythema	—	—	4	—	3	—	7	—	14
Lymphocyte count decreased*	—	—	5	—	3	—	3	—	11
Rash pustular	—	—	11	—	4	—	3	—	18

*Subject 4018 and 4024, in addition to skin redness also experienced erythema in the face and redness of arm.

* "Lymphocyte count decreased" was defined as an AE in case percentage of lymphocyte dropped below 10%.

- Overall, the incidence of treatment-emergent AEs per subject was much greater in the two higher compared to the lower dose groups. Except for skin redness, incidences at

the 2.5- and 5.0-g dose were similar and nearly identical between the two highest doses. The incidence of treatmentemergent AEs per subject in the 5-g placebo group can be considered reliable, while the low number of subjects in the other placebo groups led to more variable incidences. The incidences of treatmentemergent AEs per subject in the verum and placebo groups are shown in the following table:

	2.5 g OH-Co		5 g OH-Co		7.5 g OH-Co		10 g OH-Co	
Incidences	Verum (n=9)	Placebo (n=3)	Verum (n=66)	Placebo (n=22)	Verum (n=9)	Placebo (n=3)	Verum (n=18)	Placebo (n=6)
Including all treatment-emergent AEs	2.4	0.0	3.5	0.4	8.7	1.7	6.6	1.0
Except chromaturia and skin redness	1.4	0.0	1.5	0.4	4.7	1.7	4.6	1.0

The number of AEs by intensity and relationship is given in the following table:

	2.5 g OH-Co		5 g OH-Co		7.5 g OH-Co		10 g OH-Co		Total
	Verum (n=9)	Placebo (n=3)	Verum (n=66)	Placebo (n=22)	Verum (n=9)	Placebo (n=3)	Verum (n=18)	Placebo (n=6)	(n=136)
Mild	10	0	109	5	24	3	41	4	196
Moderate	4	0	57	6	26	2	40	3	138
Severe	9	0	68	0	10	0	37	0	124
Not related	5	0	13	6	3	1	2	7	37
Unlikely related	2	0	17	2	1	4	3	0	29
Possibly related	16	0	204	3	56	0	113	0	392
Not assessable	0	0	0	0	0	0	0	0	0
Overall	23	0	234	11	60	5	118	7	458

- Description of characteristic AEs: Chromaturia (red-colored urine): All subjects in each active dose group experienced chromaturia after OH-Co infusion. All occurrences of this event were graded as intense. In the 2.5- and 5.0-g dose groups, the duration of chromaturia ranged from 7 - 35 days, mainly lasting for 14 days. In the two higher dose groups, the range was 7 - 28 days, but most frequently the event lasted 25 – 28 days. In all dose groups, red-colored urine occurred already with the first urine sample after infusion, i.e. within the first urine collection interval of 0 - 2 hours.

  Erythema (skin redness and redness all over the body): A total of 89 (87.3%) of all verumtreated subjects, i.e., 62 in the 5.0-g dose group and all subjects at the two highest dose levels showed redness of the skin. None of the subjects in the 2.5-g group reported erythema (skin redness). The Investigator most often graded this event as moderate, but in some cases as intense. All occurrences were considered possibly study-drug related by the Investigator. Skin reddening in the 5 - lO-g dose groups mainly occurred within 10 to 33 min after star of infusion, i.e. after infusion of 3.3 - 4.3 g OH-Co. In most cases the event resolved after approximately 7 days with a total range of 1 to 15 days.

  Infusion-site reactions: At dose levels of 5.0 g, 7.5 g and 10.0 g OH-Co, injection-site reactions were reported in 14 subjects. The symptoms comprised edema, pain,

exanthema (local macula), reddening of the vein or arm at the infusion site. The symptoms redness of infusion vein or arm and local redness at infsion site in the lO-g active group were rated as mild or moderate by the Investigator, stared between about 34 and 52 min after begin of infusion and persisted for about 2 to 48 hours. None of the subjects, who received placebo, reported any injection site reaction.

Skin reactions: Pustular rash was reported in 11,4 and 3 subjects of the 5.0-, 7.5- and 1O.0-g dose groups, respectively. The Investigator mainly considered the intensity as mild. The onset of these AEs ranged from 7 to 25 days post-dose and lasted for approximately 6 to 38 days across all dose groups. The pustules were most often located in the face and neck, but also once on the chest and once on the back. This AE was not observed in any of the subjects included in the lowest dose group and in the placebo groups.

Headache: During the entire study, 22 subjects complained about headache ('headache' and 'head pressure'), 19 subjects (18.6%) after OH-Co infusion and 3 subjects (8.8%) following placebo. In addition, one subject of the verum and the placebo group each experienced headache before infusion. Overall, an increase in the number of subjects with the AE 'headache' related to the increase in dose was found in the two highest dose groups (55.6% and 33.3% in the 7.5- and l0-g groups versus 22.2% and 9.1 % in the 2.5- and 5-g dose groups). Incidences in the 2.5- and 5-g groups appeared to be similar, in particular in view of the differences in sample size.

In 9 out of 24 subjects with clinically relevant increases in blood pressure, headache was observed starting immediately after start of infusion up to about 24 h after start of infusion. In the two lower dose groups, only 1 subject suffered from headache and increased blood pressure compared to 8 subjects of the higher dose groups. Three of the 9 headaches occurred within about 2.5 hours after star of infusion, 3 within about 12 hours and the remaining from about 16 to 24 hours after start of infusion. Therefore, a direct correlation between the rise in blood pressure and headache seemed to be unlikely.

Physical examination, neurological assessments, spirometry and ECG recordings
Physical examination performed at screening follow-up and certain time points during the study did not reveal any findings, which deviated from the AEs assessments. Neurological examinations were inconspicuous at all assessments and for all subjects. None of the spirometric assessments revealed any relevant deviations for any of the parameters. Only minor deviations were recorded. Overall, none of the intervals of ECG variables were considered clinically significant by the Investigator. No ECG and telemetry finding was classified as an AE. Following confirmation by FDA, the study was finalized prematurely due to tolerability reasons.
The AEs were considered not acceptable for healthy volunteers, however the observed symptoms would not be judged as being critical for therapy in a lie-threatening situation (for further details

3. Pharmacokinetic results:

- All subjects but one (subject 4018) received their planned dose, but infusion time was nearly two-fold prolonged in one subject of the 2.5-g dosing group that led to a delayed Tmax. For free cobalamins-(III), mean tmax occurred slightly earlier compared to total cobalamins-(II) in all groups, which is due to the fast complexation of hydroxocobalamin (one form of free cobalamins-(II)) with plasma proteins. The 96-h sampling interval was sufficiently long for proper determination of the apparent terminal elimination half-life. Mean (SD) PK parameters for free and total cobalamins are listed in the summary table below:

- Total cobalamins-(II) Cmax and AUC0-t increased approximately proportionally with dose over the entire dose range, with a somewhat higher exposure in the 5.0-g group. For free cobalamins-(II), however, Cmax dose of the 2.5-g dose of OH-Co was approximately 30% to 70% higher compared to the 5-, 7.5- and 10-g doses, but no difference in group characteristics was observed for dose-normalized AUC0-inf/dose.

- Urine Concentrations of Total Cobalamins-(III) - The urine samples were stabilized by acidification and cooled immediately after sample collection and total cobalamins-(III) were determined only (for further details see Section 9.5.3.2 and Lab Manual Version 2.0). No quantifiable amounts of total cobalamins-(III) were found in the urine of any subject prior to start of infusion. Maximum urinary original concentrations were generally found within the first two collection intervals (i.e. from 0-2 and 2-- hours). Due to these high urinary concentrations, subjects had to consume a minimum of 1.5 L water from pre-dose to 6 hours post-dose. Quantifiable amounts of total cobalamins-(III) were present in all urine samples available. In isolated cases urine samples were not available during the first 12 hours, since subjects could not void urine during the corresponding collection interval. All urine samples following administration were deep red in color up to the last collection interval of 72 to 96 hours (Day 4) and in some subjects even up to Days 15 or 28.

a. Pharmacokinetic Parameters of **Free** Cobalamins-(III) Following i.v. Administration of 2.5 to 10.0 g HOCo:

Dose		C_{max} (µg eq/mL)	t_{max} (h)	AUC_{0-t} (µg eq/mL*h)	$AUC_{0-\infty}$ (µg eq/mL*h)	$t_{1/2}$ (h)	V_{ss} (L)	CL (L/h)
2.5 g (N=9)	Mean	73.1	0.142	188.4	201.3	30.3	325.8	12.5
	SD	14.5	0.041	25.5	28.1	2.4	41.0	2.1
	Min	46.8	0.125	136.1	143.6	27.5	285.5	10.8
	Max	90.8	0.250	212.9	227.6	35.0	405.2	17.2
	CV%	19.9	29.3	13.5	13.9	7.8	12.6	16.4
5 g (N=12)	Mean	112.7	0.242	366.0	394.6	30.2	349.5	12.6
	SD	20.8	0.029	37.2	36.9	6.7	99.1	1.2
	Min	69.4	0.183	311.2	335.2	23.9	243.4	10.4
	Max	150.4	0.283	446.3	473.8	49.5	629.4	14.7
	CV%	18.4	12.0	10.2	9.3	22.1	28.4	9.2
7.5 g (N=9)	Mean	128.6	0.371	562.3	593.4	27.5	333.1	13.2
	SD	46.9	0.047	167.7	171.5	2.5	91.5	2.8
	Min	78.9	0.261	411.4	440.6	25.4	152.3	7.3
	Max	235.6	0.416	973.7	1013.2	31.5	455.8	16.8
	CV%	36.4	12.8	29.8	28.9	9.0	27.5	21.4
10 g (N=11)	Mean	197.2	0.506	762.5*	813.8	25.9	280.7	12.5
	SD	40.3	0.013	141.9	153.3	2.7	62.0	2.0
	Min	125.3	0.500	612.4	647.5	22.6	203.3	8.3
	Max	249.6	0.532	1114.4	1190.7	30.0	406.6	15.2
	CV%	20.4	2.6	18.6	18.8	10.4	22.1	16.1

b. Pharmacokinetic Parameters of **Total** Cobalamins-(III) Following i.v. Administration of 2.5 to 10.0 g HOCo

Dose		C_{max} (µg eq/mL)	t_{max} (h)	AUC_{0-t} (µg eq/mL*h)	$AUC_{0-\infty}$ (µg eq/mL*h)	$t_{1/2}$ (h)	V_{ss} (L)	CL (L/h)
2.5 g (N=9)	Mean	267.6	0.225	3566.0	4018.4	32.8	25.6	0.633
	SD	32.5	0.119	636.9	729.3	2.9	4.8	0.122
	Min	253.5	0.125	2475.2	2820.3	29.6	19.9	0.464
	Max	363.2	0.375	4705.5	5322.2	38.5	35.6	0.875
	CV%	11.3	53.0	17.9	18.1	8.9	18.6	19.3
5.0 g (N=12)	Mean	579.0	0.324	8453.7	9422.9	31.0	21.8	0.566
	SD	112.6	0.110	2639.8	2991.6	2.8	5.0	0.148
	Min	441.9	0.250	6091.2	6525.1	25.6	13.1	0.333
	Max	778.8	0.500	13007.4	14826.0	35.0	28.4	0.757
	CV%	19.4	34.1	31.2	31.7	8.9	22.8	26.1
7.5 g (N=9)	Mean	740.3	0.525	10815.0	11963.6	30.5	24.3	0.644
	SD	182.7	0.118	2479.2	2640.2	4.8	5.9	0.131
	Min	541.6	0.367	7396.6	8363.3	26.8	13.9	0.418
	Max	1180.3	0.649	16439.3	17715.7	41.1	34.9	0.885
	CV%	24.7	22.5	22.9	22.1	15.2	24.3	20.3
10 g (N=11)	Mean	995.3	0.551	14271.5	15681.1	29.6	23.0	0.645
	SD	149.1	0.099	2166.5	2571.5	4.7	2.7	0.103
	Min	770.7	0.500	11863.3	12339.5	21.6	18.1	0.496
	Max	1240.6	0.750	18324.1	19824.6	39.2	26.9	0.800
	CV%	15.0	18.0	15.2	16.4	15.8	11.7	16.0

c. Summary Statistics for the Percentage Ratio of Free/Total Cobalamins-(III) for AUCo-t and AUCo-inf per Dose Group

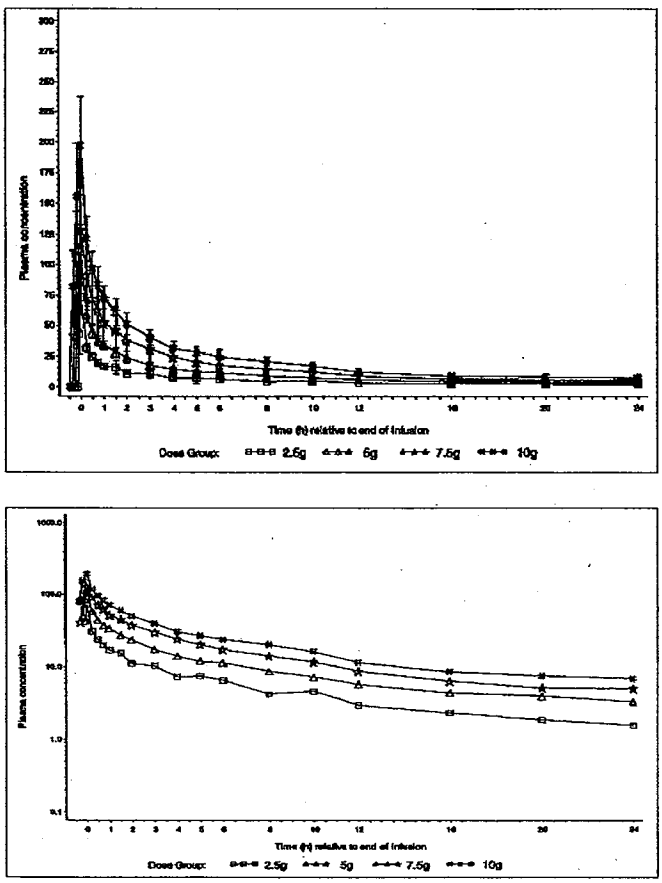
Parameter	Statistic	2.5 9 OH-Co	5.0 9 OH-Co	7.5 9 OH-Co	10.0 9 OH-Co
AUCo-t	N	9	12	9	10
	Mean	5.3	4.6	5.2	5.4
	SD	0.4	1.1	0.6	0.7
	Min	4.5	2.8	4.3	4.2
	Max	5.9	6.1	5.9	6.3
	CV%	7.7	24.7	11.4	12.2
AUCo-inf	N	9	12	9	11
	Mean	5.1	4.5	4.9	5.2
	SD	0.4	1.1	0.6	0.6
	Min	4.3	2.7	4.2	4.0
	Max	5.7	5.8	5.7	6.1
	CV%	8.7	25.2	12.0	12.3

d. Pharmacokinetic Parameters of **Total** Cobalamins-(III) in **Urine** After Administration of 2.5 to 10.0 g OH-Co

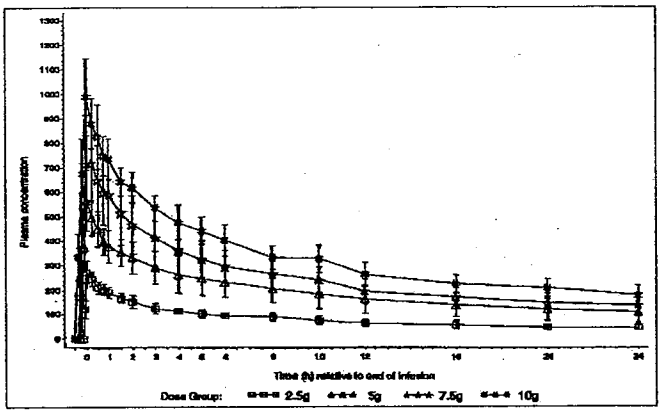
Oose		AeO-72	AeO-72	CLR	Aeo-~	Aeo-~
2.5 9	Mean	(g eq) 1.46	(%) 59.0	(LJh) 0.459	(g eq) 1.80	(%) 72.7
(N=9)	SO	0.29	11.7	0.122	0.38	15.3
	Min	0.92	37.3	0.260	1.09	44.4
	Max	1.97	79.7	0.683	2.45	99.3
5 9	CV% Mean	19.8 3.01	19.8 60.9	26.6 0.414	21.0 3.64	21.0 73.8
(N=12)	SO	0.54	11.0	0.122	0.67	13.6
	Min	2.14	43.3	0.210	2.58	52.4
	Max	3.92	79.4	0.585	4.75	96.2
7.5 9	CV% Mean	18.1 4.26	18.1 57.5	29.5 0.443	18.5 5.11	18.5 69.1
(N=9)	SO	0.42	5.7	0.092	0.54	7.3
	Min	3.67	49.5	0.299	4.35	58.7
	Max	4.81	64.9	0.567	5.79	78.2
	CV%	9.9	9.9	20.7	10.5	10.5
10 9	Mean	4.84	49.0	0.372	5.74	58.2
(N=11)	SO	1.35	13.6	0.118	1.65	16.7
	Min	2.01	20.4	0.159	2.33	23.6
	Max	6.65	67.3	0.538	7.90	80.0
	CV%	27.8	27.8	31.6	28.8	28.8

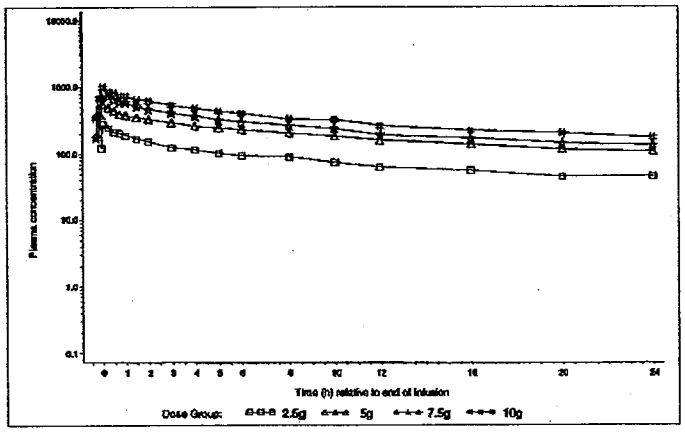
c. Figures

Free Cobalamins-(III) Mean (SD) Plasma Concentrations Over Time of Each Dose Group on a Linear (Top) and Semi-Logarithmic Scale (Bottom)

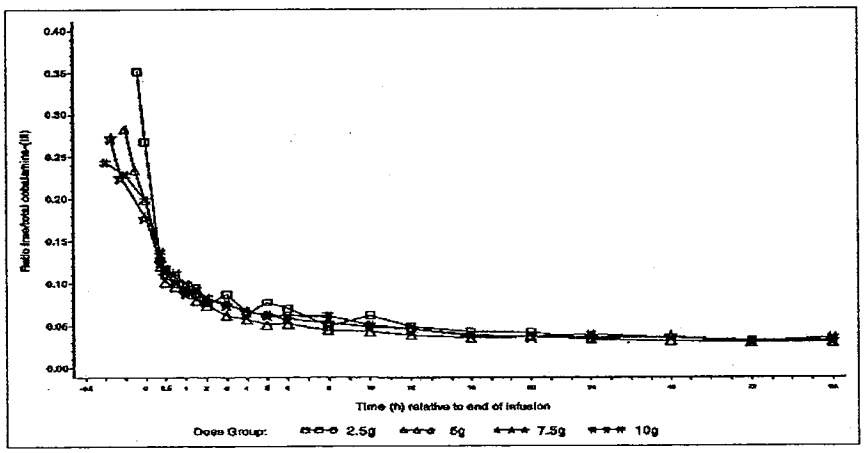


Total Cobalamins-(III) Mean (SD) Plasma Concentrations Over Time of Each Dose Group on a Linear (Top) and Semi-Logarithmic Scale (Bottom)





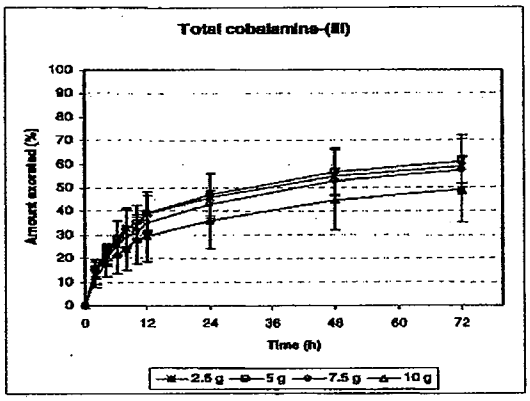
Mean Ratio of the Concentrations of Free / Total Cobalamins-(III) of Each Dose Group Versus Time



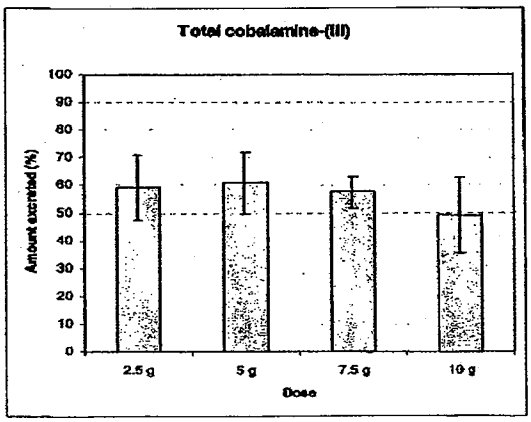
- The reason for differing dose-normalized Cmax values of free cobalamins may be explained by the time necessary to reach equilibrium between free and total cobalamins-(II), e.g. protein binding. Due to the different infusion durations in the four dose groups, the equilibration at tmax is more advanced in the high dose groups compared to the lower dose groups. Overall, the ratio of free to total cobalamins-(II) AUCO_t and AUCo_oo amounted to approximately 5% in all OH-Co dose groups.

- Total clearance of free cobalamins-(II) of approximately 12.5-13.2 L/h (or 208-220 mL/min) exceeded the normal overall glomerular filtration rate for healthy individuals (4.8-7.9 L/h or 80-132 mL/min). This may mainly be due to the rapid binding to plasma proteins. A lower urinary amount was observed during the 72-h urine sampling after the 10-g dose compared to lower doses, which may be explained by urine collection at home (e.g. impact of temperature on stability cobalamins in urine). Renal and total clearance of total cobalamins-(II) were slightly lower in female than in male subjects, which could be attributed to gender-related differences in lean body mass.

Mean (SD) Cumulative Percentage Amount of Total Cobalamins-(III) Excreted in Urine Versus Time - PK Population



Mean (SD) Ae (% of Dose) of Total Cobalamins-(III) Versus Dose - PK Population



4. Other comparisons

a. Creatinine clearance in the PK population

- As a measure of the subjects' renal elimination, in particular, their glomerular fitration rate, creatinine clearance, CLCR was determined at pre-dose on Day 1 in the PK population. Mean values were comparable across all dose groups amounting to 119.2, 127.0, 114.6 and 124.9 mL/min in the 2.5-g, 5.0-g, 7.5-g and 10.0-g OH-Co dose groups, respectively.
- Overall, subjects in the PK population showed CLCR values between 81.1 and 166.3 mL/min. Individual CLCR values below 90 mL/min were found in two subjects: for Subject 1007 (2.5-g group) and Subject 4015 (10.0-g group), CLCR values of 84.6 and 81.1 mL/min, respectively, were obtained at pre-dose Day 1. Whereas the renal clearance, CLR, of total cobalamins-(III) in Subject 1007 was identical to the mean value in the 2.5-g group (0.46 L/h), CLR in Subject 4015 (0.27 L/h) was lower than the mean value in the 1O.0-g group (0.372 L/h; Table 14.4.8). Overall there was a correlation of total clearance of free (CLfree) and total cobalamins-(III) (CLtotal) with CLCR with Pearsons' correlation coefficients of r = 0.66.

b. Pharmacokinetic parameters and safety correlations:

Figure: Mean (SD) Dose-Normalized Cmax of Free and Total Cobalamins-(III) in Plasma

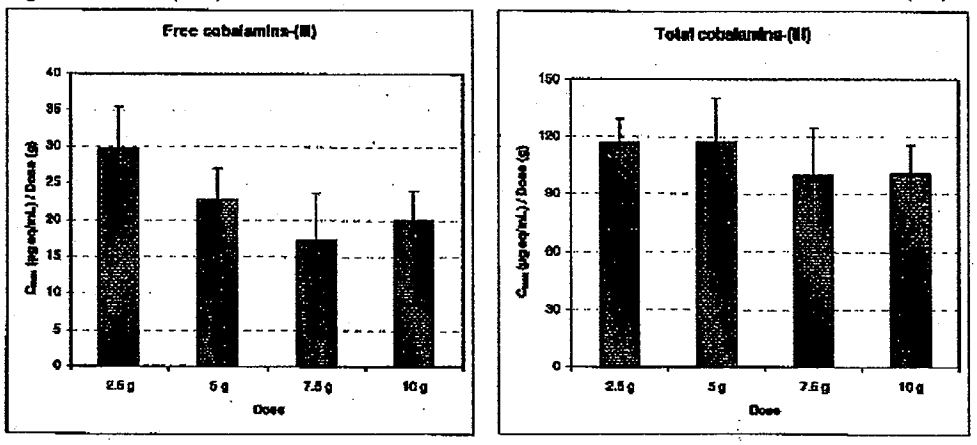


Figure: Mean (SD) Dose-Normalized AUC0-t of Free and Total Cobalamins-(III) in Plasma

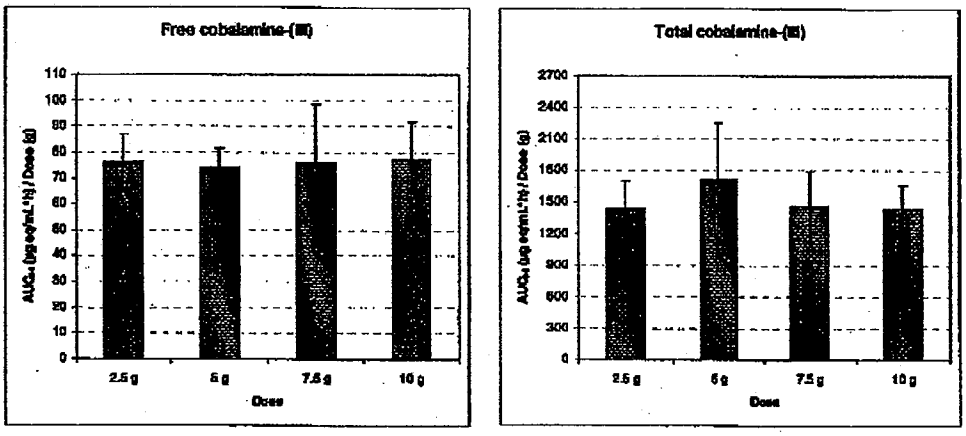


Figure: Mean (SD) tV2 of Free and Total Cobalamins-(III) in Plasma

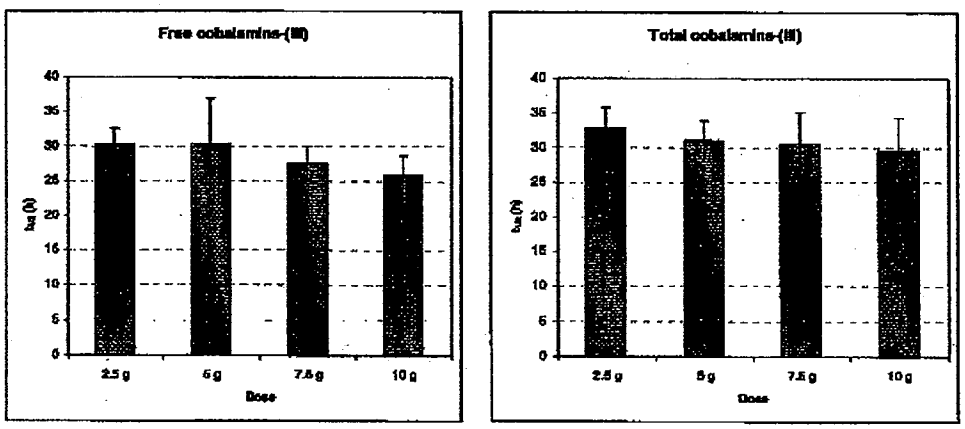


Figure: Mean (SD) Vss of Free and Total Cobalamins-(III) in Plasma

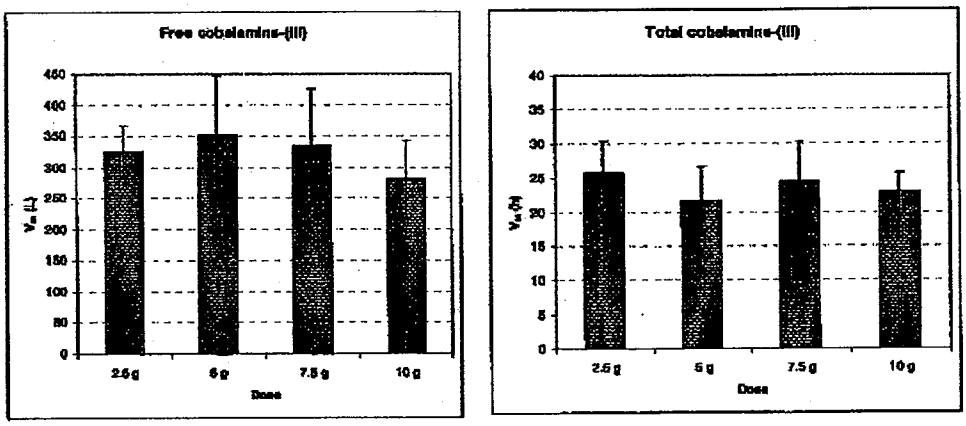
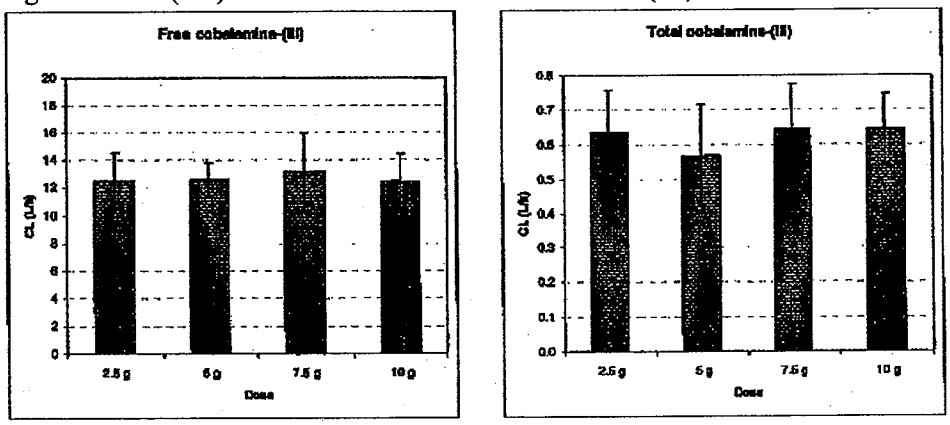


Figure: Mean (SD) CL of Free and Total Cobalamins-(III) in Plasma



c. PK Parameters of Free and Total Cobalamins-(III) in Plasma by Nicotine Consumption, Gender and Age Group

- Nicotine - As per protocol smoking was not allowed during the study for subjects belonging to the PK subgroups. Therefore, there was only one smoker, but 10 ex-smokers and 30 non-smokers in the PK population, which were unequally distributed across the dose groups. No conclusions on the effects of nicotine consumption on the pharmacokinetics of cobalamins-(III) could be drawn. In general, male and female subjects revealed no major differences in the plasma pharmacokinetic parameters of free and total cobalamins-(III) as mentioned above, except for Vss and CL, which tended to be higher in male than in female subjects. However, these differences were negligible when the parameters were related to body weight. Female subjects showed a slightly shorter mean t1/2 (27.9 and 27.5 hours) of total cobalamins-(III) than male subjects (33.8 and 31.3 hours) after 7.5 and 10.0 g OH-Co. The percentage amounts of total cobalamins-(III) excreted in 72-hour urine, Aeo-n were also slightly smaller

in female than male subjects, in particular in the highest dose group (42.2% versus 54.7%). Accordingly, renal clearance of total cobalamins-(III) was lower in female (0.322 - 0.387 L/h) than in male (0.413 - 0.532 L/h) subjects.

- There was only a low number of elderly in the PK population, which ranged from 0 to 3 subjects per dose group (Subjects 1003, 1007, 1011, 2010, 2019, 2022, 4015 and 4022). Thus, no consistent differences were found between the age groups regarding the pharmacokinetic parameters of free and total cobalamins-(III) in plasma and in urine. The two older subjects (:: 45 years) in the 10.0-g dose group (Subjects 4015 and 4022) showed slightly higher mean AUC and t1l2 values and a lower mean CL of free and total cobalamins-(III) compared with younger subjects (:S 45 years) and also showed a creatinine clearance that was below the group mean value (see Tables 14.4.17 - 14.4.19 and Table 14.4.26).

Conclusions:
Safety:
. A total of 458 AEs occurred during the study, 435 in 102 subjects during or after infusion of OH-Co and 23 AEs in 15 subjects during or after infusion of placebo. No serious AE occurred and none of the subjects died during the study. In one subject of the 1O-g dose group, the infusion was prematurely discontinued after 11.9 min due to an allergic reaction.

. The main AEs considered by the Investigator to be possibly study-drug related comprised chromaturia, erythema (skin redness), pustular rash, headache, increased diastolic blood pressure, abnormal relative lymphocyte count (below 10%) and erythema at the injection site.

. A variety of laboratory safety parameters could not be properly evaluated due to the coloration of blood, as investigated in an in-vitro study prior to this clinical study, e.g. cholesterol, creatinine, triglycerides, LDH, amylase, CK, GOT (AST) or GPT (ALT), total bilirbin and uric acid.

. In urine sediment oxalate crystals seem to be increased in a dose dependant manner following OH-Co administration mainly on days 2 to 8.

. Decreases in lymphocytes percentages to below 10% were the most prominent drug- and partly dose-related hematology abnormality starting already within 2- 4 hours and lasting on average up to 4 to 8 days after infusion, but absolute lymphocyte counts remained within the normal range in nearly all subjects. Absolute and relative neutrophils slightly increased within the reference range in a variety of subjects.

. Frequently elevated CRP may be viewed as the corresponding clinically significant biochemistry finding that was considered drug-related. No other abnormal findings in biochemistry parameters related to OH-Co infusion were identified, in spite of restrictions of interpretation.

. Blood pressure (BP) increases that were drug- and roughly dose-related amounted up to around 25 and 20 mmg for systolic and diastolic BP. This relationship was supported by changes of around 20 mmg for mean arterial BP and further PK-safety evaluations. These changes were somewhat higher for the two higher compared to the two lower doses. Blood pressure usually returned to baseline values by 4 hours after end of infusion. Pulse rates decreased correspondingly by more than 10 bpm at the higher doses.

. There were only negligible effects on body temperature and no relevant changes recorded for respiratory rate and pulse oximetry.

. Physical examination, if not recorded as an AE regarding slG reactions, did not reveal any findings. Neurological tests were all inconspicuous. Spirometry as well as 12-lead ECG also did not indicate any noteworthy changes.

Pharmacokinetics:

. Mean tmax of free cobalamins-(II) occurred slightly earlier compared to total cobalamins-(II) in all groups, which may be due to the fast binding of hydroxocobalamin (the main species of free cobalamins during infusion) to plasma proteins. At the 2.5-g dose free to total cobalamins-(II) Cmaxdose was approximately 30% to 70% higher compared to higher doses, which may be related to a different extent of equilibration with plasma proteins due to longer infusion times at the higher doses.

. Total cobalamins-(II) Cmax and AUCO-t increased approximately proportionally with dose over the entire dose range with a ratio of free to total cobalamins-(III) AUCo_t and AUCo_oo of approximately 5% in all OH-Co dose groups.

. Mean volume of distribution at steady state, Vss, appeared to be independent of dose and ranged from approximately 281 - 350 L for the free fraction and from approximately 22 - 26 L for total cobalamins-(II) across the OH-Co dosing groups.

. Mean apparent terminal half-life of free and total cobalamins-(II) ranged from approximately 26 to 33 hours overall and appeared to be independent of dose.

. Total systemic clearance of both free and total cobalamins-(II) did not change over the entire dose range. Total clearance of the free fraction approximately two-fold exceeded the glomerular filtration rate, reflecting the immediate binding of hydroxocobalamin to (plasma) proteins.

. Renal clearance appeared to decrease at the highest 1O-g OH-Co dose level without a change in total clearance. Renal and total clearance of total cobalamins-(II) were slightly lower in female than in male subjects.

. Although incidences of AEs and changes in vital signs parameters indicated a dose/exposure relationship, no dose/exposure proportionality can be concluded.

4.3 Consult Review (including Pharmacometric Reivews) – Not applicable

4.4 Cover Sheet and OCPB Filing/Review Form

Office of Clinical Pharmacology New Drug Application Filing and Review Form			
General Information About the Submission			
	Information		Information
NDA Number	22-041	Brand Name	Cyanokit
OCP Division	II	Generic Name	Hydroxocobalamin
Medical Division	HFD-170	Drug Class	Antidote
OCPB Reviewer	David Lee	Indication(s)	Suspected cyanide poisoning
OCPB Team Leader	Suresh Doddapaneni	Dosage Form	Immediate release tablet
		Dosing Regimen	Single dose
Date of Submission	6/16/06	Route of Administration	Intravenous
Estimated Due Date of OCPB Review	-	Sponsor	EMD Pharmaceuticals, Inc
Medical Division Due Date	-	Priority Classification	3P
PDUFA Due Date	12/16/06		

Clin. Pharm. and Biopharm. Information				
	"X" if included at filing	Number of studies submitted	Number of studies reviewed	Critical Comments If any
STUDY TYPE				
Table of Contents present and sufficient to locate reports, tables, data, etc.	X			
Tabular Listing of All Human Studies	X			
HPK Summary	X			
Labeling	X			
Reference Bioanalytical and Analytical Methods	X			
I. Clinical Pharmacology				
Mass balance:				
Isozyme characterization:				
Blood/plasma ratio:				
Plasma protein binding:				
Pharmacokinetics (e.g., Phase I) -				
Healthy Volunteers-				
single dose:	X	1	1	
multiple dose:				
Patients-				
single dose:				
multiple dose:				
Dose proportionality -	X			
fasting / non-fasting single dose:				
fasting / non-fasting multiple dose:				
Drug-drug interaction studies -				

In-vivo effects on primary drug:				
In-vivo effects of primary drug:				
In-vitro:				
Subpopulation studies -				
ethnicity:				
gender:				
pediatrics:				Deferral
geriatrics:				
renal impairment:				
hepatic impairment:				
PD:				
Phase 1:				
Phase 2/3:				
PK/PD:				
Phase 1 and/or 2, proof of concept:				
Phase 3 clinical trial:				
Population Analyses -				
Data rich:				
Data sparse:				
II. Biopharmaceutics				
Absolute bioavailability:				
Relative bioavailability -				
solution as reference:				
alternate formulation as reference:				
Bioequivalence studies -				
traditional design; single / multi dose:				
replicate design; single / multi dose:				
Food-drug interaction studies:				
Dissolution:				
(IVIVC):				
Bio-wavier request based on BCS				
BCS class				
III. Other CPB Studies				
Genotype/phenotype studies:				
Chronopharmacokinetics				
Pediatric development plan				
Literature References				

Filability and QBR comments		
	"X" if yes	Comments Seeking approval based on animal efficacy model.
Application filable ?	X	Reasons if the application is not filable (or an attachment if applicable) For example, is clinical formulation the same as the to-be-marketed one?

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/s/

David Lee
11/27/2006 10:17:32 AM
BIOPHARMACEUTICS

Suresh Doddapaneni
11/27/2006 10:32:54 AM
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