CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

22-041

PHARMACOLOGY REVIEW(S)

Addendum to NDA 22-41 Pharmacology/Toxicology Review

Date: Dec 15, 2006

From: L.S. Leshin

Through: D. Mellon, PharmTox Team Leader

Subject: Postmarketing Commitment and Response to Additional Information Submitted for Cyanocobalamin Study T8380
NDA 22-041
Sponsor: EMD Pharmaceuticals, Inc.
Drug: Cyanokit (hydroxocobalamin for injection)

On Dec 15, 2006, the Division received a Fax correspondence containing information about the purity of cyanocobalamin used in toxicological study T8380 of NDA 22-041. This purity of this compound and levels of impurities are adequate, allowing acceptance of this study to support the NDA.

cc: list
NDA 22-041
HFD-170Divison File
/PM/Sullivan
/PharmTox TL/Mellon
/MO/Simone

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/s/

Lawrence Leshin
12/15/2006 01:54:32 PM
PHARMACOLOGIST

R. Daniel Mellon
12/15/2006 02:22:50 PM
PHARMACOLOGIST

I concur. Therefore, the pharmacology toxicology recommendation to repeat the 14-day toxicology study of cyanocobalamin or provide the purity data as a Phase 4 Commitment is no longer necessary.

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION
Supervisor's Secondary Review

NDA NUMBER:	**22-041**
SERIAL NUMBER:	**000**
DATE RECEIVED BY CENTER:	**16-June-2006**
DRUG NAME:	**Cyanokit® (Hydroxocobalamin)**
INDICATION:	**Treatment of known or suspected cyanide poisoning**
SPONSOR:	**EMD Pharmaceuticals**
DOCUMENTS REVIEWED:	**Primary Review of Dr. L. Steven Leshin, D.V.M., Ph.D.**
REVIEW DIVISION:	**Division of Anesthesia, Analgesia and Rheumatology Drug Products (HFD-170)**
PHARM/TOX REVIEWER:	**L. Steven Leshin, D.V.M., Ph.D.**
PHARM/TOX SUPERVISOR:	**R. Daniel Mellon, Ph.D.**
PROJECT MANAGER:	**Matthew Sullivan**

Date of review submission to Division File System (DFS): December 5, 2006

EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability

From a nonclinical pharmacology toxicology perspective, NDA 22-041 is **approvable**, pending agreement on drug product labeling and proposed phase 4 commitments. The lack of adequate data to support the safety of the proposed drug substance and drug product impurity specifications will have to be weighed against the potential benefit of the drug product by the clinical team.

B. Recommendation for nonclinical studies

1. The sponsor has not completed adequate reproduction and developmental toxicology studies for hydroxocobalamin. These studies may be submitted in fulfillment of a Phase 4 Commitment. The sponsor should conduct the standard battery of reproductive toxicology studies as described in ICHM3, S5A, S5B, and S5B(M) Guidances to Industry, as follows:

    a. Segment I (Fertility and Early Embryonic Development)
    b. Segment II (Embryofetal Development) in two species
    c. Segment III (Peri- and Post-natal Development)

2. The Sponsor has not provided adequate safety qualification for the proposed specified and unspecified drug substance and drug product impurities which exceed the ICH Q3A and Q3B(R2) thresholds for qualification. Adequate safety qualification should include a minimal in vitro genetic toxicology screen (one in vitro mutagenicity assay and one in vitro assay for chromosome damage) and a toxicology study of adequate duration to support the proposed clinical trial. These studies may be submitted at a Phase 4 commitment, at the discretion of the medical review team, based upon their assessment of the existing clinical data and the potential benefit of the drug for cyanide exposure individuals.

3. The Sponsor has not completed adequate photosafety testing to support the drug product. Such studies may be submitted as a Phase 4 commitment.

4. The Sponsor has not provided adequate characterization of cyanocobalamin. In the event of cyanide poisoning and treatment with hydroxocobalamin, significant exposure to cyanocobalamin will occur. Such exposure will significantly exceed exposure to cyanocobalamin

when administered as a vitamin supplement, and therefore should be adequately characterized. Dr. Leshin's review indicates that the submitted studies do not provide information regarding the purity of the drug substance tested. Therefore, the sponsor should either provide information regarding the purity of the drug studies submitted, or conduct a new toxicology study with well-characterized cyanocobalamin chemical substance. I concur.

5. Dr. Leshin has recommended that the sponsor complete tissue distribution studies for both hydroxocobalamin and cyanocobalamin that determine the duration of time the drug remains in the tissues. He notes that these studies should be performed in both sexes and in lactating animals, to determine the extent of accumulation and elimination in the mammary glands. Such studies will allow for more definitive description of the amount time a person receiving the drug should avoid sunlight, and provide additional information regarding the use of the drug in nursing mothers. I agree and I concur with his assessment that since these studies are not critical to the product's immediate life-saving indication, they may be submitted as a Phase 4 Commitment.

C. Recommendations on labeling

Dr. Leshin has made the following recommendations on labeling. I concur with his recommendations and recommend additional changes to the Clinical Pharmacology section of the label.

Proposed Labeling (nonclinical)	**Recommended Labeling (nonclinical)**
8 USE IN SPECIFIC POPULATIONS	***8 USE IN SPECIFIC POPULATIONS***
8.1 PREGNANCY	**8.1 PREGNANCY**
Pregnancy Category C. Animal studies are insufficient with respect to effects on pregnancy and embryo-fetal development. There are no adequate and well-controlled studies in pregnant women.	Pregnancy Category C. Animal studies are insufficient with respect to effects on pregnancy and embryo-fetal development. There are no adequate and well-controlled studies in pregnant women. Cyanokit should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

b(4)

3 Page(s) Withheld

_____ Trade Secret / Confidential (b4)

✓ Draft Labeling (b4)

_____ Draft Labeling (b5)

_____ Deliberative Process (b5)

Withheld Track Number: Pharm/Tox-_____

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

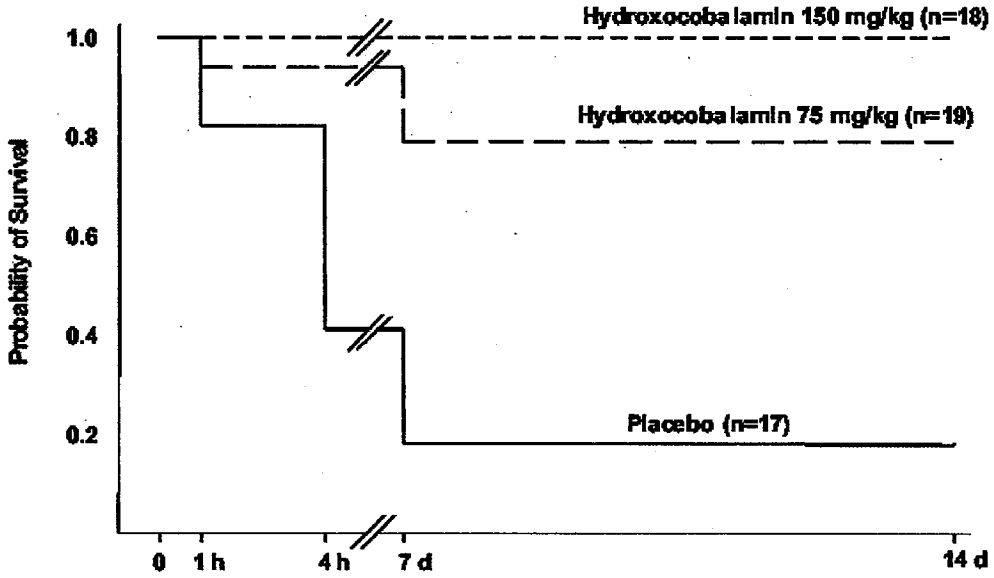
The safety and efficacy of the proposed drug product, Cyanokit (hydroxocobalamin) injection for the treatment of known or suspected cyanide poisoning, has been evaluated as per 21 CFR §314.600 (Subpart I – Approval of New Drugs When Human Efficacy Studies Are Not Ethical or Feasible). It is my opinion that the sponsor has fulfilled the criteria for the animal efficacy rule as outlined in the regulations, as follows:

1. The sponsor has conducted an adequate and well-controlled animal study that has established that the drug product is reasonably likely to produce clinical benefit to humans, and
2. the mechanism by which cyanide poisoning leads to lethality as well as the mechanism by which hydroxocobalamin leads to detoxification of neutralization of the cyanide, and
3. the effect of hydroxocobalamin has been demonstrated in multiple animal species in the literature, supported by the controlled nonclinical efficacy study in the dog model that is sufficiently well-characterized for predicting the response in humans, and
4. the endpoint of the animal efficacy study, survival or prevention of major morbidity, is clearly a desired benefit in humans, and,
5. the data or information on the kinetics and pharmacodynamics of hydroxocobalamin combined with the previous experience in humans has allowed the review team to select an effective dose in humans.

In previous discussions with the sponsor, the Division agreed that a single well-controlled animal efficacy study would likely be adequate to support the efficacy of the drug product. This decision was based in part on the existence of several early animal efficacy studies completed by the sponsor and submitted in support of their application to the French authorities. In addition, there are several well known published reports of nonclinical efficacy studies in the rat, mouse, and rabbit models, as well as previous human experience from France. The Agency reviewed the sponsors proposed animal efficacy study in the dog model via a special protocol assessment prior to initiation of the study. The Agency agreed that the dog was the most relevant model and that the intravenous route of administration of cyanide would provide the "worst-case scenario" and the most robust assessment to establish efficacy of hydroxocobalamin.

The key nonclinical efficacy study employed intravenous infusion of potassium cyanide (0.4 mg/kg/min, IV) to the beagle dog. Cyanide was infused until apnea was seen and continued for an additional 3 minutes. Three treatment groups were employed: Saline control, a low dose of hydroxocobalamin (75 mg/kg), and a high dose of hydroxocobalamin (150

mg/kg). The results of this study have recently been published. The effectiveness of the treatments has been depicted via a survival curve, reproduced from the sponsor's submission:



Dr. Leshin has reviewed the nonclinical efficacy study and has concluded that the study is adequate to support the conclusion that the administration of hydroxocobalamin to dogs treated with a lethal dose of potassium cyanide via the intravenous route of administration results in improvement in the probability of survival.

As noted in §314.600, approval of a drug under Subpart I does not address the safety evaluation for the products to which the animal efficacy rule applies. As stated in Federal Register notice RIN 0910-AC05 (Final Rule), "Products evaluated for effectiveness under subpart I of part 314 and subpart H of part 601 will be evaluated for safety under preexisting requirements for establishing the safety of new drug and biologic products."

The sponsor has submitted both single-dose and repeat-dose toxicology studies with hydroxocobalamin in the rodent and the dog model that were completed in the early 1970s. Dr. Leshin and I are in agreement with the sponsor that these early studies are insufficient to characterize the potential toxicity of the proposed high intravenous doses of hydroxocobalamin. The sponsor conducted repeat-dose toxicology studies in the dog model to provide support for this application. Specifically, the sponsor completed a 28-day repeat-dose toxicology study with hydroxocobalamin in the dog model. The target organs of toxicity have been identified as the skin, the liver, and the kidney. The study identified a NOAEL of 150 mg/kg in the study, which was defined by reversible histological changes in the liver.

The Sponsor also completed a 14-day repeat dose intravenous toxicology study in the dog model to characterize the potentially large exposure to cyanocobalamin which would result if a cyanide poisoned individual were to be treated with the drug. The sponsor's studies are not optimal; the first dose range finding study was conducted only in a single animal per dose making interpretation of any findings impossible. The second study did not contain information regarding the purity of the cyanocobalamin administered; therefore, definitive characterization of the potential toxicity can not be made. I agree with Dr. Leshin that adequate characterization of the potential toxicity to high dose cyanocobalamin can be completed as a Phase 4 Commitment if the NDA is approved on the first cycle.

The sponsor submitted two segment II (embryofetal development) studies (rat and rabbit) that were completed in the early 1970s. Although these studies did not reveal evidence of teratogenicity, Dr. Leshin concluded that these studies are not adequate by current standards and therefore should be repeated. The sponsor did not include results from these studies in their proposed labeling; rather they state that nonclinical studies are inadequate. I concur with both the Sponsor and Dr. Leshin. The sponsor did not complete either Segment I (fertility and early embryonic development) or Segment III (peri- and post-natal development) reproductive toxicology studies to date. I believe that these studies may be completed as a phase 4 commitment if the NDA is approved on the first cycle.

Genetic toxicology studies were completed to assess the mutagenic potential of hydroxocobalamin. Hydroxocobalamin was negative in the standard battery of tests (in vitro bacterial reverse mutation assay or Ames assay, in vitro mouse lymphoma assay, and the in vivo rat micronucleus assay). Carcinogenicity studies are not required for this drug, as it is not administered chronically.

Special toxicology studies were also completed for this drug product. Specifically, the sponsor submitted a Neutral Red Uptake assay using Balb/c 3T3 fibroblasts, which is an in vitro phototoxicity assay. The potential for phototoxicity was evaluated based on unexpected adverse findings during a clinical pharmacokinetic study, specifically the occurrence of a skin rash on the face. Dr. Leshin has recommended an in vivo assessment of the phototoxic potential of the drug product which can be completed as a Phase 4 Commitment. I concur with his recommendation.

The sponsor originally requested the following drug substance release specifications and drug product release and shelf life specifications. The drug is to be administered at a maximum daily dose of 5 grams via intravenous infusion which could be followed by an additional 5 grams of drug pending the clinical response. Therefore, based on ICH Q3A (drug substance) and ICH Q3Br (drug product), the following thresholds should be applied to the drug product:

Maximum Daily Dose > 2 grams/day	Reporting Threshold	Identification Threshold	Qualification Threshold
Drug Substance	0.03%	0.05%	0.05%
Drug Product	0.05%*	0.10%	0.15%

* for drug product reporting thresholds, the maximum daily dose is > 1 grams/day.

The table below indicates the proposed drug substance and drug product release specifications. The sponsor's proposed specifications significantly exceed the safety qualification threshold as per ICH Q3A and ICH Q3BR(2). I agree with Dr. Leshin that the impurities should be adequately qualified. The potential toxicity of several of these impurities at the propose levels have not been addressed by the sponsor.

Impurities	Proposed Drug Substance Release Specs (%)	Proposed Drug Product Release Specs (%)	Proposed Drug Product Shelf Life Specs (%)	Maximum Impurity level qualified for safety via Ames assay Batch 9337, 2080	28-Day Dog Toxicology Hydroxo-cobalamin Batch 2059
Related substances (%w/w)					
Specified indentified impurities					
[illegible]	[illegible]	–	–	–	–
Specified unidentified impurities					
b(4)					
Any other impurity					
Total Content of Impurities					

B. Pharmacologic activity

Hydroxocobalamin is a parenteral preparation of Vitamin B_{12a}. Hydroxocobalamin binds to cyanide resulting in the formation of cyanocobalamin (Vitamin B_{12}) which is then excreted in the urine.

C. Nonclinical safety issues relevant to clinical use

I agree with Dr. Leshin's recommendation that wording regarding the potential for photosensitization or phototoxicity has not been completely addressed by the sponsor via nonclinical studies. In light of the specific indication, information to avoid direct sun exposure for the duration of time the drug remains in the tissues should be included in the drug product label.

The sponsor has not provided adequate safety qualification for the impurities in their drug product. Specifically, as per ICH Q3B(R2), the sponsor should reduce the impurities (both identified and unidentified) to below the qualification threshold of — %. Adequate safety qualification should include a minimal genetic toxicology screen (one point mutation assay and one chromosomal aberration assay) and general toxicity study in a single species of at least 14 days duration. The sponsor states in their submission that the proposed impurity specifications are justified based on nonclinical toxicology studies conducted with batches of drugs that contained high levels of these impurities. However, the studies that were submitted were not adequate in design and did not test the drug impurities up to the levels proposed. It is not possible to delineate the potential toxicity of these impurities at the levels proposed based upon the information provided in the submission. Therefore, the risk that may be associated with the exposure to these impurities is not known. However, according to the Chemistry Manufacturing and Controls (CMC) review by Dr. Milagros Salazar-Driver, the specified unidentified impurities were tested on their UV characteristics. Dr. Salazar-Driver concluded that:

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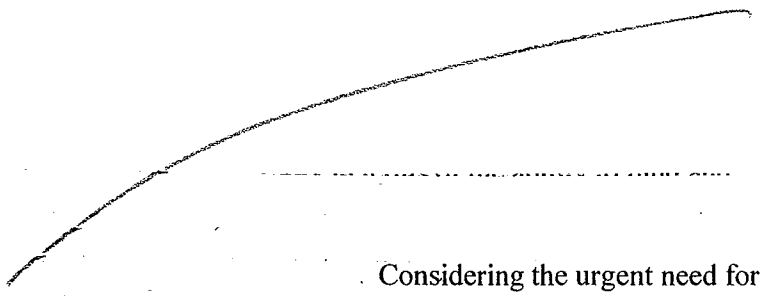
1. the spectra provide evidence that the unidentified impurities do show a and

2. the UV spectrum

b(4)



. Considering the urgent need for the availability of life-saving treatments for cyanide poisoning, the potential risk due to the lack of definitive information on these impurities would not appear to outweigh the potential benefit of the drug product. The final assessment of this risk benefit analysis, however, remains the discretion of the clinical review team.

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/s/

R. Daniel Mellon
12/5/2006 10:28:38 PM
PHARMACOLOGIST
Pharmacology Toxicology Supervisor

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	**22-041**
SERIAL NUMBER:	**000**
DATE RECEIVED BY CENTER:	**6/16/06**
PRODUCT:	**Cyanokit® (Hydroxocobalamin)**
INTENDED CLINICAL POPULATION:	**individuals with known or suspected cyanide poisoning**
SPONSOR:	**EMD Pharmaceuticals, Inc.**
DOCUMENTS REVIEWED:	**18 Vols.**
REVIEW DIVISION:	**Division of Anesthesia, Analgesia and Rheumatology Drug Products (HFD-170)**
PHARM/TOX REVIEWER:	**L. Steven Leshin, D.V.M., Ph.D.**
PHARM/TOX SUPERVISOR:	**R. Daniel Mellon, Ph.D.**
DIVISION DIRECTOR:	**Bob A. Rappaport, M.D.**
PROJECT MANAGER:	**Matthew Sullivan**

Date of review submission to Division File System (DFS): **December 5, 2006**

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EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability

From a nonclinical pharmacology toxicology perspective, NDA 22-041 is approvable. The reproductive and developmental toxicological information is incomplete and does not meet current ICH and FDA guidances. In addition, the recently proposed impurity limits (not in the original NDA submission) exceed the impurity concentrations that have been qualified through genotoxicity and acute toxicity studies.

B. Recommendation for nonclinical studies

1. The Sponsor has not completed adequate reproduction and developmental toxicology studies for hydroxocobalamin. Since these studies are not critical to the products immediate life-saving indication, it is felt that they may be submitted as a Phase 4 Commitment. The Sponsor should conduct studies to characterize the toxicity of their product in the following conditions as described in ICHM3, S5A, S5B, and S5B(M) Guidances to Industry:

   Fertility and Early Embryonic Development
   Embryo-fetal Development in two species
   Peri- and Post-natal Development

2. To support the proposed higher specification limits, genetic toxicology studies (for mutagenicity and for chromosome damage) and an acute toxicology study should be conducted with the product containing the amount of impurity at or exceeding the proposed limits, or with the isolated impurity, itself.

3. The purity of the cyanocobalamin in study T8380 was not provided, precluding the ability to draw definitive conclusions from the information submitted about the toxicity of cyanocobalamin. The sponsor should either provide information on the purity of the cyanocobalamin used in the existing toxicology studies, or conduct a new toxicology study with well-characterized cyanocobalamin chemical substance.

4. Tissue accumulation of hydroxocobalamin and cyanocobalamin occurred. However, it was not determined how long this takes to be eliminated since there were no tissue distribution studies nor measurements of tissue concentrations at multiple timepoints. Therefore, a single dose tissue distribution study should be performed to address the question of how long tissue hydroxocobalamin and cyanocobalamin is maintained in the

body and if there are associated toxicological manifestations that need to be monitored during this time period. This study should be performed in both sexes and in lactating animals, to determine the extent of accumulation and elimination in the mammary glands. Again, since these studies are not critical to the product's immediate life-saving indication, it is felt that they may be submitted as a Phase 4 Commitment.

5. An additional issue concerns potential phototoxicity and photosensitization. One of the target organs for drug accumulation was the skin. This was visibly detected within minutes after infusion and persisted for days depending on dose and frequency of administration. A European approved test for phototoxic effects was performed *in vitro* with fibroblast cells. Although the test was negative, this single study with only one of many cell types comprising the skin is not very conclusive as an overall determination of phototoxicity. Both hydroxocobalamin and cyanocobalamin discolor skin and organs and is not readily eliminated from tissues. Therefore, they should verify these findings with an *in vivo* phototoxicity study. This would help determine if treated patients should avoid sunlight and the length of avoidance, if necessary. Again, since these studies are not critical to the product's immediate life-saving indication, it is felt that they may be submitted as a Phase 4 Commitment

C. Recommendations on labeling

~~Red strikethrough~~ refers to deletion of the Sponsor's label
Blue refers to additions to the Sponsor's label

8 USE IN SPECIFIC POPULATIONS
8.1 Pregnancy

Pregnancy Category C.

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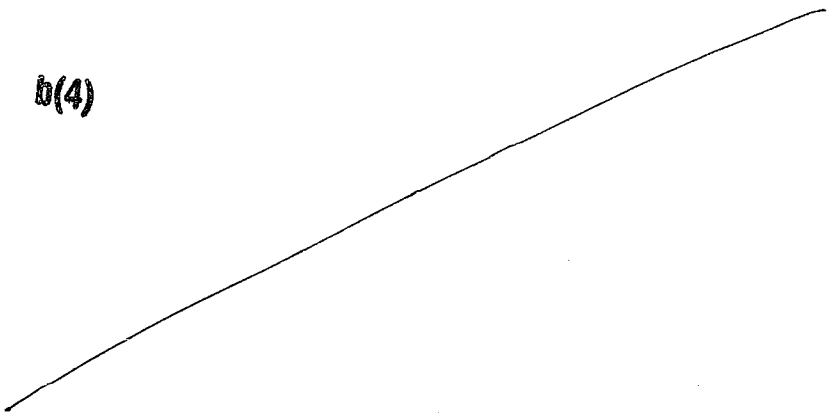

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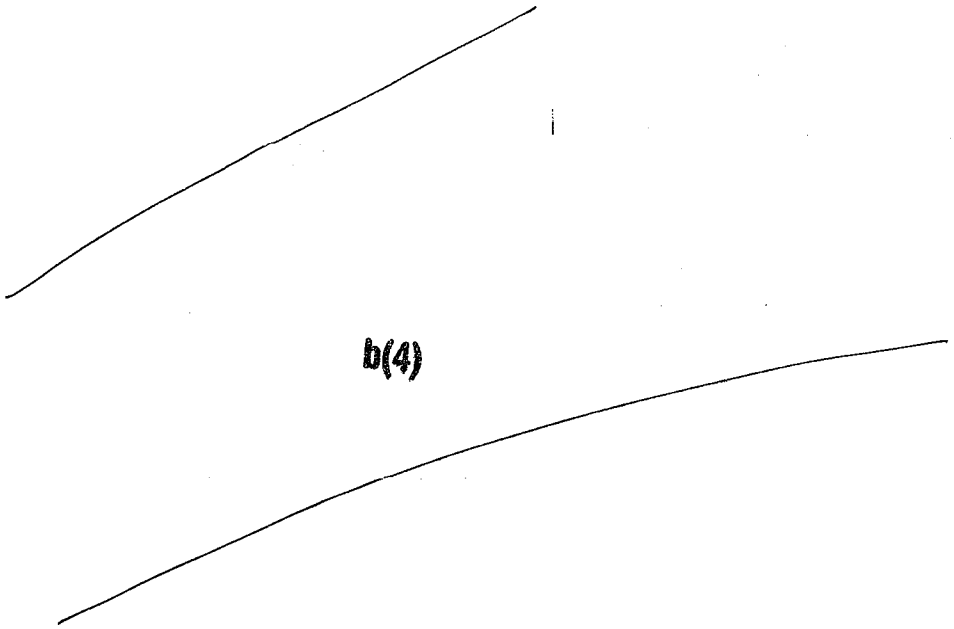
_____ Draft Labeling (b5)

_____ Deliberative Process (b5)

Withheld Track Number: Pharm/Tox-1



II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

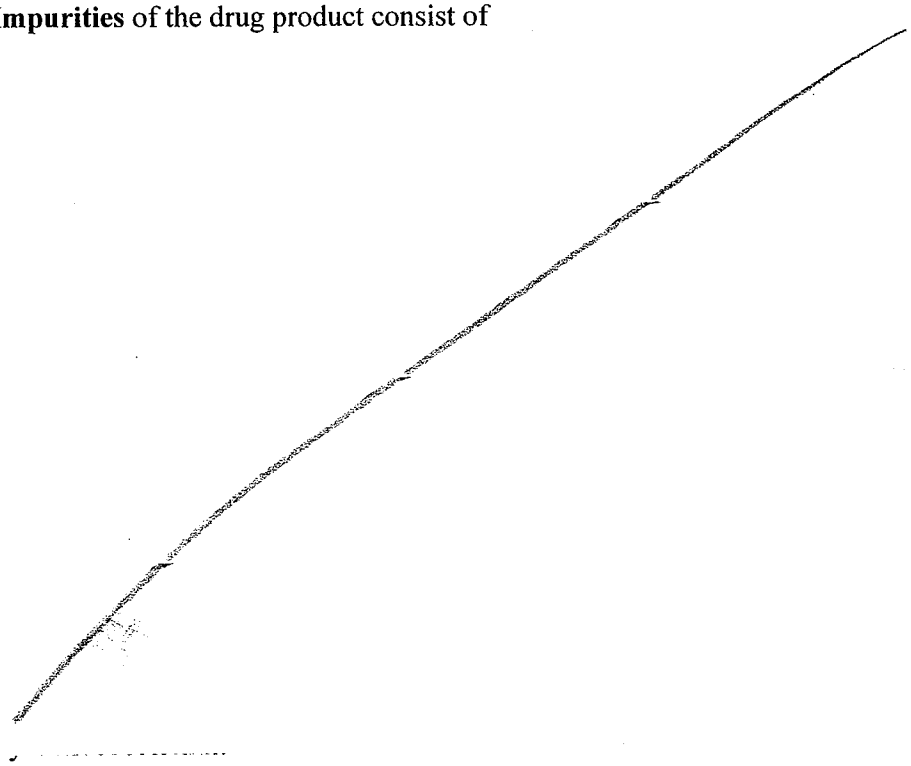
General Toxicology: Single and repeated dose toxicological studies were performed in rats and dogs. Many of these studies do not satisfy our recommended guidelines in terms of number of animals, gender of animals, length of treatment, and parameters measured. However, in total, the major toxicities could be identified.

In the single dose rat studies, hydroxocobalamin was administered intraperitoneal at doses of 75 to 1000 mg/kg. The major clinical signs in rats included dyspnea, locomotor disturbance, piloerection, incomplete eyelid closure, sunken flank and reddish urine and reddish skin. The major organ toxicities were accumulation of fluid in the lungs at 1000 mg/kg (highest dose) and death in some animals (1000 mg/kg), discolored and congested liver, discolored organs and tissues at ≥300 mg/kg including skin, abdominal fat, testes, and epididymides.

In the dog, all doses of hydroxocobalamin were administered intravenously and ranged from 75 to 1200 mg/kg. There were no deaths at any dose. The major clinical signs included reddish discoloration of the skin and mucous membranes and reddish urine at all doses. Wrinkles and or wheals about the head, swollen ears, and head edema developed, possibly signs of hypersensitivity or osmotic fluid shifts with drug distribution. At the higher

doses of 300 and 1200 mg/kg, emesis and tremors were observed. During a recovery period, all these signs resolved. Consistent changes in liver enzymes were reported, but it was also noted that the reddish hydroxocobalamin might interfere with colorimetric analyzers. Validation of these measurements was not reported. Other changes in chemistry and hematology were not of toxicological significance. There were no changes in body weight or food consumption. There were no changes in EKG parameters, heart rate or blood pressure, but the times of measurement were only at 2 hours after administration. There were no ophthalmologic examinations. The target organs were kidney, liver, bone marrow and skin. The liver of high dose dogs was characterized by edema of intrahepatic sinuses with activation of Kupffer cells, multifocal small acute necrosis, and microgranulomas. The kidney findings included multifocal tubule eosinophilic casts, focal papilla hemorrhage, multifocal tubular dilatation, and crystalline intracytoplasmic deposits in the distal tubule. In the bone marrow, there was minimal to moderate single cell necrosis that appeared to be dose-dependent in incidence and severity. These were thought to be macrophages. The ratios of hematopoietic cells were not altered. In the gall bladder, adrenals and fat tissue, hemorrhages were present. With time post-treatment the occurrence and severity of these findings decreased, but resolution was not complete by 2 weeks post-treatment.

Impurities of the drug product consist of



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b(4)

Genetic toxicology: Studies were conducted to characterize both the mutagenic and clastogenic potential of hydroxocobalamin. In bacterial reverse mutation bacteria assays (Ames test), hydroxocobalamin, at concentrations of up to 5000 µg/plate, was not mutagenic to strains of *S. typhimurium* and *E. coli* in the absence or presence of S9 mix. Hydroxocobalamin was not mutagenic at the TK locus (5-trifluorothymidine resistance) in mouse lymphoma cells using a fluctuation protocol at concentrations of 158 to 5000 µg/mL, with or without S9.

In the in vivo micronucleus assay, hydroxocobalamin was administered to male rats at doses up to 140 mg/kg, sufficient to induce clinical signs of toxicity, but did not induce micronuclei in rat bone marrow polychromatic erythrocytes and did not alter the ratio of normochromic to polychromic erythrocytes. Therefore, hydroxocobalamin was not clastogenic in this assay.

Carcinogenicity: There were no carcinogenicity studies completed, nor are they required for an acute indication.

Reproductive toxicology: Embryo-fetal toxicity studies were conducted with rats and rabbits in 1974. These studies did not meet current GLP and ICH guidelines and therefore could not be used to support the reproductive safety of the proposed product.

Local Tolerance: There were no separate studies on local tolerance. The Sponsor did not indicate abnormal clinical or histopathological findings for injection sites in the dog toxicology studies. In the rat studies, following intraperitoneal injection, some abdominal pathological findings were attributed to the injection.

In the dog studies, hypersensitivity-like swelling of regions of the head and ears and the presence of wrinkles or wheals occurred soon after intravenous administration of hydroxocobalamin and cyanocobalamin (1 male at the high dose). These reactions resolved within a few hours or by the following day, somewhat dependent on dose administered. In one study, they reoccurred with repeated dosings on subsequent days, suggesting a fluid redistribution, rather than a immunologic hypersensitivity reaction. The cause of this reaction was not identified, since the infused solution was supposedly iso-osmotic.

Special toxicology: Hydroxocobalamin was not phototoxic to *in vitro* cultures of mouse fibroblasts at doses up to 1000 µg/mL with or without exposure to UVA light wavelengths. Toxicity measures of this assay reflect only the most serious degree, that of cell death. Phototoxicity may also cause less severe cellular reactions in skin resulting in erythema, swelling, and eschar formation, altered DNA (thymidine dimmer, adduct formation) which may lead to mutagenesis and tumors, not detectable by this assay. This assay

did not test UVB wavelengths, nor does it consider other types of cells in the skin.

B. Pharmacologic activity

Hydroxocobalamin is the hydroxolated active form of vitamin B_{12} in which cobalt ion is coordinated in 4 positions by a tetrapyrol (or corrin) ring. The rationale for administering hydroxocobalamin as an antidote for cyanide poisoning is based on the high affinity of the cyanide ion for cobalt compounds. The acute toxicity of cyanides is due to the binding of cyanide to cytochrome oxidase resulting in the blockade of the respiratory chain. Since the cobalt binding site in hydroxocobalamin has a higher affinity for cyanide than the hydroxyl group of hydroxocobalamin, cyanocobalamin will form. Although not experimentally confirmed, survival results from animal studies, and from human administered hydroxocobalamin after accidental cyanide exposure, suggests that binding of hydroxocobalamin occurs for the most part in blood. Hydroxocobalamin does not appear to enter cells readily, or "pull cyanide off" of its ferric binding site of cytochrome oxidase, but this has not been empirically determined. Both cyanocobalamin and hydroxocobalamin are eliminated mostly via the kidney into urine.

Distribution studies with hydroxocobalamin were not performed. From the toxicological studies, the target organs of accumulation were the liver, kidney, bone marrow, and skin.

The main nonclinical efficacy study clearly demonstrated the pharmacological efficacy of hydroxocobalamin. In anesthetized dogs, administration of a lethal dose of cyanide, followed immediately by an infusion of 75 or 150 mg/kg hydroxocobalamin resulted in dose-related survival, achieving 100% survival in dogs administered 150 mg/kg hydroxocobalamin. A dose of 75 mg/kg resulted in 79% survival. Dogs survived to 15 days post-treatment, a time at which minimal clinical or neurological signs were observed in the dogs.

C. Nonclinical safety issues relevant to clinical use

Discussed above in Recommendations for Nonclinical Studies were the issues of impurities, the lack of information on reproduction and development toxicity, lack of complete characterization of cyanocobalamin clearance and toxicity, and potential phototoxicity or photosensitization.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

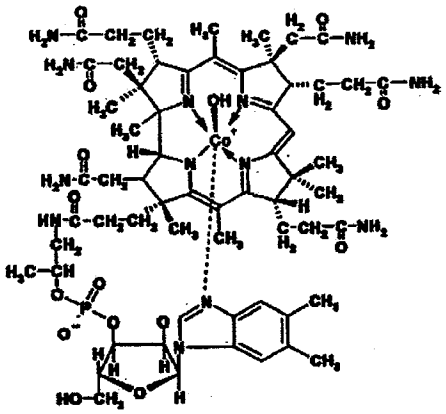
2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number:	**20-041**
Review number:	1
Sequence/date/type of submission:	000/April 13, 2006/Commercial
Information to sponsor:	Yes ( ) No ( )
Sponsor and/or agent:	**EMD Pharmaceuticals** Durham, NC 27707
Manufacturer for drug substance:	b(4)
Reviewer name:	L. Steven Leshin
Division name:	Division of Anesthesia, Analgesia and Rheumatology Drug Products
HFD #:	170
Review completion date:	December 1, 2006

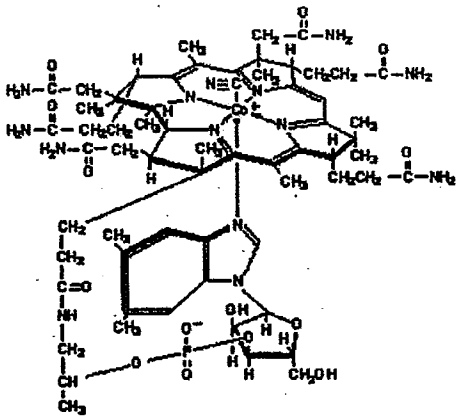
Drug:

Trade name:	**Cyanokit® (Proposed name)**
Generic name:	Hydroxocobalamin
Code name:	EMD 415722
Chemical name:	Cobinamide hydrochloride dihydrogen phosphate (ester), mono (inner salt), 3'-ester with 5,6-dimethyl-1-α-D-ribofuranosyl-1H-benzimidazole
CAS registry number:	13422-51-0
Molecular formula:	$C_{62}H_{89}CoN_{13}O_{15}P$
Molecular weight:	b(4)

Structure:



hydroxocobalamin hydrochloride



cyanocobalamin
(MW 1355.37)

Cyanide combines with hydroxocobalamin to produce cyanocobalamin. In the structural illustration above, different planar views are presented, but the only structural difference is the replacement of the hydroxyl group bound to cobalt in hydroxocobalamin with the cyanide group in cyanocobalamin. Some toxicological studies were conducted with cyanocobalamin (dog studies T8380 and T8377). The code name "Art. 524950" was used for Sponsor produced batches of cyanocobalamin used in those toxicological studies.

Relevant INDs/NDAs/DMFs:

preIND 67,151 (Cyanokit, hydroxocobalamin)

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Drug class:	Cyanide poison antidote, Vitamin B12
Intended clinical population:	Individuals with known or suspected cyanide poisoning
Clinical formulation:	Lyophilized powder for intravenous (IV) infusion, 2.5 g of lyophilized hydroxocobalamin per vial, administration of 2 vials required (5 g initial dose, with a second dose also 5 g, 2 vials, if necessary, for a total potential dose of 10 g)

First marketed in France in 1996 as
Cyanokit® Lyophilizate for parenteral use

Reviewer's Comments on the Impurities in the Clinical Formulation: The impurity

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Route of administration: Intravenous infusion

Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

Regulatory History

- Approval would be under the Animal Rule (21CFR314.600)
- Fast Track Status granted March 24, 2006 (FD&C Act Section 506(a) amended by FDA Modernization Act of 1997)
- Orphan Drug Designation granted Nov 25, 2003 (21CFR316), thus user fees waived

Studies reviewed within this submission:

Primary Pharmacodynamics **Efficacy Study**	
Pilot Study	
N106341 Mod. 4, Vol. 2, Tab 4.2.1.1.4, N106341	Pilot Study of Intravenous Cyanokit® Administration Following Intravenous Poisoning with Potassium Cyanide in Adult Beagle Dogs
DMPK 140-04 Mod. 4, Vol. 3, Tab 4.2.1.1.5, DMPK 140-04	HPLC-UV Determination of Total Cobalamins-(III) and Cyanocobalamin in Beagle Dog Plasma Samples Collected During a Pilot Efficacy Study
DMPK 190-04 Mod. 4, Vol. 3, Tab 4.2.1.1.6, DMPK 190-04	Determination of Cyanide in Dog Whole Blood Samples Using Liquid Chromatography with Tandem Mass Spectrometric Detection
Main study	
N106342 Mod. 4, Vol. 1, Tab 4.2.1.1.1, N106342	**Efficacy of Intravenous Hydroxocobalamin (Cyanokit®) Administration Following Intravenous Poisoning with Potassium Cyanide in Adult Beagle Dogs**
DMPK 166-04 Mod. 4, Vol. 1, Tab 4.2.1.1.2, DMPK 166-04	HPLC-UV Determination of Total Cobalamins-(III) and Cyanocobalamin in Beagle Dog Plasma Samples Collected During the Study N106342
DMPK 30-05 Mod. 4, Vol. 1, Tab 4.2.1.1.3, DMPK 30-05	Determination of Cyanide in Dog Whole Blood Samples Obtained During the Dog Efficacy Study N106342 [redacted] Using Liquid Chromatography with Tandem Mass Spectrometric Detection
Secondary Pharmacodynamics	
PhD/0001 Mod. 4, Vol. 3, Tab 4.2.1.2.1, PhD/0001	Hydroxocobalamin (Cyanokit®): NO-Trapping as Cause of Haemodynamic Effects in Anaesthetized Rabbits
PhD/0002 Mod. 4, Vol. 3, Tab 4.2.1.2.2, PhD/0002	Cyanocobalamin: Characterization of Haemodynamic Effects in Anaesthetized Rabbits
Safety pharmacology	
III.F Mod. 4, Vol. 12, Tab 4.2.3.7.7.3.1, III.F	Safety Pharmacology and Primary Pharmacodynamic Studies in the Mouse, Rabbit, Rat, Guinea Pig and Dog [redacted]
Pharmacokinetics	
Analytical Methods and Validation Reports: Total and Free Cobalamins	
DMPK 208-04 Mod. 4, Vol. 3, Tab 4.2.2.1.1.1, DMPK 208-04	Reactions of Hydroxocobalamin in Human Plasma and Urine: Explanation of a New Bioanalytical Strategy
DMPK 25-04 Mod. 4, Vol. 3, Tab 4.2.2.1.1.2, DMPK 25-04	EMD 415722 -Exploratory Investigation of Hydroxocobalamin Stability in Rat, Dog and Human, Plasma at Different Conditions
DMPK 130-03 Mod. 4, Vol. 3, Tab 4.2.2.1.1.3, DMPK 130-03	EMD 415722 -Exploratory Investigation of Hydroxocobalamin Stability in Human Urine at Different Conditions
DMPK 03-05 Mod. 4, Vol. 3, Tab 4.2.2.1.1.4, DMPK 03-05	Validation of an HPLC-UV Assay for the Determination of Total Cobalamin-(III) in Rat Plasma
DMPK 04-05 Mod. 4, Vol. 3, Tab 4.2.2.1.1.5, DMPK 04-05	Validation of an HPLC-UV Assay for the Determination of Free Cobalamin-(III) in Rat Plasma
DMPK 96-04	Validation of an HPLC-UV Assay for the Determination of Total

Mod. 4, Vol. 3, Tab 4.2.2.1.1.6, DMPK 96-04, p. 1	Co-(III)-Cobalamins in Dog Plasma
DMPK 122-04 Mod. 4, Vol. 3, Tab 4.2.2.1.1.7, DMPK 122-04	Validation of an HPLC-UV Assay for the Determination of "Free Cobalamin (III) Derivatives" in Dog Plasma
DMPK 205-05 Mod. 4, Vol. 3, Tab 4.2.2.1.1.8, DMPK 205-05, DMPK 205-05	Comparison of Two Bioanalytical Methods (Houeto /Merck KGaA) for Measurement of Total Cobalamins-(III) and Cyanocobalamin in Human Plasma
Cyanide in Dog Whole Blood	
DMPK 209-04 Mod. 4, Vol. 4 Tab 4.2.2.1.2.1, DMPK 209-04	Determination of Cyanide in Dog Blood Samples in the Presence of Hydroxo- and Cyanocobalamin
DMPK 165-04 Mod. 4, Vol. 5, Tab 4.2.2.1.2.2, DMPK 165-04	EMD 415722-Validation of an HPLC-MS Method for the Quantification of Cyanide in Dog Blood in the Presence of Hydroxocobalamin, Cyanocobalamin and Thiocyanate
Cyanocobalamin in Dog Plasma	
DMPK 135-04 Mod. 4, Vol. 5, Tab 4.2.2.1.3.1, DMPK 135-04	Validation of an HPLC-UV Assay for the Determination of Cyanocobalamin in Dog Plasma
Validation of Hydroxocobalamin in Dog Plasma	
DMPK 133-03 Mod. 4, Vol. 5, Tab 4.2.2.1.4.1, DMPK 133-03	Validation of an LC/MS-MS Method for the Quantification of Hydroxocobalamin and Cyanocobalamin in Dog Plasma
MPK/Hydroxo.03.01 Mod. 4, Vol. 6, Tab 4.2.2.1.4.2, MPK/Hydroxo. 03.01, Mod. 4	Validation of an HPLC-UV Method for the Quantification of Hydroxocobalamin in Dog Plasma
Toxicology: Rat Studies	
Single-Dose	
III.A.1 Mod. 4, Vol. 12, Tab 4.2.3.7.7.4.1, III.A.1	Single-Dose Toxicity Studies in the Mouse and Rat [redacted]
T15741 Mod. 4, Vol. 6, Tab 4.2.3.1.1, T15741	EMD 415 722 -Acute Toxicity Study in Rats after Intraperitoneal Administration
T15765 Mod. 4, Vol. 6, tab 4.2.3.1.2, T15765	EMD 415722 -Acute Toxicity Study in Rats after Fractionated Intraperitoneal Administration of 1000 mg/kg
T15096 Mod. 4, Vol. 6, Tab 4.2.3.1.3, 15096	EMD 415722 (Hydroxocobalamin) –Single Intraperitoneal Injection in Rats
High Impurity Studies	
T15948 Mod. 4, Vol. 11, Tab 4.2.3.7.6.1 T15948	EMD 415722 (Cyanokit® 2.5 g Batch 9337) - Acute Toxicity Study in Rats after Intraperitoneal Administration
T16400 Mod. 4, Vol. 12, Tab 4.2.3.7.6.2 T16400	EMD 415722 (Cyanokit® 2.5 g, Batch 2080) - Acute Toxicity Study in Rats after Intraperitoneal Administration
Repeat-Dose	
III.A.2.1 Mod. 4, Vol. 12, Tab 4.2.3.7.7.5.2, III.A.2.1	3-Month Toxicity Study in the Rat ([redacted])

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Dog Studies	
Single Dose	
T8374 Mod. 4, Vol. 7, Tab 4.2.3.1.4, T8374	EMD 415722 -Intravenous Tolerance Study in Beagle Dogs
Repeat-Dose: Hydroxocobalamin	
T8355 Mod. 4, Vol. 8, Tab 4.2.3.2.1.1, T8355	EMD 415722 -Intravenous Tolerance Study in Beagle Dogs
T8348 Mod. 4, Vol. 9, Tab 4.2.3.2.1.2, T8348	EMD 415722 -4 Week Intravenous Toxicity Study with an 8 Week Recovery Period in Beagle Dogs
T8347 Mod. 4, Vol. 9, Tab 4.2.3.2.1.3, T8347	EMD 415722 -Intravenous Tolerance Study in Beagle Dogs
III.A.2.2 Mod. 4, Vol. 12, Tab 4.2.3.7.7.5.1, III.A.2.2	4 Week Toxicity Study in the Dog ([redacted] , b(4)
Repeat-Dose: Cyanocobalamin	
T8380 Mod. 4, Vol. 10, Tab 4.2.3.2.2.1, T8380	Art. 524950 (Cyanocobalamin) -2 Week Intravenous Toxicity Study in Beagle Dogs
T8377 Mod. 4, Vol. 11, Tab 4.2.3.2.2.2, T8377	Art. 524950 (Cyanocobalamin) –Intravenous Tolerance Study in Beagle Dogs
Genotoxicity	
Mutgenicity Assays	
T15570 Mod. 4, Vol. 11, Tab 4.2.3.3.1.1, T15570	EMD 415 722 (Cyanokit®) –Bacterial Mutagenicity Assay, Salmonella Typhimurium and Escherichia Coli
T15950 Mod. 4, Vol. 11,Tab 4.2.3.3.1.2, T15950	EMD 415722 (Batch 2070) –Bacterial Mutagenicity Assay, Salmonella Typhimurium and Escherichia Coli
T15575 Mod. 4, Vol. , Tab 4.2.3.3.1.3, T15575	EMD 415722 (Cyanokit®) -In Vitro Mammalian Cell Gene Mutation Test (L5178Y trK+/-)
Clastogenicity Assays	
T15574 Mod. 4, Vol. 11, Tab 4.2.3.3.2.1, T15574	EMD 415722 (Cyanokit®) -Micronucleus Test in Rats after Intravenous Administration
Impurities: Mutagenicity Assays	
T15917 Mod. 4, Vol. 12, Tab 4.2.3.7.6.3, T15917	EMD 415722 (Batch 9337) –Bacterial Mutagenicity Assay, Salmonella Typhimurium and Escherichia Coli
T16401 Mod. 4, Vol. 12, Tab 4.2.3.7.6.4, T16401	EMD 415722 (Batch 2080) -Bacterial Mutagenicity Assay, Salmonella Typhimurium and Escherichia Coli

Reproductive Toxicology	
III.C.1 Mod. 4, Vol. 12, Tab 4.2.3.7.7.6.1, III.C.1	Embryofetal and Perinatal Toxicity Study in the Rat (——)
III.C.2 Mod. 4, Vol. 12, Tab 4.2.3.7.7.6.2, III.C.2	Embryofetal and Perinatal Toxicity Study in the Rabbit (——)
Phototoxicity	
70/212 Mod. 4, Vol. 12, Tab 4.2.3.7.7.2.1, 70/212	EMD 415722(Cyanokit®2.5g) -Evaluation of In Vitro Phototoxicity on Balb-c/3T3 Fibroblasts Using the Neutral Red Uptake Assay

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Studies not reviewed within this submission:

The following sections were determined to be Not Applicable by the Sponsor and no studies were submitted:

Safety Pharmacology **Dependence**
Pharmacodynamic Drug Interactions
Absorption, Distribution, Metabolism, Excretion
Pharmacokinetic Drug Interactions (Nonclinical)
Other Pharmacokinetic Studies
Carcinogenicity
Other Studies
Reproductive and Developmental Toxicity **Fertility and Early Embryonic Development** **Embryo Fetal Development** **Prenatal and Postnatal Development, Including Maternal Function** **Studies in Which the Offspring are Dosed and/or Further Evaluated**
Other Toxicity Studies **Local Tolerance** **Antigenicity** **Immunotoxicity**
Other Mechanistic Studies

Reviewer's Comments: Nonclinical information on the topics above should not be considered Not Applicable. They are necessary for the appropriate extrapolation of the findings in animal studies to human use, especially in terms of dosing and potential toxicities.

The numerous literature references were included in the submission. Although these studies were examined for this submission, a detailed analysis has not been prepared for this review as the information in them was not necessary for approval of this NDA application. These references; however, are listed in Appendix 2.

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

Hydroxocobalamin is the hydroxolated active form of vitamin B_{12} in which cobalt ion is coordinated in 4 positions by a tetrapyrol (or corrin) ring. The rationale for administering hydroxocobalamin as an antidote for cyanide poisoning is based on the high affinity of the cyanide ion for cobalt compounds. The acute toxicity of cyanides is due to the binding of cyanide to cytochrome oxidase resulting in the blockade of the respiratory chain. Since the cobalt binding site in hydroxocobalamin has a higher affinity for cyanide than the hydroxyl group of hydroxocobalamin, cyanocobalamin will form. Cyanocobalamin is then eliminated mostly through the urine.

2.6.2.2 Primary pharmacodynamics

MECHANISM OF ACTION

Hydroxocobalamin is the hydroxolated active form of vitamin B_{12} in which cobalt ion is coordinated in 4 positions by a tetrapyrol (or corrin) ring. The rationale for administering hydroxocobalamin (OHCo) as an antidote for cyanide poisoning is based on the high affinity of the cyanide ion for cobalt compounds, as depicted in the sponsor's chemical reaction diagramed below:

$$\mathbf{OHCo + HCN \rightleftharpoons CNCo + H_2O}$$

Cyanide affects virtually all body tissues, attaching to ubiquitous metalloenzymes and rendering them inactive. Following absorption, the cyanide ion rapidly diffuses to cells and binds ferric iron of heme a_3 of cytochrome oxidase (cytochrome a3) in inner membrane of mitochondria to form a reversible complex. When cyanide binds to cytochrome oxidase, the electron flow from cytochrome c to O_2 is blocked. This prevents proton pumping across the mitochondria membrane, reducing the cellular redox potential, and thus uncoupling oxidation and phosphorylation. This leads to cytotoxic anoxia, which shifts the cell to anaerobic metabolism, resulting in lactic acid production.

Hydroxocobalamin, or Vitamin B_{12a}, is an antagonist of cyanide intoxication that binds cyanide directly. In vitro, the reaction of hydroxocobalamin with cyanide results in the displacement of the hydroxo ligand by the cyanide ion to form cyanocobalamin (CNCo), another form of vitamin B_{12}. The rationale for administering hydroxocobalamin (OHCo) as an antidote to cyanide poisoning is based on the high affinity of the cyanide ion for cobalt compounds.

Cyanide detoxification can be represented by the diagram below, reproduced from the Sponsor's submission. b(4)

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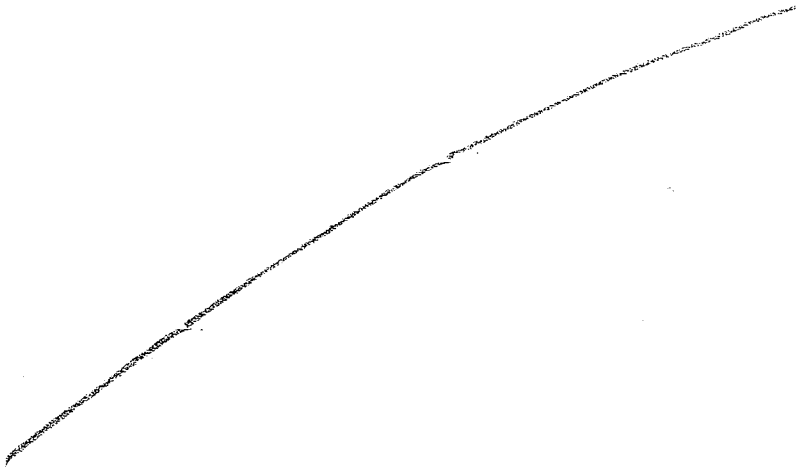

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CYANIDE DETOXIFICATION

DRUG ACTIVITY RELATED TO PROPOSED INDICATION

Study title: Safety Pharmacology and Primary Pharmacodynamic Studies in the Mouse, Rabbit, Rat, Guinea Pig and Dog (

Key study findings: These studies investigated the effects of hydroxocobalamin on various types of experimental acute and chronic cyanide poisoning.

Study no.: III.F
Vol. 12, Tab 4.2.3.7.7.3.1 III.F, p. 1
Conducting laboratory and location:

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Date of study initiation: study period in 1974
GLP compliance: no
QA report: no
Drug, lot #, and % purity:

Hydroxocobalamin, Batch RL 23918 (active substance), Purity: no information
Preparation for injection Batch 210 bis (HOCo 15000)
Hydroxocobalamin base 15 mg
Buffered aqueous solution QSP 2ml for 1 vial

Sodium acetate	3.89 mg
Sodium chloride	8.20 mg
Acetic acid QSP pH 5.8	(about 2.68 mg)
Water for injection QSP	2 mL

1) Acute Poisoning in the Mouse

Methods

Male mice (24-25 g) were administered hydroxocobalamin or saline (IP, 0.4 mL/20 g) either 15 minutes before or immediately after ingesting 6.5 mg/kg of potassium cyanide (250 mg KCN/100 mL water; 0.2 mL20 g; a previously determined mouse oral LD_{100}). Also the effects of different forms of cobalamins (methylcobalamin, de-oxyadenosylcobalamin) at equimolar doses were examined.

Results

Hydroxocobalamin provided effective protection against the lethal dose (LD_{100}) of cyanide.

-Treatment immediately after the cyanide: ED_{50} = 83 mg/kg
-Treatment 15 minutes before the cyanide: ED_{50}= 54 mg/kg

These doses correspond to 0.061 and 0.039 mM/kg respectively, and the dose of cyanide corresponds to 0.250 mM/kg, but it should be noted that the toxic agent was administered by digestive route, whereas after injection by intraperitoneal route, the hydroxocobalamin was diffused much more rapidly into the bloodstream.

Table I: Protective effects of hydroxocobalamin by intraperitoneal route against the toxic effects of 6.5 mg/kg of cyanide administered *per os* in the mouse.

DOSE	PROTECTION (% survivors)	
	Treatment 15 min before cyanide	Treatment immediately after cyanide
200 mg/kg	80%	80%
100 mg/kg	90%	50%
50 mg/kg	40%	30%
10 mg/kg	15%	0%
2.5 mg/kg	0%	0%

Protective effect of various cobalamins used at equimolar doses by intraperitoneal route versus poisoning with 6.5 mg/kg of cyanide administered per os in the mouse. Hydroxocobalamin was the most effective of the three compounds.

SUBSTANCE	DOSE	PROTECTION (% survivors)	
		Treatment 15 min before cyanide	Treatment immediately after cyanide
Hydroxocobalamin	150 mg/kg	70%	50%
Methylcobalamin	150 mg/kg	20%	5%
De-oxyadenosyl-cobalamin	176 mg/kg	10%	0%

2) Acute Poisoning in the Rabbit

Methods

Rabbits (~2.8 kg) were anesthetized with ethyl carbamate (1 g/kg, IP) and prepared for carotid artery blood pressure monitoring and a tracheal cannula for respiratory amplitude and frequency. Potassium cyanide (0.1 mg/kg/minute of CN^-, 1 mL/ minute) was infused into the saphenous vein. One minute after the onset of primary apnea, 50 mg/kg hydroxocobalamin was injected into the saphenous vein.

Results

Poisoning resulted in hypertension accompanied by an increase in respiratory amplitude, followed by apnea then agony-like respiratory movements or gasps that eventually ceased (Table, below). During the latter stages, arterial pressure steadily fell until the animal died.

In animals injected with hydroxocobalamin 1 min after the onset of apnea, respiratory movement was resumed and arterial pressure restored. However, as cyanide infusion was continued, arterial pressure and respiration decreased resulting in death.

Table III: Impact of hydroxocobalamin (50 mg/kg IV) on acute cyanide poisoning in the anesthetized rabbit

GROUP	NUMBER OF ANIMALS	APNEA I	BREATHING RESUMED	APNEA II	GASPS	TERMINAL APNEA	DEATH	DOSE OF CN^-
Control	13	4 min 33 ± 1 min 13			5 min 37 ± 1 min 26	9 min 29 ± 1 min 12	12 min 41 ± 1 min 30	1.27 mg ± 0.15
Hydroxo-cobalamin treated (50 mg/kg)	15	4 min 23 ± 56 sec	6 min 01 ± 1 min	10 min 41 ± 1 min 35	11 min 02 ± 1 min 30 *	16 min 03 ± 2 min 27 *	19 min 06 ± 3 min 12 *	1.91 mg ± 0.32 *

* $p < 0.01$: significantly different from the control group

3) Chronic Poisoning in the Rat

The effects of hydroxocobalamin were investigated in chronic poisoning with cyanide and an organic cyanide derivative acetonitrile, CH_3CN. It is thought that acetonitrile releases cyanide into the body by the following mechanism:

$$CH_3 - CN \rightarrow CH_2OH - CN \xrightarrow[\text{nitrilase}]{OH_2} OH_2 + HCHO + HCN$$

Methods

Male Wistar rats (~330 g) were divided into 5 groups of 6 rats which received the following treatments IP, for 28 days over 6 weeks, with hydroxocobalamin administered 6 hours after cyanide or acetonitrile. Urine was collected 6 days per week, and pooled within group day for analysis of free cyanides and acetonitrile. On day 40 all animals were sacrificed for toxicology assessment of organs and histopathology of brain, kidney, liver, spleen, pancreas, and testes.

- **Group 1 (Controls), each rat received:**
  - **1 ml of physiological saline at 10 a.m.,**
  - **1 ml of physiological saline at 4 p.m.**
- **Group 2, each rat received:**
  - **1 ml of 50 mg/ml acetonitrile solution at 10 a.m.,**
  - **1 ml of physiological saline at 4 p.m.**
- **Group 3, each rat received:**
  - **1 ml of 50 mg/ml acetonitrile solution at 10 a.m.,**
  - **1 ml of 5 mg/ml solution of hydroxocobalamin**
- **Group 4, each rat received:**
  - **1 ml of 1.12 mg/ml solution of potassium cyanide at 10 a.m.,**
  - **1 ml of physiological saline at 4 p.m.**
- **Group 5, each rat received:**
  - **1 ml of 1.12 mg/ml solution of potassium cyanide at 10 a.m.,**
  - **1 ml of hydroxocobalamin solution at 4 p.m.**

Results

The KCN-poisoned animals, received during the study: 31.36 mg KCN per rat, i.e. 12.5 mg CN^- (0.48 mmoles). The acetonitrile-poisoned animals received 1400 mg per rat (34.14 mmoles). The hydroxocobalamin treated animals, received 140 mg per rat (0.104 mmoles).

Only a very small percentage of acetonitrile (3 to 4%) was excreted by the renal route, most of the toxic substance being excreted by pulmonary route and in the feces. In contrast, in the case of cyanide, renal excretion is a major route of excretion which accounts for more than 20% of the dose administered.

For the poisonings with acetonitrile, the excretion of free cyanide was the same regardless of whether the group had received hydroxocobalamin; the total excretion in terms of combined cyanides and of acetonitrile was slightly higher in group 3, which was treated with hydroxocobalamin, but the mean daily excretion values were not significantly different (investigated using Student's "t" test). Table IV (reproduced from the sponsor's submission) shows the urinary elimination data for free and acetonitrile-

combined cyanides, expressed in terms of the mean excretion per group and per day, the total excretion, and the percentage of the dose administered.

Table IV: Urinary excretion of the free and combined cyanides and of acetonitrile.

CODE	FREE CYANIDES			COMBINED CYANIDES			ACETONITRILE		
	Eliminat. per day µg	Total eliminat. mg	% dose administered	Eliminat. per day µg	Total eliminat. mg	% dose administered	Eliminat. per day µg	Total eliminat. mg	% dose administered
Group 1 Control	0.03 ± 0.004	0.001		57.05 ± 6.8	1.940				
Group 2 Aceto-nitrile	32 ± 4	1.101	0.019	4395 ± 355	149.353	2.70	2262 ± 265	76.935	0.91
Group 3 Aceto-nitrile + Hydroxo-coba-lamin	29 ± 4	1.017	0.018	5164 ± 335	175.202	3.16	2557 ± 296	86.942	1.03
Group 4 Cyanide	39 ± 3	1.335	1.70	480 ± 35	16.326	20.88			
Group 5 Cyanide + Hydroxo-cobal-amin	31 ± 3	1.183	1.51	530 ± 39	18.030	28.38			

The quantities of free and combined cyanides were relatively low in most organs, but they were always higher than in the control group, even though the analyses were done two days after the last injection.

In groups 2 and 3, which had been poisoned with acetonitrile, higher levels of free cyanides occurred in most of the organs of the rats that had received hydroxocobalamin treatment. Only the kidneys and the brain had a lower level of cyanides in the hydroxocobalamin-treated group. In contrast, the combined cyanides were found mainly in the heart, the lungs, the stomach, the testes, but above all in the organs of excretion, the intestine, and the kidneys.

In groups 4 and 5, the hydroxocobalamin-treated animals had higher levels of free hydrocyanic acid in their organs than the untreated, poisoned animals. However, all the organs of the animals in group 5 had combined cyanide concentrations which were higher than in group 4, which can be related to the action of the detoxicant used. The table below was reproduced from the sponsor's submission:

Table V: Disposition of free and combined hydrocyanic acid in the various organs (expressed in μg of hydrocyanic acid), (mean per rat).

Organs	GROUP 1 (Control)		GROUP 2		GROUP 3		GROUP 4		GROUP 5	
	Free HCN	Combined HCN	Free HCN	combined HCN	Free HCN	Combined HCN	Free HCN	Combined HCN	Free HCN	Combined HCN
Heart	0	3	1.3	8.5	1.7	7.5	2.5	3.1	2.2	5.5
Lungs	0	3	2	9.3	2.2	8.7	1.5	5.5	2.7	4.8
Liver	0.3	1.8	2.3	9.1	3.3	8.8	1.5	5	1.8	5
Kidneys	0.2	1.2	5.7	11	1.2	23	1.3	4.4	2.2	5.5
Intestine	0.3	3.6	1	25	1.2	20	2.5	3.1	2	4.6
Testes	0	0.6	1.9	8.5	2.2	7.4	1.5	5	3.1	5.7
Skin	0	2.1	1.5	21.5	2	8.6	1.3	6.5	1.2	17.8
Muscle	0	2.3	1.5	6.3	2.7	3.6	1.8	6	2	5.3
Brain	0.2	3.5	2.3	8.2	1	5.6	3.9	4.1	8.3	5.5
Spleen	0	1.8	0.7	6.1	2	4.3	2.5	5	2.8	6
Stomach	0.2	12	3.2	33	2	36	1.8	16.5	1.5	22.5

The changes seen consisted of picnotic nuclear images with hyperchromatophilic cytoplasm. The cells were counted on 15 to 17 sections per animal. Table VI (reproduced from the sponsor's submission) shows the total number of abnormal Purkinje cells per group, the mean number of damaged cells per section and the percentage of sections with no abnormal cell; the Ki test was used for the statistical analysis of this latter value.

Table VI: Effect of the poisoning and treatment with hydroxocobalamin on the Purkinje cells of the brain.

	GROUP 1 Control	GROUP 2 Acetonitrile	GROUP 3 Acetonitrile + Hydroxocobalamin	GROUP 4 Cyanide	GROUP 5 Cyanide + Hydroxocobalamin
Total number of abnormal Purkinje cells	93	584	166	794	122
Percentage of abnormal Purkinje cells per section	1.85	9.08	2.60	15.53	2.46
Percentage of sections with no abnormal cells	59.2%	28.5%*	54.7%	27.4%	52.1%

* $p < 1\%$ versus group 1.

Degenerative changes were seen in the kidneys (chronic nephrosis) and testes (some seminiferous tubules were empty), and chronic infectious changes (foci of lymphoplasmocytic infiltration of the renal interstitial tissue, inflammatory lymphocytic infiltrations in the hepatic parenchyma). The incidence of these lesions was the same in all groups. Chronic poisoning with either an organic derivative of cyanide or an alkaline cyanide induced lesions in the central nervous system. Inhibition of the respiratory enzymes led to anoxic phenomena and the Purkinje cells provided an indicator of anoxic distress: these cells maintained their metabolic activity at a preferential energy level for

some time, but if the anoxia was prolonged, they were amongst the most severely affected cells.

Hydroxocobalamin treatment restored the normal appearance of the Purkinje cells. The site of impact of hydroxocobalamin seems to be located in the structures that are sensitive to cyanides, i.e. the central nervous system. The presence or absence of treatment seemed to have no impact on tissue disposition, or global urinary excretion; the most that was seen in response to hydroxocobalamin was a slight increase in combined cyanides in the excretory organs: kidneys and intestine, and in the urine.

4) CHRONIC POISONING IN THE RABBIT

Method

Male and female rabbits (2.5 to 3 kg) were poisoned by orally administered potassium cyanide in biscuits or inhaled cigarette smoke. The total cyanide content of a biscuit was 0.254 µg/kg of CN^-. Blood samples were taken by puncturing the marginal ear vein twice a week. The blood levels of free cyanides and thiocyanates; in the case of the animals treated with hydroxocobalamin, we also assayed hydroxocobalamin and cyanocobalamin. Urine concentrations were also tested. Rabbits were sacrificed at the end of the experiments and the levels of free cyanides and thiocyanates were assayed in the liver, kidney, heart, lung, brain, eye, optic nerve, optic chiasma, sciatic nerve, muscle, adipose tissue and sometimes in the gastric contents. Control rabbits (3 males, 1 female) were used to determine baseline levels of free cyanides and thiocyanates in blood, urine, and organs.

Results

Free cyanides in blood, urine, and organs were generally not detectable, but a low baseline level of thiocyanates was relatively stable for an individual animal. The table below was reproduced form the sponsor's submission:

Table VII: Levels of free and total cyanides in the blood, urine and the main organs in the normal rabbit

	Free cyanides expressed as CN^-	Total cyanides (in the form of thiocyanates) expressed as CN^-
Blood	0	1.48 ± 0.15 mg/l
Urine	0	2.34 ± 0.27 mg/l
Liver	0	0.32 mg/kg
Kidneys	0	0.115 mg/kg
Heart	0	0.22 mg/kg
Lungs	0	0.20 mg/kg
Brain	0	0.086 mg/kg
Muscle	0	0.190 mg/kg

Poisonings were administered to 4 rabbits: 2 groups of 2 rabbits of the same sex and similar bodyweight were used. Both rabbits, monitored for a few days in the laboratory,

received progressive doses of potassium cyanide in successive dose levels, each successive dose being maintained for a few days until a fixed dose was reached which was then maintained throughout the rest of the experiment. These doses of cyanide were administered daily.

In each group, one of the rabbits was treated with hydroxocobalamin 15000, according to the conditions in Table VIII; in this way, the progress of the 2 animals was compared in each group.

Group I
The progress of these 2 rabbits was very similar until treatment with hydroxocobalamin began.

Blood levels:

Free cyanides: blood levels of free cyanides were initially zero, as in the control group of rabbits. Free cyanides began to appear in the blood as soon as poisoning began, at relatively low levels: 20 to 30 micrograms per liter. Levels then rose as the doses administered increased, to reach 120 to 130 micrograms per liter before the animal was sacrificed. The free cyanides did not return to zero again throughout the study.

Thiocyanates: initial blood levels of thiocyanates were similar to those in the control rabbits, with a mean of 2 mg/L expressed in terms of CN^-, which is equivalent to about 4.3 mg/L expressed in terms of SCN^-. From day 1 of cyanide administration, the concentration increased and reached a plateau after a few days when the same dose of cyanide was administered. This level then rose again to another plateau when the dose administered was increased.

Urinary excretion:

Free cyanides: before poisoning, the urine did not contain free cyanides, but the day after poisoning, free cyanides appeared in the urine at levels which increased with the daily dose of cyanide administered. The levels rose from 20 to 30 µg/L, within the first few days of the poisoning (dose administered: 1.2 mg expressed as CN-), to 120-130 mg/L, at the end of the study (dose of cyanides administered 4.8 mg). The levels of free cyanides in the urine during poisoning were the same as the level of free cyanides found in the blood during the same period.

Thiocyanates: the base level of urinary thiocyanates was initially equivalent to that in the control rabbits (about 3 mg/L expressed in CN^-). The level of thiocyanates rose rapidly after cyanide poisoning. However, in contrast to the blood levels resulting from poisonings caused by administering low doses of CN^-, when the level of urinary thiocyanates rose over time even though the same daily dose was administered, whereas, when higher doses of KCN were administered, urinary excretion soon reached a plateau which remained stable throughout the poisoning. The table below was reproduced from the sponsor's submission:

Table VIII: Conditions of KCN poisoning *per os* and treatment with HOCo 15000 *per os* in the Rabbit; the doses of CN^- correspond to the total dose administered per day to each animal.

Group	N°	Sex	Initial weight	Final weight	Poisoning received	Treatment
I	32	M	2920 g	4300 g	1.2 mg CN^- for 6 days + 2.4 mg CN^- for 6 days + 3.6 mg CN^- for 26 days + 4.8 mg CN^- for 102 days	No treatment Sacrificed at the end of the poisoning
	33	M	3010 g	4070 g	Total duration of poisoning: 140 days	Treated with HOCo 15000: 1.5 mg/l for the last 15 days of poisoning. Sacrifice
II	20	F	3700 g	4310 g	1.2 mg CN^- for 3 days + 1.8 mg CN^- for 6 days + 2.4 mg CN^- for 55 days + 3 mg CN^- for 22 days	No treatment Sacrificed 40 days after the end of poisoning
	19	F	3800 g	4325 g	3.6 mg CN^- for 34 days Total duration of poisoning: 120 days	Treated with HOCo 15000: 3 mg/kg for 40 days after the end of the poisoning.

Table IX: Tissue disposition of free and total cyanides in the bodies of rabbits sacrificed without stopping the poisoning, with or without treatment with HOCo 15000, 1.5 mg/kg/day *Per os*.

	RABBIT 32 Poisoned, untreated		RABBIT 33 Poisoned, treated with HOCo 15000	
ORGAN	Free cyanides µg/kg in CN^-	Total cyanides in CN^- µg/kg	Free cyanides µg/kg in CN^-	Total cyanides in CN^- µg/kg
Liver	115	720	0	630
Kidneys	96	720	0	594
Heart	48	585	0	285
Lungs	42	720	0	510
Sciatic nerve	10	700	0	294
Muscle	12	165	0	270
Adipose tissue	15	315	0	168
Gastric contents	30	990	0	288

Free cyanides were present in all organs, (whereas the organs of control rabbits, which had not been poisoned, did not contain free cyanides). The liver alone contained levels of free cyanides of the same order as those found in the blood and urine. The other organs contained much lower levels. The nerve tissue (sciatic nerve) contained free cyanides and thiocyanates. The level of thiocyanates in the various organs was much higher than in the control rabbits, but in much smaller proportions in the case of the blood and urine (2 to 3 times more in the organs, 10 times in the blood and urine).

Within the first few days of treatment, free cyanides disappeared from the blood, despite the administration of KCN per os. The level of thiocyanates fell markedly.

Cyanocobalamin soon appeared, but some hydroxocobalamin remained in the blood that had not been converted to cyanocobalamin, despite the continued presence of a considerable quantity of thiocyanate.

As in the blood, the free cyanides soon disappeared from the urine, and the level of thiocyanates fell, whereas cyanocobalamin appeared and the total quantity of cyanide excreted (thiocyanates + cyanocobalamin) was much higher than in the corresponding poisoned animal. Unconverted hydroxocobalamin was excreted in the urine.

Tissue disposition
After necropsy, free cyanide was found to be absent from the organs. The level of thiocyanates in the organs was also lower than in the corresponding rabbit not treated with hydroxocobalamin (Table IX -rabbit 33).

Group II
As in group I, the 2 rabbits displayed similar changes, until treatment with hydroxocobalamin.

Blood levels:
During the poisoning, the changes in the levels of free cyanides and of thiocyanates were similar to those in group I. After the poisoning had stopped in untreated rabbit 20, free cyanides remained in the blood. This phenomenon can only be accounted for by the "formation" of free cyanides from thiocyanates present in the body of these rabbits by the reversible reaction $CN \longleftrightarrow SCN^-$. The level of thiocyanates began to fall slowly without returning to the starting level of 40 days after the poisoning had stopped.

Urinary excretion:
Free cyanides: The situation observed was the same as for the blood, i.e. the free cyanides persist in the urine, even when the poisoning had been stopped, until they were sacrificed.

Urinary thiocyanates: Again, the same phenomenon was observed as in the blood. The level of urinary thiocyanates fell after the poisoning stopped, but did not return to baseline, even 40 days after the poisoning had stopped. Here too, and although the poisoning had been stopped for a fairly long time, the organs contained large quantities of free cyanides at levels similar to those in the rabbits sacrificed, without the poisoning being stopped. The most probable explanation for this phenomenon is the "formation" of free cyanides from thiocyanates present in the organs of the rabbits, according to the reversible reaction: $CN \longleftrightarrow SCN^-$. High levels of thiocyanates were also present in the organs. These levels were similar to those of the rabbits poisoned and sacrificed without stopping the poisoning.

Table X: Tissue disposition of free and total cyanides in the organs of rabbits sacrificed 40 days after poisoning had been stopped, with or without treatment with HOCo 15000, 3 mg/kg/day *per os*.

	RABBIT 20 Poisoned, untreated		RABBIT 19 Poisoned, treated with HOCo 15000	
ORGANS	Free cyanides in µg/kg CN^-	Total cyanides in µg/kg CN^-	Free cyanides in µg/kg CN^-	Total cyanides in µg/kg CN^-
Liver	40	117	0	106
Kidneys	40	72	0	30
Heart	30	28	0	120
Lungs	60	90	0	135
Muscle	120	72	0	75
Adipose tissue	-	-	0	0
Gastric contents	35	180	0	690

Treatment with hydroxocobalamin (3 mg/kg/day per os) after stopping KCN poisoning

Blood levels
Free cyanides: disappeared from the blood very soon after starting the treatment with HOCo 15000; the level of thiocyanates fell very sharply to baseline.

Cyanocobalamin: Cyanocobalamin was detected from the first day of treatment and remained at high levels until the animal was sacrificed, but some unconverted hydroxocobalamin remained.

Urinary excretion
The pattern of change was very similar to that observed in the blood (Figure 12, appended), but it should be noted that the level of thiocyanates remained below baseline.

Tissue disposition (Table X - rabbit 19)
No organ contained free cyanides and the level of thiocyanates was lower than in the control animals (see Table VII).

Treatment with hydroxocobalamin during per os potassium cyanide poisoning therefore resulted in the disappearance of free cyanides from the blood, the urine and the organs, even if the poisoning was continued; it is therefore clear that hydroxocobalamin binds cyanides in the body, as a result of its high affinity for them. The level of thiocyanates fell sharply when the treatment was started, and it returned to normal only if the poisoning was stopped.

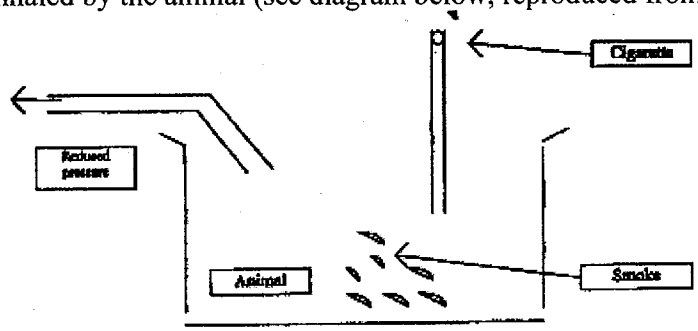
Administration of hydroxocobalamin, during chronic cyanide poisoning, therefore leads to a change in the normal metabolic pathway of the cyanides (thiocyanates) to form

cyanocobalamin, however, the thiocyanate formation pathway continues since thiocyanates were always present. It is difficult to explain why some hydroxocobalamin persisted, even though thiocyanates were still present, because the cyanides could still be bound by hydroxocobalamin.

With regard to the pathology examinations of organs, such as the liver, kidney, lung, no difference was found between the different groups, images of hypertrophic reticulate pneumonia (lung) or of vascular congestion (liver) were insignificant and unrelated to the poisoning or the treatment. In contrast, in the case of nervous structures, cyanide poisoning led to images of demyelinization, and treatment with hydroxocobalamin sometimes produced some normalization, particularly of the optic nerve. It should be noted, however, that the prolongation of poisoning resulted in injuries which were only reversible with great difficulty (unlike the biochemical injuries).

POISONING BY CIGARETTE SMOKE INHALATION

Poisonings were carried out in two groups of two rabbits of the same sex and similar bodyweights. The rabbit was placed in a Plexiglas metabolism cage into which cigarette smoke was "released", by inducing a slight lowering of pressure which "drew" on the lighted cigarette: this was therefore the "secondary current" of the cigarette which was inhaled by the animal (see diagram below, reproduced from the sponsor's submission).



Four sessions were carried out per day, during which the animal inhaled the smoke of 2 to 3 cigarettes. In each group, one of the rabbits was treated with HOCo 15000, as shown in Table XI (reproduced from the sponsor's submission).

Table XI: Poisoning by inhalation of cigarette smoke and treatment with HOCo 15000 *per os* in the rabbit.

Group	N°	Sex	Poisoning	Treatment
III	29	F	4 sessions x 3 cigarettes x 217 days	No treatment. Sacrificed at the end of the poisoning.
	28	F	4 sessions x 3 cigarettes x 193 days	Treated with HOCo 15000 1.5 mg/kg, for 20 days, as soon as the poisoning ended.
IV	18	F	4 sessions x 3 cigarettes x 140 days + 4 sessions x 2 cigarettes x 40 days Total duration of poisoning: 180 days	No treatment Sacrificed at the end of the poisoning
	15	F		Treated with HOCo 15000 3 mg/kg, for the last 30 days of the poisoning

Free cyanides appeared in blood within the first few days of poisoning and persisted throughout the poisoning at relatively constant levels (about 120 µg/L). Urinary excretion of free cyanides paralleled the pattern seen in blood. Free cyanides were found in organs of the rabbit not treated with HOCo 15000.

Thiocyanates increase steadily for about 20 days, then plateaued until the end of poisoning. Urinary thiocyanates paralleled those seen in blood. Concentrations of thiocyanates were high in all organs examined (table below from sponsor's submission).

Table XII: Tissue disposition of free and total cyanides in the organs of rabbits sacrificed after poisoning by cigarette smoke, with or without treatment HOCo 15000, 1.5 mg/kg/day *per os*.

	RABBIT 29 Poisoned, untreated		RABBIT 28 Poisoned, treated with HOCo 15000	
ORGAN	Free cyanides in µg/kg CN^-	Total cyanides in µg/kg CN^-	Free cyanides in µg/kg CN^-	Total cyanides in µg/kg CN^-
Liver	30	504	0	306
Kidneys	24	300	0	285
Heart	66	150	0	150
Lungs	60	320	0	285
Sciatic nerve	86	260	0	0
Muscle	5	180	0	45
Adipose tissue	15	60	0	15

Treatment with hydroxocobalamin (1.5 mg/kg/day per os) after stopping poisoning by inhalation of cigarette smoke.

In this group, the free cyanides disappeared as soon as treatment with hydroxocobalamin began. As the level of thiocyanates fell, cyanocobalamin concentrations increased. In urine, as free cyanides concentration decreased, the level of thiocyanates decreased also, reaching a baseline on about day 15 of treatment. Cyanocobalamin and hydroxocobalamin appeared rapidly in urine. No free cyanides were found in the organs examined and thiocyanates were lower than in the untreated rabbit.

Changes in Group 4 rabbits

Free cyanides appeared from the beginning of poisoning and thiocyanates increased rapidly to reach a plateau. Reducing the number of cigarettes reduced the level of thiocyanates. Urinary excretion of free cyanides and thiocyanates paralleled those in blood. Both free cyanides and high concentrations of thiocyanates were found in the organs examined.

Treatment with hydroxocobalamin (3 mg/kg/day per os) during poisoning by inhalation of cigarette smoke

Free cyanide disappeared from blood and the level of thiocyanates fell rapidly. There were still large amounts of cyanocobalamin and unconverted hydroxocobalamin. In urine, free cyanides disappeared, thiocyanates decreased and cyanocobalamin and hydroxocobalamin were still present. There were no free cyanides in the organs examined. The tissue levels of thiocyanates were lower than in untreated animals (see table below, reproduced from the sponsor's submission).

Table XIII: Tissue disposition of the free and total cyanides in the organs of rabbits sacrificed without stopping the poisoning by inhalation of cigarette smoke, with or without treatment with HOCo 15000, 3 mg/kg/day *per os*.

	RABBIT 18 Poisoned, untreated		RABBIT 15 Poisoned, treated with HOCo 15000	
ORGAN	Free cyanides in µg/kg CN^-	Total cyanides in µg/kg CN^-	Free cyanides in µg/kg CN^-	Total cyanides in µg/kg CN^-
Liver	30	630	0	276
Kidneys	12	555	0	240
Heart	120	290	0	164
Lungs	118	530	0	297
Adipose tissue	30	120	0	28

Thus, cigarette smoke lead to the appearance of high levels of free and combined cyanides in the blood, urine and organs in the rabbit, similar to that following potassium cyanide poisoning per os. Hydroxocobalamin produced similar effects in both poisoning models.

EFFICACY STUDY (ANIMAL RULE, CFR 314.600)

The effect of hydroxocobalamin on survival following a lethal infusion of potassium cyanide was investigated in beagle dogs. The primary endpoint was the survival at 14 days post-treatment. Secondary endpoints included cardiovascular, electrocardiogram (ECG), and respiratory measurements, clinical chemistry, hematology, clinical observations, and histopathology.

Dog Efficacy Model: Intravenous Route of Administration

In the clinical setting, especially for treatment of smoke inhalation victims, the exposure to cyanide occurs mainly via inhalation. In the dog efficacy study, animals were exposed intravenously to cyanide. The main reasons for the selection of this route of cyanide administration in the animal model were:

- Cyanide poisoning by all routes results in similar signs and symptoms, as well as similar distribution pattern. The main difference with regard to the route of administration is the rate of onset of poisoning, which is also dose-dependent.
- Animals were dosed IV with cyanide until 3 minutes after the onset of apnea, which was defined as a tidal volume of less than 4 mL/kg, to ensure a potentially lethal exposure. Exposure via inhalation would not have allowed such a controlled exposure scenario.
- Inhalation studies, while of interest due to the importance of this route in clinical cyanide poisoning, require onerous experimental conditions for the protection of laboratory personnel, and are consequently generally limited to small animals (mice, rats, guinea pigs).
- The oral absorption rate of cyanide is quite variable, rendering predictability of the degree of intoxication in a particular time frame quite difficult.

Dog Efficacy Model: Summary

The Sponsor summarized the dog IV infusion method of cyanide poisoning as an excellent model to examine potential cyanide antidotes due to complete absorption of the toxicant, ability to titrate the dose to a specific lethal (if untreated) effect, opportunity to progressively observe the development of toxicity and monitor the effect of antidote therapy, as well as obtaining toxicokinetic and safety profiles of the experimental therapy, in this case, hydroxocobalamin.

PILOT STUDY

Study title: **Pilot Study of Intravenous Cyanokit® Administration Following Intravenous Poisoning with Potassium Cyanide in Adult Beagle Dogs**

Study no.: N106341
Vol. 2, Tab 4.2.1.1.4, p. 1

This study included Toxicokinetic Quantification of Total Cobalamins, Cyanocobalmin and Cyanide, which were reported in the following studies:

> **Study title:** **HPLC-UV Determination of Total Cobalamins-(III) and Cyanocobalamin in Beagle Dog Plasma Samples Collected During a Pilot Efficacy Study**
>
> **Study no.:** DMPK 140-04
> Vol. 3, Tab 4.2.1.1.5, DMPK 140-04, p. 1
>
> **Study title:** **Determination of Cyanide in Dog Whole Blood Samples Using Liquid Chromatography with Tandem Mass Spectrometric Detection**
>
> **Study no.:** DMPK 190-04
> Vol. 3, Tab 4.2.1.1.6, DMPK 190-04, p. 1

Key study findings: An experimental model to test the effects of hydroxocobalamin on cyanide poisoning in the dog was developed.

Conducting laboratory and location: [redacted] b(4)
Date of study initiation: March 16, 2005
GLP compliance: yes
QA report: yes

Drug, lot #, and % purity:
Hydroxocobalamin, Batch 2081, Purity 94.4%
Vehicle: 0.9% sodium chloride for injection, USP,
Active Agent:
Potassium Cyanide (KCN), reagent grade, batch 00813TA ([redacted]; b(4)
prepared for use as (1 mg/mL)

Methods
Major considerations that were examined during model development included potassium cyanide dose rate and duration of the apnea period to produce a lethal cardiovascular and respiratory dysfunction. The anesthetic was changed from propofol to isoflurane due to propofol's chemistry as a lipid emulsion, interfering with the separation of blood components for the hematology and assays.

The infusion rate of potassium cyanide was increased stepwise from 0.08 mg/kg/minute (N=1), 0.1 mg/kg/minute (N=3), 0.2 mg/kg/minute (N=1) to a final selected infusion rate of 0.4 mg/kg/minute (N=12),(see Table 1). Potassium cyanide was administered for 1.5 to 4 minutes after the first apnea was noted. A 3 minute period of apnea provided the appropriate time to achieve a fatal dose of KCN in conjunction with an administration rate of 0.4 mg/kg/min. Apnea was defined as complete cessation of breathing or a reduction in tidal volume to less than 4 mL/kg body weight. At the end of the apneic period, each dog was mechanically ventilated with a small animal ventilator at a rate of 10 to 20 breaths/minute and tidal volume of ~15 mL/kg (parameters were set to approximate the baseline minute volume values of the dog), using supplemental oxygen at FiO_2 of 1.0, and continued for 15 minutes. The design of the study is depicted in the table below, reproduced from the sponsor's submission:

Table 1. Experimental Design Summary

Animal ID	Day 1 BW (kg)	KCN Infusion (mL/min)	Date	Anesthetic	KCN Dose Rate mg/kg/min	Total KCN (mg/kg)	Time to Apnea (min)	Apnea Period (min)	Cyanokit Dose (mg/kg)	Outcome	Comments
101	12	1	11/2/04	Propofol	0.08	1.7	20.0	1.5	0	Survived	
151	8.2	0.82	11/3/04	Propofol	0.1	1.10	9.0	2.0	0	Survived	Increase apnea period
102	9.6	0.96	11/4/04	Propofol	0.1	1.40	11.0	3.0	0	Survived	Increase apnea period
152	7.9	0.79	11/15/04	Propofol	0.1	2.00	16.0	4.0	0	Survived	Increase apnea period
103	11.9	2.4	11/17/04	Propofol	0.2	1.80	5.0	4.0	0	Survived	Increase KCN rate
153	10.2	4.1	11/22/04	Propofol	0.4	2.77	2.9	4.0	0	Died 11-22-04	Increase KCN rate
104	9	3.6	11/22/04	Propofol	0.4	2.80	3.0	4.0	0	Died 11-22-04	
154	8.9	3.6	11/29/04	Propofol	0.4	2.60	2.5	5.0	150	Died 11-29-04	5 min total apnea due to Cyanokit delayed infusion start (1 min)
105	13.6	5.4	12/2/04	Propofol	0.4	2.50	2.8	3.5	0	Died 12-2-04	Reduce apnea period
152[a]	8	3.2	12/2/04	Propofol	0.4	2.36	2.9	3.0	0	Died 12-2-04	Repeat dosing for this dog after washout. Reduce apnea period
155	8.1	3.2	12/3/04	Propofol	0.4	2.52	3.3	3.0	150	Died 12-3-04	Initial positive response to Cyanokit, then CV collapse
156	8.5	3.5	12/8/04	Isoflurane	0.4	2.59	3.3	3.0	150	Survived	
157	7.9	3.2	12/9/04	Isoflurane	0.4	2.40	3.0	3.0	150	Survived	
106	10.6	4.2	1/3/05	Isoflurane	0.4	2.60	2.5	4.0[b]	0	Died 1/3/05	Brain dead, CV survival to 3.5 HR(bpm)s
107	9.7	3.9	1/7/05	Isoflurane	0.4	2.20	2.5	3.0	0	Died 1/7/05	Died acutely
108	10.1	3.8	1/10/05	Isoflurane	0.4	2.28	3.0	3.0	75	Survived	
109	9.6	3.8	1/11/05	Isoflurane	0.4	2.20	2.5	3.0	75	Survived	

a. Began using a volume controlled ventilator instead of a pressure controlled ventilator.
b. Due to an error in timing, this dog had an extended period of apnea. Since total KCN dose was similar to other dogs, this was not considered an impact to study interpretation.

The animals were removed from the ventilator at the end of the 15 minute period from end of apnea and allowed to breathe room air or medical grade air, depending on the anesthetic regimen, via the endotracheal tube. In dogs anesthetized with propofol, repeated brief cycles of assisted ventilation were often necessary to wean the surviving animals from the ventilator whereas surviving dogs anesthetized with isoflurane did not typically require a ventilator weaning period. These weaning cycles allow carbon dioxide to accumulate, thus provoking physiologic resumption of spontaneous

ventilation. Animals that required weaning were treated as follows: if apnea persisted for 45 seconds, artificial ventilation was reinstated for 15 seconds then stopped. The assisted ventilation was repeated, 6 times maximally, during the weaning period. This weaning protocol did not represent an attempt at resuscitation from poisoning, but rather support of resumption of spontaneous breathing in surviving animals. Failure to resume spontaneous ventilation (or tidal volume greater than 4 mL/kg) after 6 cycles of weaning was viewed as a treatment failure consistent with animal death. Once weaned from the ventilator, the animals breathed room air via the endotracheal tube throughout the monitoring period following vehicle or test article dosing.

After the cyanide model was developed, the dogs received either vehicle (6 mL/kg 0.9% saline, IV) or hydroxocobalamin at a low-dose (75 mg/kg Cyanokit, IV) or high-dose (150 mg/kg Cyanokit, IV) over 7.5 minutes via the cephalic vein. For the final model, the dogs were dosed according to the following table as a study design, reproduced from the sponsor's submission:

Pilot Study of Intravenous Cyanokit (Cyanokit®) Administration Following Intravenous Poisoning with Potassium Cyanide in Adult Beagle Dogs

Exposure Group	Total Potassium Cyanide (mg/kg)	Potassium Cyanide Infusion Rate mg/kg/min	Apnea Duration[1] (minutes)	Cyanokit Dose (mg/kg)	Cyanokit or vehicle Infusion Volume (mL/kg)	Animal Numbers
1 Vehicle (saline)	2.2 to 2.6	0.4	3[2]	0	6	106, 107
2 (Cyanokit)	2.2 to 2.3	0.4	3	75	3	108, 109
3 (Cyanokit)	2.4 to 2.6	0.4	3	150	6	156, 157

[1] Apnea was defined in this study as complete cessation of breathing or a reduction in tidal volume to less than 4 mL/kg body weight.
[2] One of the two dogs (Animal Number 106) had an extended apnea period of 4 minutes.

A summary of the pilot studies are presented below (tables reproduced from the sponsor's submission).

Appendix III Summary of the dog experiments

Table 8 Summary of the dog experiments performed at Battelle

Animal ID	BW (kg)	Anesthetic	KCN infusion (mL/min)	KCN dose rate mg/kg/min	Total KCN dose (mg/kg)	Time to apnea (min)	Apnea period (min)	OHCo or placebo	outcome
				model adjustment phase					
101	13.0	Propofol	1.00	0.05	1.79	20.0	1.5	placebo	survived
151	6.2	Propofol	0.62	0.10	1.16	6.0	2.0	placebo	survived
102	9.6	Propofol	0.96	0.10	1.40	12.0	3.0	placebo	survived
152	7.9	Propofol	0.79	0.10	2.00	18.0	4.0	placebo	survived
103	11.9	Propofol	2.38	0.20	1.80	6.0	4.0	placebo	survived
153	10.2	Propofol	4.16	0.40	2.77	2.9	4.0	placebo	died
154	9.0	Propofol	3.60	0.40	2.80	3.0	4.0	placebo	died
104	9.9	Propofol	3.96	0.40	2.80	2.5	4.0	OHCo, 100 mg/kg	died
105	13.6	Propofol	5.44	0.40	2.80	2.9	3.5	placebo	died
152[a]	8.0	Propofol	3.20	0.40	2.36	2.5	3.0	placebo	died
155	8.1	Propofol	3.24	0.40	2.82	3.3	3.0	OHCo, 100 mg/kg	died
				final treatment schedule					
156	8.5	Isoflurane	3.89	0.40	2.59	3.3	3.0	OHCo, 100 mg/kg	survived
157	7.9	Isoflurane	3.16	0.40	2.40	3.0	3.0	OHCo, 100 mg/kg	survived
158	10.6	Isoflurane	4.24	0.40	2.80	2.5	4.0	placebo	died
107	9.7	Isoflurane	3.88	0.40	2.20	2.5	3.0	placebo	died
108	10.1	Isoflurane	3.54	0.40	2.28	3.0	3.0	OHCo, 75 mg/kg	survived
109	9.8	Isoflurane	3.84	0.40	2.20	2.5	3.0	OHCo, 75 mg/kg	survived
mean	9.4		3.74	0.40	2.38	2.8	3.2		
S.D.	1.9		0.37	0.00	0.18	0.3	0.4		
CV%	11		0.9	0.0	7.7	12	13		

a: Repeat dosing for this dog after washout.

Blood samples for determination of whole blood concentrations of cyanide and plasma concentrations of cyanocobalamin and 'total cobalamins-(II)' were collected at:

- **T0: before initiation of the cyanide infusion**
- **T10: 10 minutes after initiation of the cyanide infusion (development phase dogs only)**
- **A0: at the end of apnea, immediately (10-20 seconds) prior to start of infusion of saline or Cyanokit**
- **Inf-5: 5 minutes after initiation of Cyanokit or saline infusion**
- **A10, A15, A20, A30, A60, A120, A240: minutes after the end of Cyanokit or saline infusion**
- **A8, A12, and A24: hours after the end of Cyanokit or saline infusion**

For calculations, nominal sampling times were transferred into a progressing time scale which set the end of cyanide infusion / start of Cyanokit0 infusion as t = 0 min and which was effectively equivalent to A0. In order to establish a common time scale for six dogs, the average time to apnea (2.8 min), average apnea period (3.2 min) and the hydroxocobalamin infusion interval of 7.5 min were used for calculation. The tables below were reproduced from the sponsor's submission:

Table 1 Time scale for PK data evaluation

Nominal sampling time	T0	A0	Inf-5	A10	A15	A20	A30	A60	A120	A240	A8	A12	A24
Time scale (min)	-5.0	0.0	5.0	17.5	22.5	27.5	37.5	67.5	127.5	247.5	487.5	727.5	1447.5
Time scale (h)	-0.100	0.000	0.083	0.292	0.375	0.458	0.625	1.125	2.125	4.125	8.125	12.125	24.125

Concentration units used

Analyte	Cyanide	CNCo	Co_{tot}
analytical unit	ng/mL	µg eq/mL	µg eq/mL
PK evaluation unit	nmol/mL	µM (nmol/mL)	µM (nmol/mL)
MW	26.018	1329.3	1329.3

Pharmacokinetic Results

Initial cyanide whole blood concentrations were >100 nmol/mL which were lethal in dogs not receiving the hydroxocobalamin. Five minutes after the start of infusion cyanide concentration decreased to <50 nmol/mL. Correspondingly, the mean cyanide $AUC_{inter(5 - 67.5 min)}$ in hydroxocobalamin treated dogs was about 25% (in the 75 mg/kg group) and 20% (in the 150 mg/kg group) of the AUC_{inter} values in the placebo group. In parallel with the initial decrease in cyanide, formation of cyanocobalamin was observed and was considered proof that cyanide had been successfully trapped by hydroxocobalamin. Fast distribution of the cyanocobalamin formed was indicated by the observed rapid drop of its plasma concentration. The figures below, reproduced from the sponsor's submission, depict the pharmacokinetic results from this pilot study.

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ON ORIGINAL

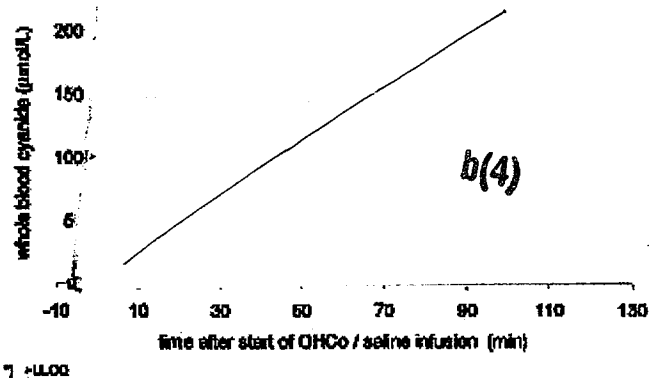

Figure 1 **Concentration time curves (-6 – 127.5 min) of whole blood cyanide concentration in cyanide poisoned dogs treated with Cyanokit®: 0 mg/kg (Dog No 106/107), 75 mg/kg (Dog No 108/109), and 150 mg/kg (Dog No 156/157)** b(4)

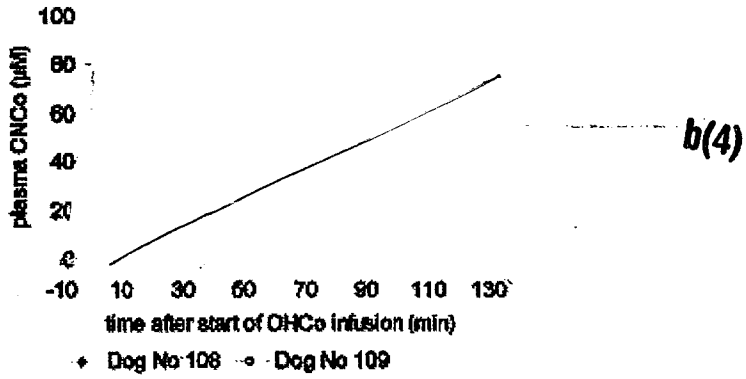

Figure 2 **Concentration time curves (0 – 127.5 min) of cyanocobalamin in cyanide poisoned dogs treated with Cyanokit®: 75 mg/kg**

In agreement with the decrease of whole blood cyanide (Figure 1), cyanocobalamin was formed rapidly in plasma reaching a mean C_{max} of 85 µM observed at five minutes after start of antidote infusion (Figure 2, Table 5).

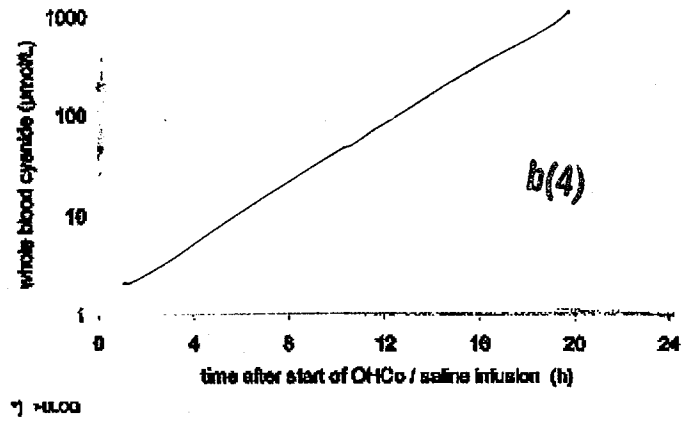

Figure 4 **Concentration time curves (-0.1 – 24.2 h) of whole blood cyanide concentration in poisoned dogs treated with Cyanokit®: 0 mg/kg (dog 106/107), 75 mg/kg (dog 108/109), and 150 mg/kg (dog 156/157)**

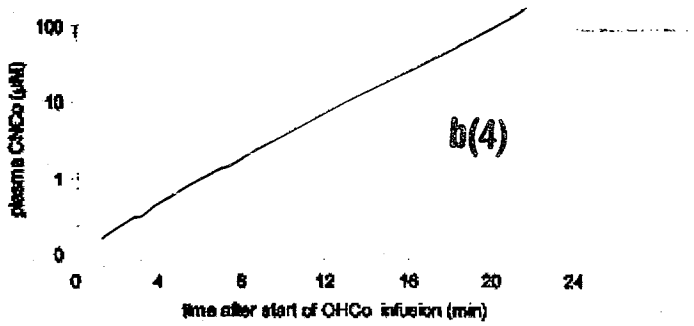

Figure 5 **Concentration time curves (-0.1 – 24.2 h) of cyanocobalamin in cyanide poisoned dogs treated with Cyanokit®: 75 mg/kg**

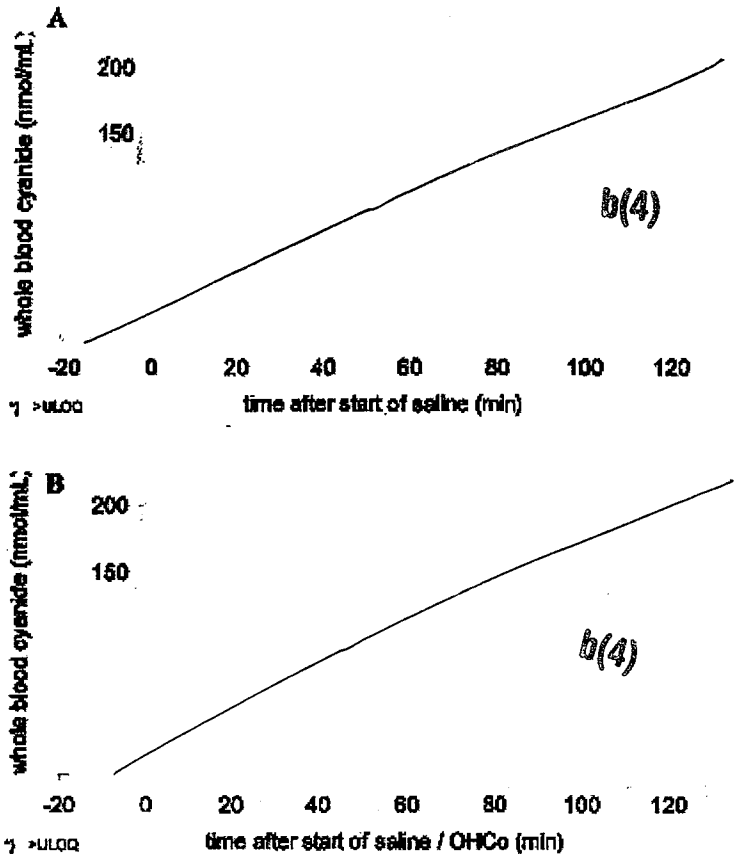

Figure 7 **Concentration time curves of whole blood cyanide after low (A: 0.08 – 0.2 mg/kg/min) and high (B: 0.4 mg/kg/min) cyanide infusion rates**

Individual infusion rates and study outcome are given in *Table 8*. Dogs No 154 and 155 received high dose Cyanokit® infusion as detailed in *Table 8*.

DOG EFFICACY STUDY: MAIN STUDY

Study title: **Efficacy of Intravenous Hydroxocobalamin (Cyanokit®) Administration Following Intravenous Poisoning with Potassium Cyanide in Adult Beagle Dogs**

Study no.: N106342
Vol. 1, Tab 4.2.1.1.1, N106342

The above study included Toxicokinetic Quantification of Total Cobalamins, Cyanocobalmin and Cyanide in the following studies:

Study title: **HPLC-UV Determination of Total Cobalamins-(III) and Cyanocobalamin in Beagle Dog Plasma Samples Collected During the Study N106342**

Study no.: DMPK 166-04
Vol. 1, Tab 4.2.1.1.2, DMPK 166-04
Study title: **Determination of Cyanide in Dog Whole Blood Samples Obtained During the Dog Efficacy Study N106342 (———) Using Liquid Chromatography with Tandem Mass Spectrometric Detection** b(4)

Study no.: DMPK 30-05
Vol. 1, Tab 4.2.1.1.3, DMPK 30-05

Key study findings: Intravenous hydroxocobalamin, administered to dogs at 75 or 150 mg/kg, immediately following a lethal exposure to intravenously administered potassium cyanide, resulted in a dose-dependent survival and recovery of dogs. Hydroxocobalamin, at 150 mg/kg, approximately twice the molar amount of cyanide exposure, resulted in 100% survival rate. This was associated with clinical observations in the vehicle dogs that did not survive beyond the 4-hour post-dose period which were primarily neurological, ranging from unresponsiveness to stupor. Dogs in vehicle and 75 mg/kg dose groups that survived past 4 hours but were euthanized by day 4 also had neurological signs (lethargy, ataxia, and paresis). All dogs that survived to day 15 had minimal clinical observations. There were no significant findings in the clinical pathology or hematological parameters.

Summary of Survival and Pharmacokinetic Findings (Reviewer created table)

Dose		Vehicle 0.9% Saline, IV		Hydroxocobalamin 75 mg/kg, IV		Hydroxocobalamin 150 mg/kg, IV	
Gender		**M**	**F**	**M**	**F**	**M**	**F**
Body Weight Day 1 (kg)		10.3 ± 2.1	7.6 ± 0.3	10.0 ± 1.3	8.2 ± 1.0	9.9 ± 1.5	8.1 ± 0.6
Total KCN (mg/kg)		2.3 ± 0.1	2.3 ± 0.2	2.4 ± 0.2	2.3 ± 0.2	2.2 ± 0.2	2.3 ± 0.2
Overall by Treatment		2.3 ± 0.2		2.4 ± 0.2		2.2 ± 0.2	
		KCN doses were within 88% of target dose of 2.5 mg/kg KCN					
cyanide C_{max} (nmol/mL)		128 ± 19		120 ± 34		114 ± 28	
Time to Apnea (min)		2.8 ± 0.3	2.8 ± 0.5	2.9 ± 0.6	2.9 ± 0.4	2.4 ± 0.4	2.8 ± 0.5
SURVIVAL							
Total N		**8**	**9**	**10**	**9**	**9**	**9**
Incidence and Time of Death	<4 hr	**6**	**4**		**1**		
	1						
	2		**2**				
	3	**1**			**1**		
	4	**1**		**1**	**1**		
% Survival to day 15, by gender and treatment		**0% n=0**	**33.3% n=3**	**90% n=9**	**66.7% n=6**	**100% n=9**	**100% n=9**
% Survival to day 15, by treatment		**17.6% n=3**		**78.9% n=15**		**100% n=18**	

[1] Mean ± SD

Reviewer's Comments:

1. The Division also reviewed the protocol for this study prior to the initiation of the study via a special protocol assessment. The review of the protocol was completed by Dr. Arthur Simone (Medical Officer) and Dr. Dan Mellon (Pharmacology Toxicology Supervisor).
2. The Division requested that this study be inspected by the Division Scientific Investigations due to the critical nature of this study to determine efficacy via the animal rule. Dr. Mark Seaton inspected the ——— facility in ——, and concluded that the study passed inspection. His review will be attached as part of the Agency's official action on this NDA review.

b(4)

Conducting laboratory and location: ——————————— b(4)
Date of study initiation: March 16, 2005
GLP compliance: yes
QA report: yes

Drug, lot #, and % purity:
Hydroxocobalamin, Batch 2081, Purity 94.4%
Vehicle: 0.9% sodium chloride for injection, USP,
Active Agent:

Potassium Cyanide (KCN), reagent grade, batch 00813TA [redacted]; b(4)
prepared for use as (1 mg/mL)
(Analysis of KCN prepared for use yielded concentrations of 110 to 111% of the target concentration.)

METHODS

Doses: 75 and 150 mg/kg Hydroxocobalamin (Cyanokit)
(Data indicating analytical verification of the concentrations of hydroxocobalamin used for dosing was not included. However, the dosing solution was prepared as suggested in labeling of the approved marketed product in France.)

The doses were based on the knowledge that hydroxocobalamin binds cyanide in a 1:1 molar ratio and a preliminary study (N106341) in which 75 and 150 mg/kg Cyanokit were efficacious in enabling survival after a lethal dose of cyanide to anesthetized dogs. Animals were anticipated to receive 2.5 mg/kg KCN (1.2 mg/kg CN^- ion) corresponding to a dose of 0.046 mmol/kg. A dose of 75 mg/kg Cyanokit is equivalent to 0.056 mmol/kg, providing a molar ratio of 1.2:1; the dose of 150 mg/kg provides a molar ratio of 2.4:1.

Species/strain: Beagle dogs

Number/sex/group or time point (main study):

Group	Cyanide Dose (mg/kg)	Hydroxocobalamin Dose (mg/kg)	Number of Animals	
			Males	Females
1	2.5	0 (vehicle)	**8**	**9**
2	2.5	75	**10**	**9**
3	2.5	150	**9**	**9**

Due to animal assignment error, 8 males were dosed in the vehicle group and 10 males were dosed in the low-dose group. The Sponsor noted that two females received a wrong dose, were removed from the study and replaced with two additional female dogs. *(The reviewer finds this replacement acceptable).*

Route, formulation, volume, and infusion rate: Intravenous infusions as follows:

- Potassium cyanide at 0.4 mg/kg/min, for approximately 6 minutes
- Hydroxocobalamin (Cyanokit®, 2.5 g lyophilized powder diluted with 100 mL saline);
  - 75 mg/kg dose: infusion volume was 3 mL/kg, infused over 7.5 minutes
  - 150 mg/kg dose: infusion volume was 6 mL/kg, infused over 7.5 minutes

Satellite groups used for toxicokinetics or recovery: Samples for toxicokinetics were obtained from each animal.
Sampling times: blood samples were collected at 11 time points during day 1

- T0: before initiation of the cyanide infusion
- A0: at the end of three minutes of apnea (start of hydroxocobalamin or vehicle infusion)

- A4, A7.5, All, A15, A20, A30, A60, A120: minutes after the end of test article or vehicle infusion
- A24: hours after the end of test article or vehicle infusion

Age: 7-8 months of age
Weight: on day 1, males: 7.6 - 13.8 kg, females: 6.5 - 9.4 kg

Unique study design or methodology:
One dog was tested per day on the study days between March 25, 2005 and July 8, 2005.

Dogs were fasted overnight prior to dose administration. Dogs were anesthetized (10 mg/kg ketamine, IV; 0.5 mg/kg diazepam IV), intubated and maintained on isoflurane (1.3-2.0%) in medical grade air (21% O_2) at a surgical stage III, in which there was a lack of sensory reflexes and gross motor movement, while allowing steady cardiac and respiratory rates.

Upon stabilization under anesthesia, the animals were instrumented as follows:

- ECG leads were attached to distal limbs
- a pneumotach with pressure transducer was placed in-line with the air supply for respiratory monitoring
- a catheter was placed percutaneously in each cephalic vein (or in a saphenous vein, if unsuccessfully placed in the cephalic vein)
  right side for KCN dose delivery
  left side for vehicle or hydroxocobalamin delivery
- saphenous arterial catheter (by cutdown incision)for arterial blood gas monitoring
- left femoral vein catheter (by cutdown incision) for blood sample collection
- left femoral artery catheter (by cutdown incision) threaded into the aorta pressure monitoring

Baseline data for ECG, respiration and blood pressure were collected for at least 15 minutes, then each dog was administered intravenous potassium cyanide at 0.4 mg/kg/min until the first apnea episode was observed. Apnea was defined as complete cessation of breathing or a reduction in tidal volume to less than 4 mL/kg body weight. At the first apnea event, the potassium cyanide infusion continued for an additional three minutes. Immediately after the conclusion of the potassium cyanide, either test article or vehicle was infused over 7.5 minutes. Concurrent with the termination of the cyanide infusion, each dog was mechanically ventilated with a ventilator at a rate of approximately 10 breaths/minute and tidal volume of approximately 15 mL/kg (parameters were set to be similar to respiratory parameters measured at baseline), using supplemental oxygen (FiO_2 100%), and continued for 15 minutes.

The animals were removed from the ventilator at the end of the 15-minute ventilation period and allowed to breathe medical grade air via the endotracheal tube. Repeated brief cycles of assisted ventilation were necessary in some dogs to wean the animal from the ventilator. This cyclical weaning procedure was as follows: if apnea persisted for 45 seconds, ventilation was reinstated for 15 seconds then stopped. The cyclic assisted

ventilation was repeated, 6 times maximally, during the weaning period. Once weaned from the ventilator, the animals spontaneously breathed medical grade air by endotracheal tube for two hours. The Sponsor mentioned that "this weaning protocol did not represent an attempt at resuscitation from poisoning, but rather support of resumption of spontaneous breathing in surviving animals." *(The reviewer agrees with this assessment).*

Animals surviving two hours after the hydroxocobalamin or vehicle infusion were weaned from anesthesia, catheters and monitoring equipment removed, had catheterized vessels ligated, and incisions closed. Animals were allowed to recover, then returned to their home cages and maintained for 14 days. Dogs that failed to recover consciousness within two hours following cessation of anesthesia were euthanized. Necropsy of surviving dogs was performed on Day 15. Necropsy was also performed on each animal dying after KCN administration.

> ***Reviewer Comments:*** Although it is difficult and somewhat subjective to determine when a dog should be euthanized given a chance they might recover with additional time, the Sponsor set up predetermined criteria to minimize this subjectivity. During the treatment phase and recovery from anesthesia, those in monitoring and recording data were blinded to the treatments, as animals were identified with only a sequential animal number. It was never mentioned who actually knew which dose to prepare and administer, but adhering to these predetermined criteria should have greatly minimized bias. The observer of clinical signs and the veterinarian who performed pretreatment general examinations and post-treatment neurological examinations were both blinded to the treatments. The hierarchy of who actually made the final decision to euthanize a dog in the days post-treatment was not mentioned and therefore it is not known if that was a blinded decision. However, the clinical observations seem to justify those decisions.

RESULTS

Mortality: checked twice daily prior to dosing, at least once daily after dosing

Indicated in the table below, following cyanide administration, all 8 vehicle-treated males and 6 of 9 females were dead or euthanized by day 4. In the 75 mg/kg hydroxocobalamin group 1 of 10 males and 3 of 9 females were dead or euthanized by day 4. All animals in the 150 mg/kg hydroxocobalamin group animals survived until the end of the study. There were no deaths after day 4 post-treatment.

Mortality of Dogs after KCN Administration

Dose	Survival (days)	Vehicle 0.9% Saline, IV		Hydroxocobalamin 75 mg/kg, IV		Hydroxocobalamin 150 mg/kg, IV	
Gender		M	F	M	F	M	F
Total N		8	9	10	9	9	9
Incidence and Time	<4 hr	6	4		1		
	1						

of Death	2		2				
	3	1			1		
	4	1		1	1		
Number of Survivors	15	0	3	9	6	9	9

Table 2. Mortality Rate Analysis in Beagle Dogs Receiving Vehicle or Cyanokit (75 or 150 mg/kg i.v.) as a Treatment after KCN Poisoning

Timepoint	Vehicle (N= 8 male, 9 female) No. Dead (%)	Low Dose (75 mg/kg) (N= 10 male, 9 female) No. Dead (%)	High Dose (150 mg/kg) (N= 9 male, 9 female) No. Dead (%)	Overall Significance among Dose Groups[a]
Hour 1	3 (17.6%)	1 (5.3%)	0 (0%)	No
Hour 4	10 (58.8%)	1 (5.3%)*	0 (0%)*	Yes
Day 8	14 (82.4%)	4 (21.1%)*	0 (0%)*	Yes
Day 15	14 (82.4%)	4 (21.1%)*	0 (0%)*	Yes

a. Separate analyses were performed for male data, female data and both sexes combined. Fisher exact tests were used to determine whether there was a Overall Significance in mortality rates among the dose groups at a 0.05 level at each time point. If there was a Overall Significance among the dose groups at a given time point, Fisher exact tests were used to compare the low or high dose with the vehicle for the given time point at the 0.025 level. * indicates data points that were Significantly different from vehicle group.

The above table was reproduced from the sponsor's submission.

Clinical signs: Clinical signs were checked twice daily prior to dosing and at least once daily after dosing. Detailed clinical observations were obtained at least once daily pre dose and at least once daily after the 2 hour post dose data collection period, and daily during recovery from anesthesia. The observers were blinded to the study treatments.

On day 1, dogs recovering from anesthesia exhibited lethargy, disorientation, ataxia and tremors following survival of the cyanide toxicity were considered by the Sponsor to have been related primarily to recovery from anesthesia due to their occurrence in all dose groups. Dogs euthanized on days 2, 3, and 4 exhibited neurological signs of lethargy, disorientation, and ataxia that compromised their ability to move and potentially to eat and drink. They were euthanized as moribund.

> ***Reviewer's comment:*** All dose groups also received cyanide, so their justification for attributing day 1 signs of lethargy, disorientation, ataxia, and tremors primarily to anesthesia is unlikely. From the initiation of the cyanide infusion, almost 6 minutes elapsed before hydroxocobalamin was infused into two of the three groups. It is more likely that cyanide had a profound effect, possibly synergistic with anesthetic, to involve the recovery behavior. It is noteworthy that those treated with hydroxocobalamin were not as severely affected, suggesting an early sign of effectiveness.

Discoloration was noted in the body and urine of most dogs receiving either the low- or high-dose of hydroxocobalamin. Body color and urine of most dogs receiving hydroxocobalamin was a reddish color. The Sponsor attributed this to dark red-purple color of the hydroxocobalamin solution, since there was no hematology, chemistry or urine values suggesting a reason for this discoloration.

Reviewer's comment: In previous toxicological studies, this reddish color was also noted, was somewhat dose-related and attenuated with days from treatment supporting an effect just due to the presence of hydroxocobalamin (and/or cyanocobalamin) in the tissues of the animal and not necessarily due to a pharmacological or toxicological mechanism.

Neurological Observations: The neurological exam was conducted prior to day 1 and on days 2, 8, and 15 by a clinical veterinarian blinded to the study treatments. A Neurological Deficit Scoring system (below) was used as summarized in the Sponsor's table below:

Attachment A – Neurological Deficit Score

Parameter	Score			
	0	30	60	100
Level of Consciousness	Normal	Reduced	Stupor	Coma
	0	50	100	
Respiratory Pattern	Normal	Abnormal	Apnea	
	0	2	5	
Pupil Size	Normal (4-10 mm)	Abnormal (>10 mm)	Severe Abnormal (>14 mm)	
Pupillary Response	Normal	Reduced	Nonreactive	
Eye Position	Normal	Moderately abnormal	Severely abnormal	
Eyelid Reflex	Normal	Reduced	Absent	
Corneal Reflex	Normal	Reduced	Absent	
Auditory Reflex	Normal	Blinking only	No response	
Gag Reflex	Normal	Reduced	Absent	
	0	5	10	25
Muscle Stretch Reflex	Normal		Increased or reduced in 1-3 limbs	Absent in all limbs
Response to Pain Stimulus	Normal	Reduced	No response	Coma
Right Lateral Positioning	Normal		Abnormal – intermittent running motion	Markedly abnormal
Muscle Tone	Normal		1-2 limbs stiff or flaccid	3-4 limbs stiff or flaccid
	0	15	30	
Drinking	Normal	Abnormal		
Sitting	Normal	Abnormal		
Walking	Normal	Ataxia	Cannot walk	

Four dogs (3 males, 1 female) had abnormal neurological scores on day 2 and were euthanized by day 4 due to the severity of their impairment. In the 75 mg/kg

hydroxocobalamin group 2 females were euthanized by day 4 due to neurological impairments. There were no neurological impairments in the 150 mg/kg hydroxocobalamin group.

Physical exams: conducted by a clinical veterinarian blinded to the study treatments, prior to day 1 and on days 2, 8, and 15.

There were no physical exam abnormalities in surviving dogs. Dose group mean body temperature, heart rate, and respiratory rates in surviving dogs on day 2, 8 and 15 were mostly similar between groups and within the normal range for dogs.

Abnormal Clinical and Neurological Signs of Dogs Euthanized through Day 4
*(Reviewer's Table)

Treatment/Dose	Vehicle 0.9% Saline, IV	Hydroxocobalamin 75 mg/kg, IV
Day 1 **Dogs Euthanized by Hour 4 and reason (post dose observations)**	**Males** **102** no recovery (lethargic, prostate, unresponsive, respiratory sounds, labored respiration, tremors) **106** no recovery (unconscious) 108 no recovery (prostate, unresponsive) **112** no recovery (prostate, unresponsive, salivation, mucoid feces, convulsive) **126** no recovery (lethargic, prostate, unresponsive, salivation, muscle fasciculation, disoriented/circling, ataxic, convulsive} **127** dead at A15-A20 (no observations) **Females** **166** no recovery (unconscious) **171** dead at A15-A20 (unconscious) **173** no recovery (prostate, unresponsive) **177** dead at A15-A20 (no observations)	**Females** **176** dead at A15-A20 (no observations)
Day 2	**Females** **160** severe neurological signs (day 1 lethargic, prostate, unresponsive, ataxic, salivation, mucoid feces, day 2 lethargic, prostate, unresponsive, ataxic, mucoid feces, reduced/absent feces, neuro exam score = 101) **178** severe neurological signs (day 1: lethargic, prostate, unresponsive; day 2: mucoid feces, diarrhea, disoriented/circling, ataxic)	
Day 3	**Males** **116** severe neurological signs, exam score = 75;	**Females** **165** severe neurological signs

	(day 1: lethargic, ataxic day 2: lethargic, prostate, emesis, mucoid feces ataxic, neuro exam score = 75 day 3: lethargic, emesis, reduced/absent feces)	(day1: Hyperactive, rapid respiration, disoriented/circling, body discoloration day 2: Prostate, rapid respiration, urine-reddened, limb weakness, tremors, neuro exam score = 45 day 3: Salivation, reduced/absent feces, limb weakness, head-tilt, tremors, muscle fasciculation, disoriented/circling, ataxic)
Day 4	**124** severe neurological signs (day1: tremors, disoriented/cycling, ataxic, convulsive day 2: ataxic, neuro exam score = 30 day 3: lethargic day 4: lethargic, disoriented, ataxic)	**Males** **118** severe neurological signs (day 1: Lethargic, urine-reddened, salivation day 2: Reduced/absent feces, urine-reddened, head-tilt, ataxic, convulsive, neuro exam not recorded day 3: ataxic day 4: head tilt. muscle fasciculation, disoriented/circling, ataxic **Females** **170** severe neurological signs (day 1 Rapid respiration, salivation, ataxic day 2: salivation, ataxic, neuro exam score = 20 day 3: salivation, ataxic day 4: ataxic

* All dogs in the 150 mg/kg dose group survived. The bolded numbers in the table above are the individual animal numbers in the study.

Abnormal Clinical and Neurological Signs of Dogs Surviving to Day 15

Treatment/Dose	Vehicle 0.9% Saline, IV	Hydroxocobalamin 75 mg/kg, IV	Hydroxocobalamin 150 mg/kg, IV
Day 1	**Females** **151** lethargic shivering **163** No abnormal observations **172** lethargic, ataxic	**Males** **103** Salivation, tremors, body discoloration, urine-reddened **104** Urine-reddened **107** Lethargic, urine-reddened **110** Lethargic tremors, ataxic, body discoloration, urine-reddened, seizure **111** Lethargic, ataxic **114** Lethargic, tremors, discoloration **119**Urine-reddened, eyes/ears reddened **122** Ataxic, body discoloration	**Males** **101** Urine-reddened, body discoloration **105** Urine-reddened, body discoloration, salivation, emesis **109** Lethargic, urine-reddened **113** Emesis, ataxic, body discoloration **115** Salivation, diarrhea, tremors, ataxic, body discoloration **117** Tremors, ataxic, urine-reddened **120** Tremors, ataxic, urine-reddened, body discoloration **121** Lethargic, tremors, ataxic,

		125 Ataxic, urine-reddened **Females** **154** Urine-reddened **156** ataxic **158** ataxic **162** Lethargic, salivation, ataxic, emesis **168** Lethargic, salivation **175** Ataxic, urine-reddened	urne-reddened, body discoloration, salivation **123** Ataxic, mucoid feces, urine-reddened, body discoloration **Females** **152** Urine-reddened, body discoloration **153** Urine-reddened, body discoloration **157** Tremors, urie-reddened, body discoloration **159** Lethargic, salivation, body discoloration, ataxic **161** Tremors, mucoid feces, reduced/absent feces, body discoloration, urine-reddened **167** Ataxic, urine-reddened **169** Ataxic **174** Ataxic, body discoloration, diarrhea, urine reddened **179** Lethargic, ataxic, body discoloration
Day 2	**Females** **163** reduced/absent feces **172** diarrhea	**Males** **103** Reduced/absent feces, urine-reddened **104** Urine-reddened **107** Salivation, urine-reddened **111** Urine-reddened **114** Urine-reddened **122** Urine-reddened soft feces **Females** **154** Urine-reddened **156** Urine- reddened **158** Urine- reddened **162** Reduced/absent feces urine-reddened **168** Reduced/absent feces, urine-reddened **175** Urine-reddened	**Males** **101** Urine-reddened **105** Urine-reddened **109** Salivation **113** Urine-reddened, reduced/absent feces **115** Mucoid feces, diarrhea **117** Urine-reddened **120** Urine-reddened **121** Urine-reddened **123** Urine-reddened **Females** **152** Urine-reddened **157** Urine-reddened **161** Urine-reddened **167** Urine-reddened **169** Urine-reddened **174** Urine-reddened **179** Urine-reddened
Day 3		**Females** **156** Urine-reddened **158** Urine-reddened	**Males** **117** Urine-reddened **Females** **167** Urine-reddened **169** Urine-reddened

Body weights: Body weights were recorded on the day of treatment, prior to dose administration and at necropsy. Body weight was not measured during the 14 days recovery/maintenance period.

Body Weight Summary (Reviewer's created table)

Dose	Vehicle 0.9% Saline, IV		Hydroxocobalamin 75 mg/kg, IV		Hydroxocobalamin 150 mg/kg, IV	
Gender	**M**	**F**	**M**	**F**	**M**	**F**
Number of dogs	**8**	**9**	**10**	**9**	**9**	**9**
Body weight*, day 1, (kg)	10.3 ± 2.1 (8.2-13.8)	7.6 ± 0.3 (7.3-8.1)	10.0 ± 1.3 (7.9-12.2)	8.2 ± 1.0 (6.5-9.4)	9.9 ± 1.5 (7.6-12.2)	8.1 ± 0.6 (7.1-9.2)
Day 15 Number of Dogs	**0**	**3**	**9**	**6**	**9**	**9**
Body weight*, d 15 (kg)		7.4 ± 0.2	9.2 ± 0.4		9.1 ± 0.3	

* mean ± s.d. (range); day 15 data were not separated by gender

Food consumption: This was not monitored.

Ophthalmoscopy: An eye exam (reflex responses) was performed as part of the neurological assessments on days-1, 2, 5, and 8. There was no further ophthalmoscopy examination.

EKG: EKG was monitored during the 2 hour anesthesia period that included the cyanide and hydroxocobalamin treatments.

Heart Rate: During the 2 hours of monitoring under anesthesia, heart rates were similar among treatment groups. Bradycardia developed approximately 1 minute before the onset of apnea and declined through the period of apnea. Heart rate recovered at the end of cyanide infusion, plateaued at 85 to 110 bpm at 2 to 15 minutes from the end of apnea (during hydroxocobalamin infusion and mechanical ventilation). In vehicle treated dogs, heart rate plateaued at 120 to 125 bpm. After mechanical ventilation was stopped, heart rate increased in all groups to 140 to 150 bpm, and then returned to baseline. The return to baseline was quicker in the 75 and 150 mg/kg hydroxocobalamin groups (50 min post-apnea), compared to the vehicle treated group (80 min post-apnea).

Systolic blood pressure: Cyanide infusion resulted in elevated systolic blood pressure to 135 - 145 mmHg during the first minute from the defined apnea episode. This declined to approximately baseline level at the end of apnea.

Diastolic blood pressure: Diastolic blood pressures were characterized by a slight decrease in pressure (up to ~15 mm Hg) during the middle of KCN infusion, and at the start of apnea. Through the end of apnea, there was a return to near baseline values then a decline (diastolic pressure 45-55 mmHg).

Mean blood pressure: Mean blood pressures were also characterized by a slight decrease in pressure (up to ~15 mm Hg) during the middle of KCN infusion, and at the start of

apnea with a return to near baseline values then a decline (diastolic pressure 45-55 mmHg) through the end of apnea.

Vehicle dogs had little changed in blood pressures through the infusion and ventilation period. Dogs receiving 75 or 150 mg/kg hydroxocobalamin had a dose dependent increase in all blood pressures that began at 1 to 2 minutes of infusion and peaked at 4 to 6 minutes of infusion. Following the end of ventilation, blood pressures in vehicle dogs increased to a normal range but remained at values less than baseline until the end of the two-hour post-dose period. Starting at the end of infusion, blood pressures in hydroxocobalamin treated dogs returned to baseline levels through the end of ventilation and remained steady throughout the post-dose monitoring period.

Pulse pressures in all groups were similar through the KCN infusion and apnea period with marked increases due to the increased systolic pressure and concurrent decline in diastolic pressure. Pulse pressure declined at the end of apnea as both systolic and diastolic pressure declined. Dogs receiving low- or high-dose Cyanokit had a dose dependent increase in pulse pressures that began at 1 to 2 minutes of infusion and peaked at 4 to 6 minutes of infusion, whereas vehicle dogs had no changes in pulse pressure in response to infusion. Pulse pressures returned to baseline levels at the by the end of ventilation in hydroxocobalamin treated dogs. Vehicle treated dogs exhibited an increase in pulse pressure for approximately 20-25 minutes following the end of ventilation. The following diagrams were reproduced from the sponsor's submission:

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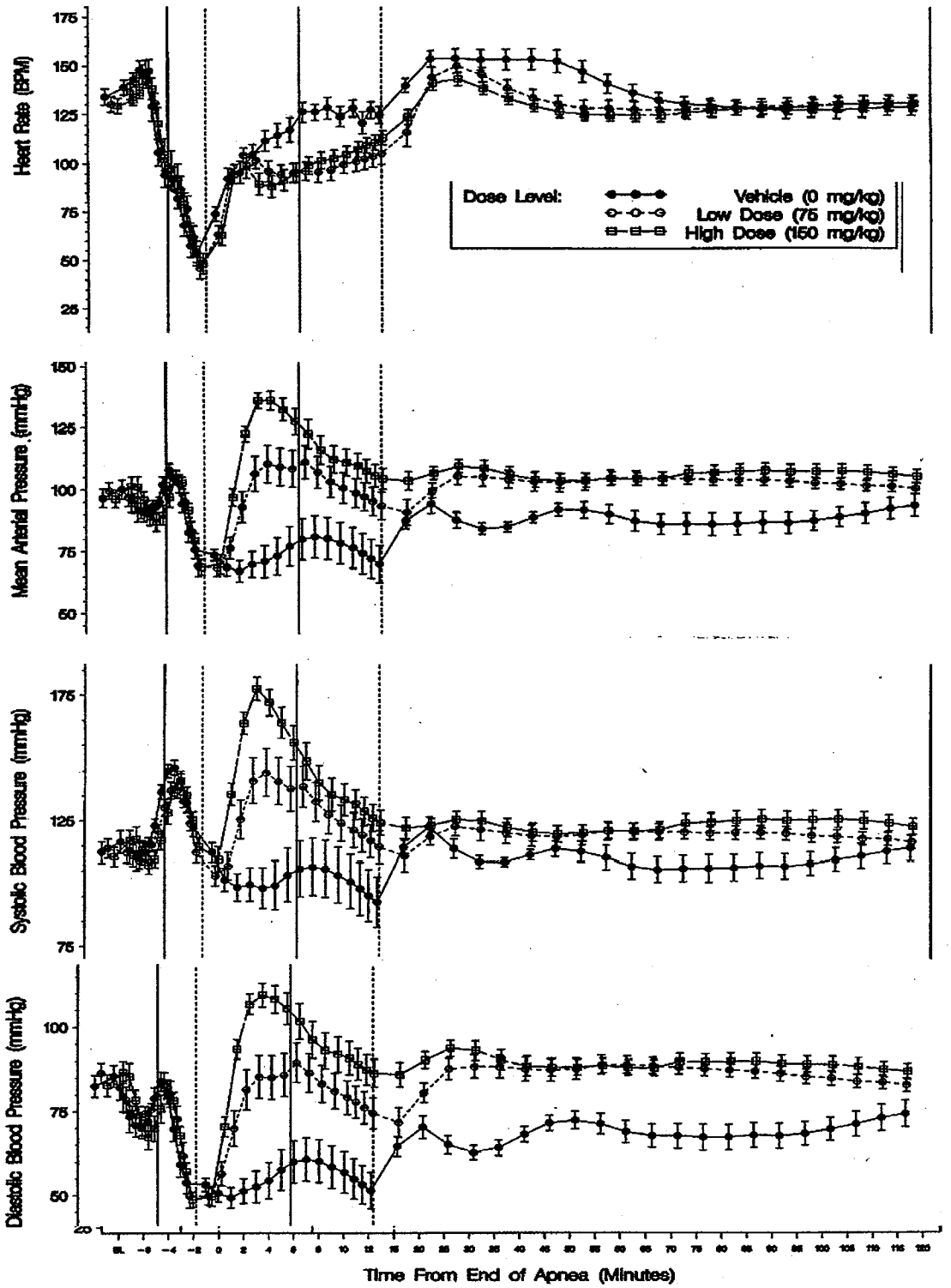


BL = 15-minute mean (SE) baseline data. First vertical line = Start of apnea. Second vertical line = end of apnea (A0) and KCN infusion, start of test article or vehicle infusion and mechanical ventilation. Third vertical line = end of test article or vehicle infusion. Fourth vertical line = end of mechanical ventilation.

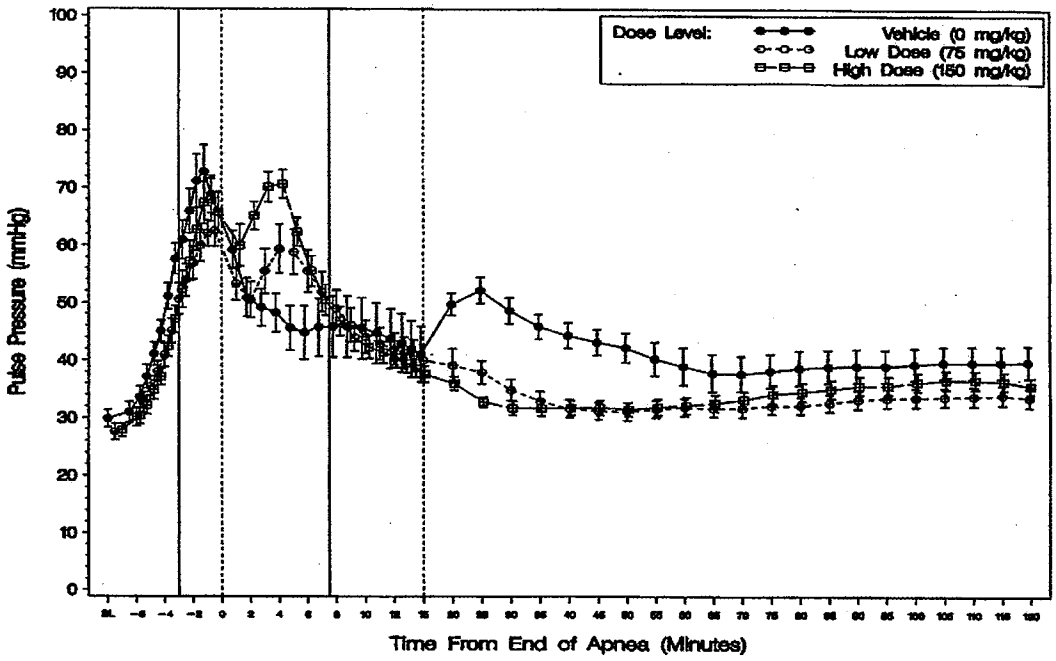
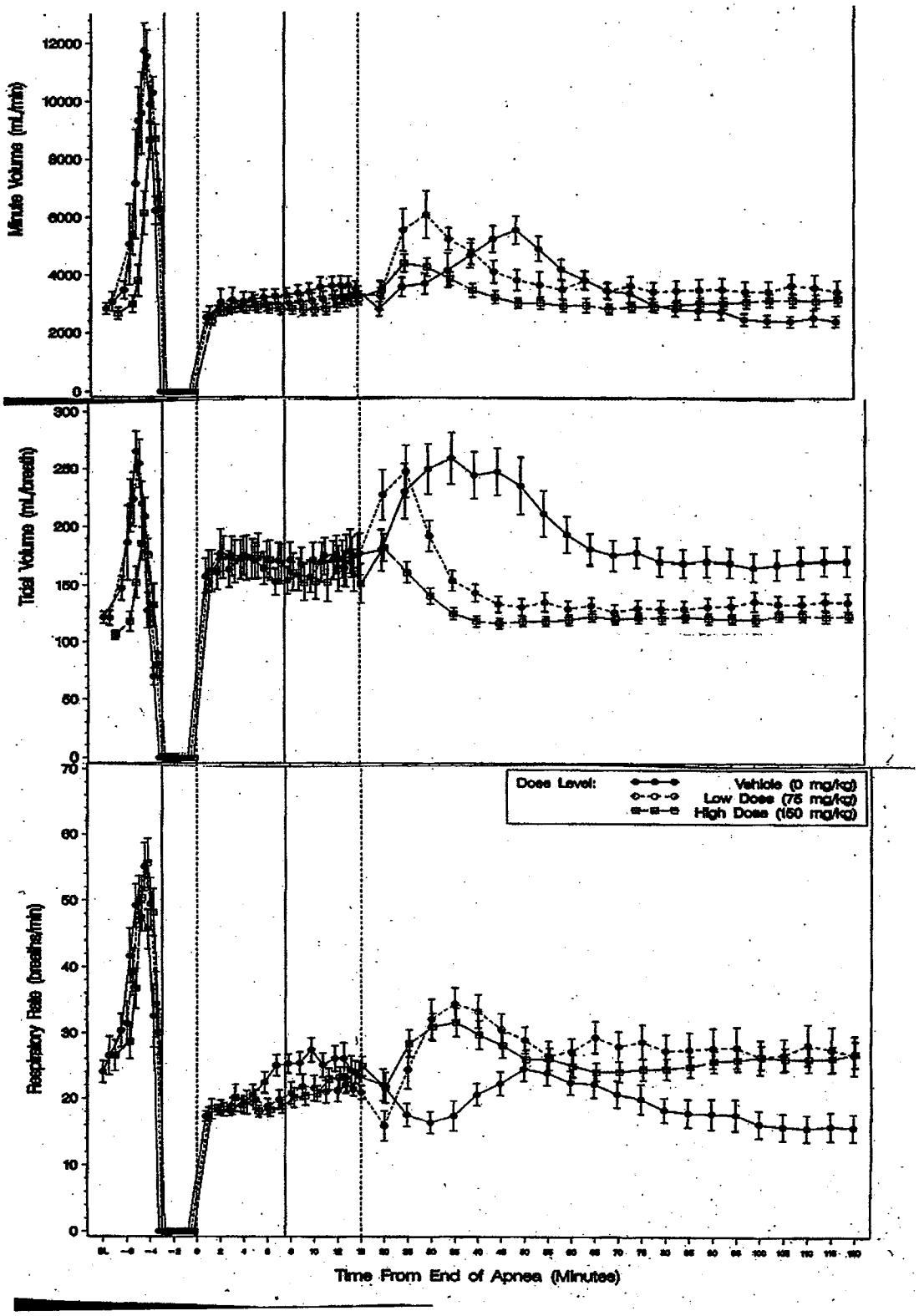


Figure 5. **Pulse Pressure (mean ± SE) in Beagle Dogs Receiving Cyanokit (0, 75 or 150 mg/kg I.V.) as Treatment after Cyanide (KCN) Poisoning.**

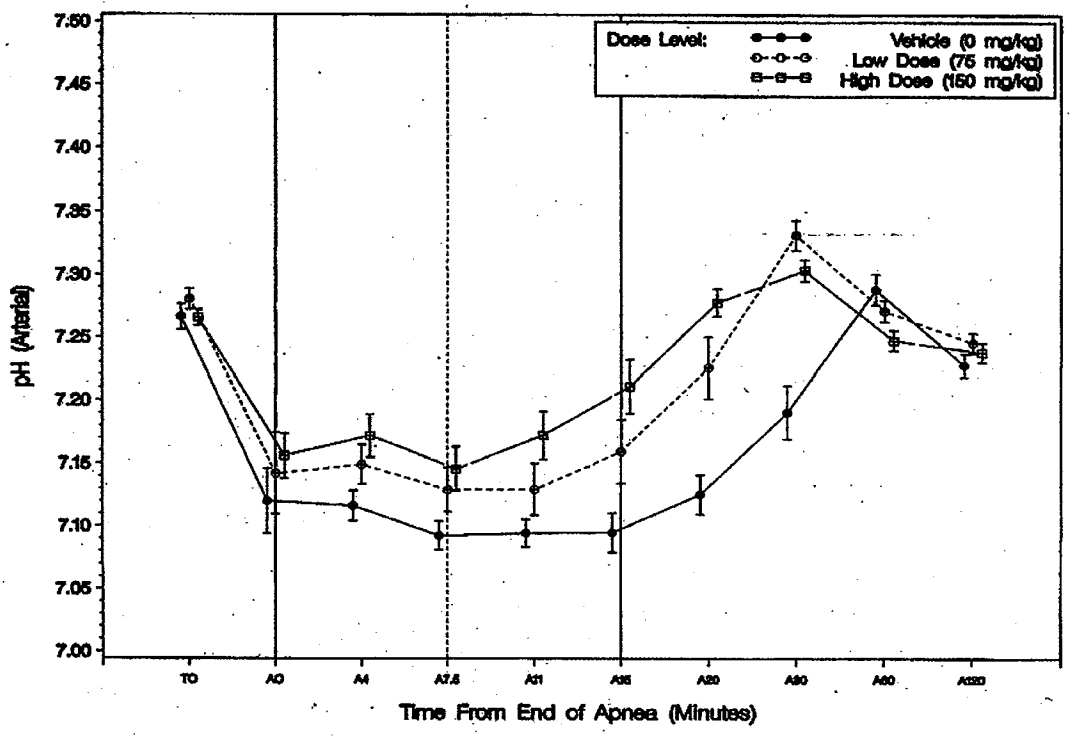
BL = 15-minute mean (SE) baseline data. First vertical line = Start of apnea. Second vertical line = end of apnea (A0) and KCN infusion, start of test article or vehicle infusion and mechanical ventilation. Third vertical line = end of test article or vehicle infusion. Fourth vertical line = end of mechanical ventilation.

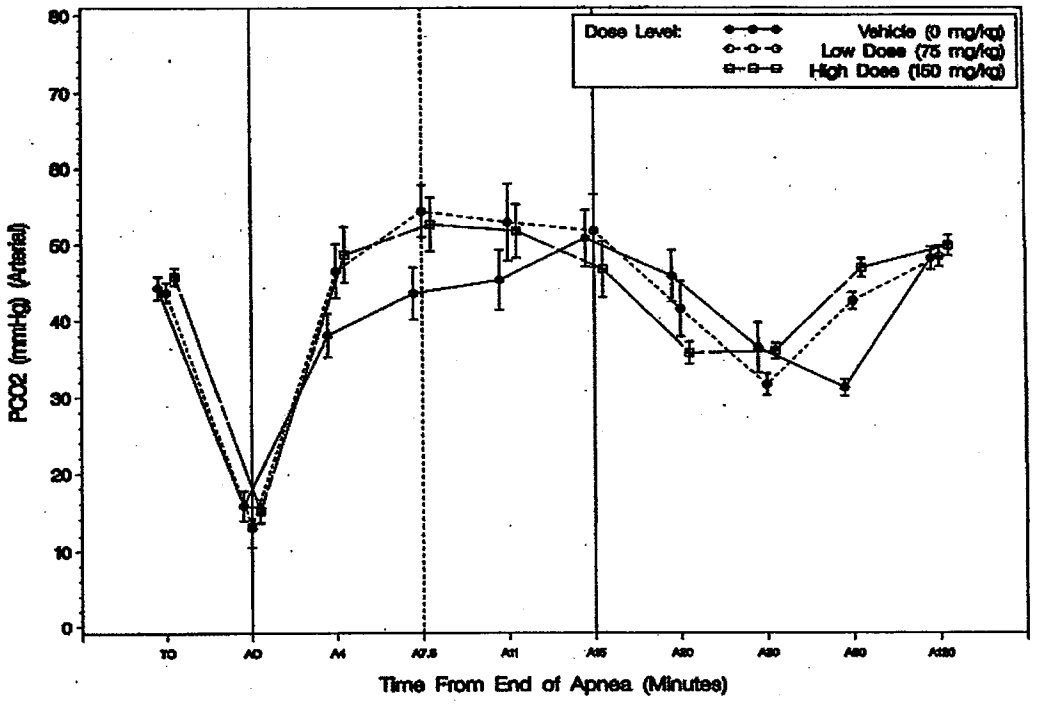
Respiration Parameters: Respiratory rate, tidal volume, and minute volume had similar changes in all dogs surviving the cyanide infusion until the occurrence of apnea. Respiratory rate and tidal volume began to increase within 30 to 60 seconds of the start of cyanide infusion and had a peak of approximately 2 to 3 times baseline values at approximately 1 to 2 minutes prior to onset of apnea. Minute volume increased approximately 3-4 times baseline values with a similar time course of change. Mechanical ventilation produced similar ventilatory values in all dose groups. It was noted that vehicle dogs had a slight increase in respiratory rate in second half of the ventilation period. This was due to the dogs beginning to spontaneously breath in addition to the ventilator respirations. Almost all dogs returned to spontaneous ventilation at the end of mechanical ventilation without requiring a weaning period. Two dogs in the vehicle group required 1 to 2 minutes of weaning before resuming spontaneous respiration. During the post-ventilation period, dogs receiving vehicle had lower respiratory rates and larger tidal volumes for up to 50 minutes from the end of ventilation. Hydroxocobalamin treated dogs had brief increases in rate, tidal volume, and minute volume for 20 to 30 minutes from the end of ventilation, and then returned to baseline values. The increase in tidal volume and minute volume was larger for low dose than for high-dose dogs. The following diagrams were reproduced from the sponsor's submission:

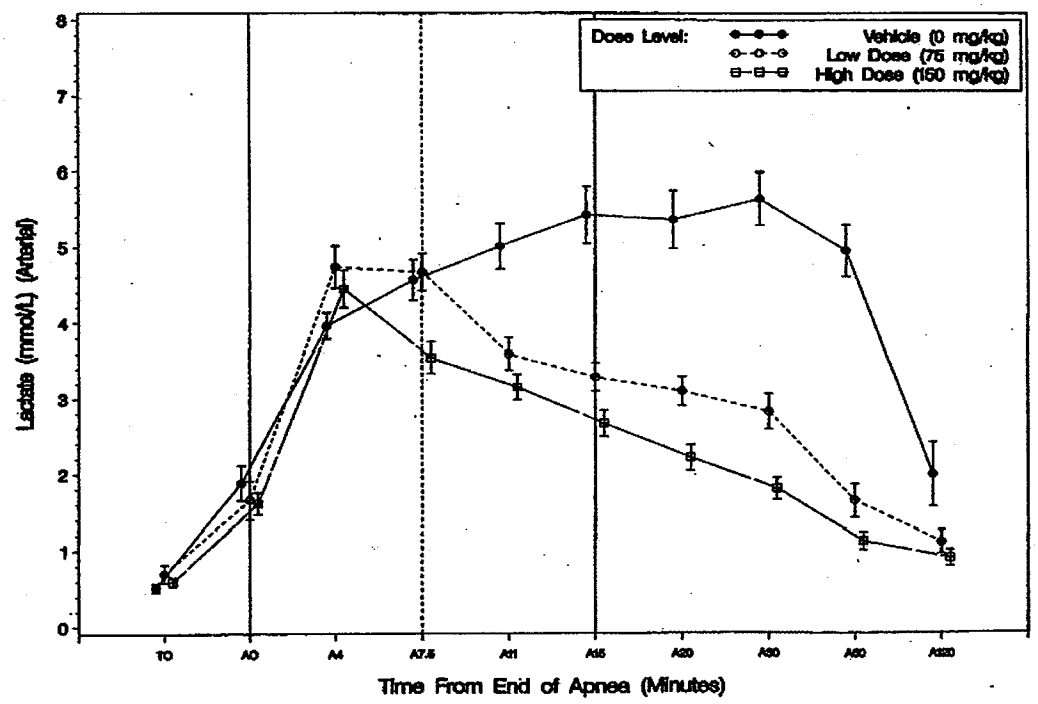

Minute Volume (mL/min)
Tidal Volume (mL/breath)
Respiratory Rate (breaths/min)
Dose Level:
Vehicle (0 mg/kg)
Low Dose (75 mg/kg)
High Dose (150 mg/kg)
Time From End of Apnea (Minutes)

Blood O_2, CO_2 and Lactate: Samples were obtained prior to initiating cyanide infusion (T0), at end of cyanide infusion (A0), during infusion of hydroxocobalamin or vehicle (A4, 4 minutes after the start of infusion, and at A7.5 (the end of infusion), A11, A15, A20, A30, A60 and A120 minutes after the start of hydroxocobalamin or vehicle infusion.

Lactate: Lactate in venous and arterial samples from dogs was significantly less in animals receiving hydroxocobalamin from approximately end of test article infusion to 60 minutes from end of apnea. Arterial and venous pH in dogs was significantly greater in animals receiving hydroxocobalamin from approximately 7.5-15 minutes to 30 minutes from end of apnea. PCO_2 was sporadically significantly higher in venous blood from dogs receiving hydroxocobalamin but the occurrences were sporadic and probably due to random variation.

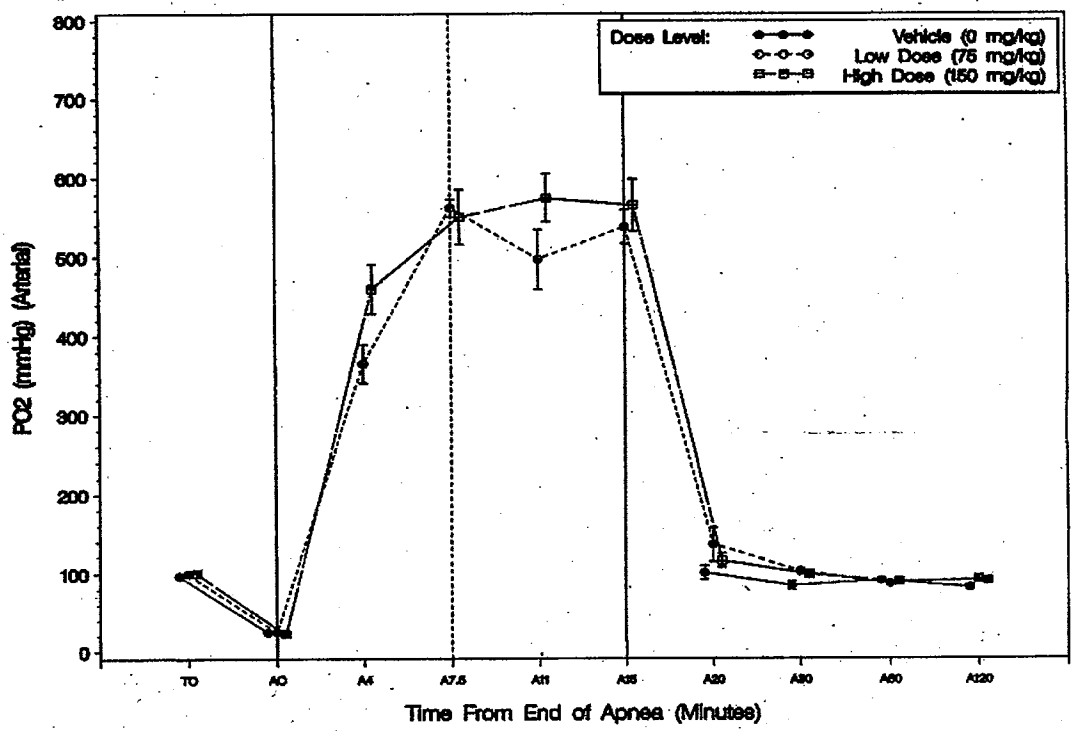






Arterial PO_2 was only measured in a few samples from vehicle dogs at the A4 to A15 time points. The number of samples that were successfully analyzed was reduced for arterial PO_2 samples in the hydroxocobalamin groups at A0 as well. The analyzer was

fully calibrated and functional so the cause of the missed analyses was uncertain. The inability of the analyzer to measure the values was interpreted to be due to an interference with the analyzer by the high levels of free cyanide in the samples. At all other time points, arterial PO_2 was not significantly different between the dose groups. Evaluation of the arterial oxygen saturation (SO_2) indicated that sufficient oxygen was supplied to saturate the hemoglobin. Venous PO_2 was significantly greater at various points from A4 to A15 in dogs receiving 75 mg/kg or 150 mg/kg hydroxocobalamin. The calculated arterial PO_2-venous PO_2 had no significant differences at time points outside of the A4 to A15 periods.



Hematology: Blood samples were obtained prior to the treatment day, day 2, and day 15 just prior to necropsy. Measurements were obtained for hematocrit, hemoglobin, white blood cell counts, and platelet counts. Also, hematocrits were obtained at T0, A7.5, A60, and A120.

> ***Reviewer's Comment:*** The reddish hydroxocobalamin and cyanocobalamin compounds are known to interfere with colorimetic assays used in automated analyzers. This was noted in earlier toxicological studies. It is not known if the values reported here for hemoglobin are from validated procedures. However, the inability to accurately measure some clinical laboratory parameters due to the color of the drug substance does not negate the validity of the study.

There were no significant differences among the treatment groups in hematology parameters. There was a slight increase in hematocrit in dogs in all dose groups at the

A7.5 time point. This appears to have been a response to the KCN poisoning as it occurred in vehicle animals as well as those receiving hydroxocobalamin.

Clinical chemistry: Blood samples were obtained prior to the treatment day, day 2 and day 15, just prior to necropsy. Measurements were obtained for electrolytes, blood urea nitrogen, creatinine, AST, ALT, bilirubin, LDH, glucose, and total protein.

> ***Reviewer's Comment:*** The reddish hydroxocobalamin and cyanocobalamin compounds are known to interfere with colorimetic assays used in automated analyzers. This was noted in earlier toxicological studies. It is not known if the values reported here for ALT, AST, LDH, bilirubin, glucose, total protein, and creatinine are from validated procedures. However, the inability to accurately measure some clinical laboratory parameters due to the color of the drug substance does not negate the validity of the study.

There were no significant differences among the treatment groups in clinical chemistry parameters.

Urinalysis: This was not performed.

Gross pathology: Necropsies were performed on each dog dying after infusion of KCN administration to examine for CNS damage related to KCN administration. All surviving dogs to day 15 were necropsied also. Following an overnight fast, dogs were weighed, then anesthetized with an intravenous barbiturate (not identified), and exsanguinated. Each necropsy included examination of the external surface of the body, all orifices, the cranial, thoracic, abdominal, and pelvic cavities, and their contents. Necropsies were supervised by a board certified veterinary pathologist. The kidney, heart, lungs, liver, spleen, adrenals, bone marrow, and CNS were examined both grossly and microscopically. Tissues were preserved in ten percent neutral buffered formalin.

Gross findings included discolorations of the cerebrum, which usually correlated with cerebral edema or necrosis, and herniated cerebellar vermis, which was present in cases with necrosis and/or edema of the cerebellum. Both gross findings were restricted to vehicle dogs that died within the first 4 days post-administration. The presence of clotted blood at the tentorium cerebelli in four dogs (three vehicle and one low-dose), although apparently associated with potassium cyanide poisoning, had no microscopic correlate upon examination.

Organ weights: Weighed organs included kidneys, heart, brain, adrenals, liver, and spleen.

Absolute brain weights were greater in dogs receiving vehicle or low-dose that died within four days of dose as compared to brain weights in dogs in all groups at Day 15. Brain/body weight ratio was also larger in vehicle dogs at most time points that data was collected including Day 15 as compared to low- or high-dose dogs that died at any time

point. Other organ weight data in vehicle or low-dose dogs that died prior to Day 15 showed some minor differences as compared to Day 15 data in each of the dose groups, but due to the small sample sizes at these points, they cannot be distinguished from biological variability. The table below was reproduced from the sponsor's submission.

Table 22. Organ Weight/Body Weight Ratio (g/kg) of Beagle Dogs Receiving Vehicle or Cyanokit (75 or 150 mg/kg i.v.) as a Treatment After KCN Poisoning

Organ	Day of Death	Mean (SE) [N] Vehicle (0 mg/kg)		Low Dose (75 mg/kg)		High Dose (150 mg/kg)	
ADRENAL GLAND	1	0.11 (0.01)	[10]	0.13 (.)	[1]		[0]
	2	0.14 (0.00)	[2]		[0]		[0]
	3	0.10 (.)	[1]	0.13 (.)	[1]		[0]
	4	0.11 (.)	[1]	0.11 (0.02)	[2]		[0]
	15	0.13 (0.01)	[3]	0.10 (0.00)	[15]	0.10 (0.00)	[18]
BRAIN	1	9.00 (0.57)	[10]	8.46 (.)	[1]		[0]
	2	10.58 (0.69)	[2]		[0]		[0]
	3	7.38 (.)	[1]	8.80 (.)	[1]		[0]
	4	11.19 (.)	[1]	8.37 (0.20)	[2]		[0]
	15	10.08 (0.55)	[3]	8.34 (0.31)	[15]	8.09 (0.26)	[18]
HEART	1	9.28 (0.34)	[10]	9.42 (.)	[1]		[0]
	2	9.27 (1.08)	[2]		[0]		[0]
	3	8.33 (.)	[1]	9.68 (.)	[1]		[0]
	4	8.27 (.)	[1]	8.69 (0.15)	[2]		[0]
	15	8.96 (0.56)	[3]	8.51 (0.24)	[15]	8.13 (0.16)	[18]
KIDNEY	1	5.75 (0.28)	[10]	5.53 (.)	[1]		[0]
	2	5.37 (0.28)	[2]		[0]		[0]
	3	5.99 (.)	[1]	6.29 (.)	[1]		[0]
	4	5.75 (.)	[1]	5.39 (0.39)	[2]		[0]
	15	4.89 (0.20)	[3]	4.92 (0.14)	[15]	5.09 (0.12)	[18]
LIVER	1	36.97 (2.22)	[10]	40.90 (.)	[1]		[0]
	2	36.96 (8.80)	[2]		[0]		[0]
	3	28.94 (.)	[1]	41.23 (.)	[1]		[0]
	4	32.43 (.)	[1]	36.86 (4.27)	[2]		[0]
	15	33.02 (1.86)	[3]	31.45 (0.90)	[15]	31.17 (0.76)	[18]
SPLEEN	1	4.06 (0.57)	[10]	2.71 (.)	[1]		[0]
	2	7.00 (1.78)	[2]		[0]		[0]
	3	5.36 (.)	[1]	2.05 (.)	[1]		[0]
	4	4.79 (.)	[1]	4.48 (2.03)	[2]		[0]
	15	6.25 (0.17)	[3]	6.20 (0.47)	[15]	7.04 (0.46)	[18]

Reviewer comments: The increase in brain weight was probably due to edema.

Histopathology: Adequate Battery: NO (see reviewer comment below)
Peer review: YES, by the Sponsor's representative
performed at

Reviewer's Comment: The major organs of toxicity identified from the toxicological studies were examined, but most organs and tissues of the body were not examined. Lacking human pathological data from previous experience with this product, it would have been prudent for the Sponsor to gather as much pathological information as possible from this pivotal study, since a similar mg/kg dose would be administered to humans. Toxicological histopathology was obtained from a 28-day hydroxocobalamin dosing study and a 14 day cyanocobalamin study, both conducted in dogs, which can be used for

comparison. The lack of complete histopathology, however, does not impact the utility of the study to demonstrate efficacy in this model.

Histopathological evaluation of the kidney, heart, lung, liver, spleen, adrenal, bone marrow, and CNS were conducted by a board-certified veterinary pathologist on all dogs that had a necropsy conducted. CNS examinations included cerebrum, cerebellum, medulla oblongata, and spinal cord (grey and white matter). Kidney, heart, lungs, liver, spleen, adrenals, bone marrow and CNS (cerebrum 3 sections; cerebellum 2 section; medulla oblongata; spinal cord, cervical, thoracic and lumbar levels) were examined grossly and microscopically.

Lesions judged to be related to administration of potassium cyanide in brain included edema, necrosis, hemorrhages, lymphocytic perivascular cuffs, and occasionally, inflammation. Edema was diagnosed based upon presence of well-defined areas where the neuropil was vacuolated and neurons shrunken but usually basophilic (occasionally pale). The capillaries in such areas often had very prominent (reactive) endothelial cells. Necrosis was diagnosed where neurons had diffuse eosinophilia (dead red neurons); often the nuclei of affected neurons were marginated and cytoplasm was shrunken.

Edema and necrosis were graded based upon the amount of tissue affected, as follows:

1. minimal, represented a lesion that was unilateral and focal to multifocal, but small
2. mild, represented a lesion that was bilateral and more than focal
3. moderate, where more than one brain site was affected (e.g. bilateral cerebral necrosis affecting multiple sections), or in the case of the caudate nucleus and substantia nigra (only 1 slide contained each), where 50-75% of at least one side was affected
4. marked, represented a bilateral and widespread lesion (e.g., cerebrum or cerebellum), or virtually all of a site affected (e.g., >75% of caudate nucleus or substantia nigra).

The entire spectrum of lesions was represented in the vehicle group: edema was observed in dogs that died or were euthanized up to the third day; necrosis was present in dogs as early as 1 hour post-dosing and also in dogs at terminal sacrifice; lymphocytic perivascular cuffs were present at each time point represented; hemorrhages were present in dogs dying between 1 hour and 4 days post-dosing; and inflammation was present in dogs that died at Days 2 and 4.

Inflammation was diagnosed in three animals (that died on Day 2 and 3), and was characterized by predominantly neutrophilic infiltration associated with moderate to marked areas of necrosis. Fewer high-dose dogs had lesions compared with the vehicle and low-dose groups (6/18 high-dose dogs compared with 17/19 low-dose and 16/17 vehicle dogs). The two dogs without brain lesions in the low-dose group survived to final sacrifice.

Where there was moderate-to-marked necrosis or edema of the caudate nucleus, it often extended into the thalamus (when present in the section). Both edema and necrosis were sometimes associated with the presence of reactive microglia (rod cells), which was not separately diagnosed, and with lymphocytic perivascular cuffs (diagnosed separately). The lymphocytic perivascular cuffs present were rarely purely lymphocytic. Areas with severe edema and/or necrosis were occasionally associated with hemorrhage(s) (diagnosed separately).

Edema was only present in vehicle group dogs. Edema would be expected to resolve or develop into necrosis within 2-3 days; since only 2 dogs died within 3 days of dosing in the low-dose group (and none in the high-dose group). Edema was not present in hydroxocobalamin treated groups.

In cerebral cortex, caudate nucleus, and/or substantia nigra (taken together), when necrosis was present it was of moderate-to-marked severity somewhat more often in vehicle than low-dose animals (71% of diagnoses in vehicle-treated dogs compared with 59% of low-dose dogs), a difference considered of equivocal biological significance. In contrast, in high-dose dogs, necrosis (when present) was graded moderate-to-marked only half as frequently as in the low dose dogs. Hemorrhages were present in 12 vehicle dogs (moderate-to-marked in 33%), three low-dose dogs (moderate-to-marked in none), and in no high-dose dogs. Focal gliosis was present occasionally in the medulla of Cyanokit-treated dogs and was interpreted to represent small foci of necrosis ~~that had~~ already largely resolved; this is occasionally observed in control (untreated) laboratory beagles. The absence of gliosis in the vehicle group may have reflected their early death (before healing).

Minimal to mild infarcts were present in the kidneys of three animals that were euthanized on Day 15 (Animals 121 and 169 from the 150 mg/kg group, and Animal 151 from the vehicle treated group), and were considered related to hypoxia, but may have also been related to the microtip catheter that was present in the aorta. Lesions in other organs were considered typical of spontaneous findings in laboratory beagles.

Toxicokinetics: Venous blood samples were collected for cyanide, cyanocobalamin, and total cobalamins at T0, A0, A4,A7.5, A11, A15, A20, A30, A60 and A120 minutes and A24 (24 hours after the end of the hydroxocobalamin or vehicle infusion.

Blood Cyanide Analysis:

The concentration of cyanide at the time of start of vehicle or hydroxocobalamin treatment was equivalent between the dose groups. At the end of the cyanide infusion period, exposure to potassium cyanide was comparable between dose-groups based on whole blood mean (± SD) Cmax values (vehicle, 128 ± 19 nmol/mL; 75 mg/kg, 120 ± 34 nmol/mL; 150 mg/kg, 114 ± 28 nmol/mL). These cyanide concentrations resulted in similar cardiovascular deterioration and time to apnea in all groups.

At the end of the hydroxocobalamin infusion, the whole blood cyanide concentration had decreased to 30 to 40 nmol/mL while in the non-treated vehicle group cyanide concentrations remained at levels of about 70 nmol/mL. Thus, the concentration at the end of hydroxocobalamin infusion in treated dogs was about 50% lower than in vehicle dogs. This is in accordance with results from a study in rabbits chronically poisoned with potassium cyanide, which showed that hydroxocobalamin complexes free cyanides continuously in vivo, resulting in the disappearance of free cyanide from the blood, the urine, and the organs.

In parallel to the initial decrease in cyanide, the considerable amounts of cyanocobalamin formation were detected. The mean C_{max} of plasma cyanocobalamin was 27% higher in dogs receiving the 150 mg/kg dose of hydroxocobalamin compared to the dogs receiving the 75 mg/kg dose. Cyanocobalamin obtained a mean C_{max} (±SD) of 78.1 ± 10.5 µM after 7.5 minutes in the low-dose group, and a mean C_{max} of 99.3 ± 15.4 µM after 4 minutes in the 150 mg/kg dose group. Individual C_{max} values of total cobalamins-(III) usually occurred at the end of hydroxocobalamin infusion, i.e., at 7.5 minutes. The mean concentrations (±SD) at the end of infusion were 424 ± 7 6 µM (low-dose hydroxocobalamin) and 904 ± 127 µM (high-dose hydroxocobalamin), indicating approximate dose linearity in this dose range.

The cyanocobalamin amount formed during the dog efficacy study after hydroxocobalamin administration has been roughly estimated to represent detoxification of about 2 hours of the cyanide burden. The pathophysiological significance of the above is clearly reflected by the mortality data in the different dose-groups. Within the first 4 hours after potassium cyanide poisoning, approximately 60% (n=10) of the vehicle dogs died, whereas only 5% (n=l) of the dogs treated with 75 mg/kg dose of hydroxocobalamin died. No dog treated with the 150 mg/kg dose of hydroxocobalamin died within 4 hours of potassium cyanide poisoning.

Plasma Total Cobalamins-III and Cyanocobalamin:

Mean plasma Cmax values were similar across treatment group, indicating similar exposures to cyanide in all groups (Cmax values: vehicle, 128 ± 19 nmol/mL; 75 mg/kg, 120 ± 34 nmol/mL; high-dose, 114 ± 28 nmol/mL). By the end of hydroxocobalamin infusion, cyanide concentrations decreased to 41.1 and 29.2 nmol/mL in the 75 and 150 mg/kg dose groups, respectively, while in the saline group cyanide concentrations decreased only to 70 nmol/mL. Thus, C_{EOI} (end of infusion) in treated dogs was about 50% lower than in vehicle dogs. Correspondingly, the mean cyanide $AUC_{-0.1\ to\ 2h}$ in Cyanokit treated dogs was reduced by 59% (in the 75 mg/kg group) and 73% (in the 150 mg/kg group) compared to the vehicle group.

In parallel to the initial decrease in cyanide, cyanocobalamin concentrations increased. However, rather than a 2-fold increase of cyanocobalamin that one might expect from treatment with 2-fold amount of hydroxocobalamin, the mean C_{max} and AUC_{0-2h}, of cyanocobalamin was only 27% and 15% higher, respectively, in dogs receiving 150

mg/kg hydroxocobalamin compared to the dogs receiving the 75 mg/kg hydroxocobalamin. The table below was reproduced from the sponsor's submission:

Reviewer's Comments: Samples were only collected for a 2 hour period. A more comprehensive study would have facilitated better extrapolation to humans.

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

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2.6.2.3 Secondary pharmacodynamics

Study title: **Hydroxocobalamin (Cyanokit®): NO-Trapping as Cause of Haemodynamic Effects in Anaesthetized Rabbits (EMD 415722)**

Key study findings: Hydroxocobalamin infusion produced moderate hemodynamic effects in the anesthetized rabbit, including increased in blood pressure, decreased cardiac output, and increased systemic vascular resistance. Although both L-NAME and angiotensin II increased blood pressure alone, the increase in blood pressure produced by hydroxocobalamin infusion was blocked by pretreatment with the NO-synthase inhibitor, L-NAME, but not angiotensin II. These findings support the hypothesis that the blood pressure increases and cardiovascular changes following infusion of hydroxocobalamin are due to the production of nitric oxide.

Study no.: PhD/0001
Vol. 3, Tab 4.2.1.2.1, PhD/0001
Conducting laboratory and location: Cardiometabolic Research Darmstadt Merck KGaA
Date of study initiation: November 30, 2005
GLP compliance: Unspecified
QA report: no
Drug, lot #, and % purity:

Hydroxocobalamin, batch 2059 (exp. date 8/2005) and batch 2066 (exp. Date 4/2006)
Vehicle: physiological saline
L-NAME: [redacted], Prod. No. N-5751
Angiotensin II: [redacted], H-1705, Lot 0570829, in saline containing 0.1% bovine serum albumin.
Fentanyl: Janssen, Reg. No. 6762282.01.00
Pancuronium bromide: Organon, Reg. No. 6077468.00.00
Pentobarbital sodium: Narcoren, Reg. No. 6088986.00.00

b(4)

Methods: Previous findings have suggested that high dose hydroxocobalamin infusion resulted in an in crease in blood pressure in the dog model. To further characterize the potential for hydroxocobalamin to alter blood pressure, the drug (75 mg/kg/ 15 min, IV) was infused into anesthetized (fentanyl followed by pentobarbital sodium-Narcoren) male White New Zealand rabbits both with an without prior treatment with an endothelial NO-synthase inhibitor, L-NAME (L-N^{ω}-nitro-L-arginine methyl ester, 30 mg/kg, IV) or angiotensin II (0.05 ug/kg/min, IV).

Results: Infusion of hydroxocobalamin alone resulted in increased MAP (mean arterial pressure, +17%), increased TPR (total peripheral resistance, +29%), decreased CO (cardiac output, -9%), and small reduction in HR and SV (heart rate and stroke volume, -

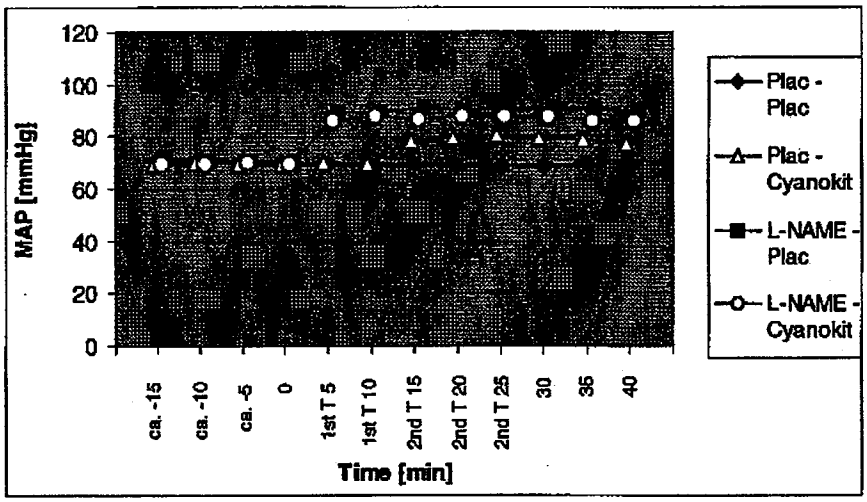
5%). An equal volume of solvent alone did not alter MAP or HR; however, CO and SV increased (+8%, +9%, respectively) and TPR decreased (-7%).

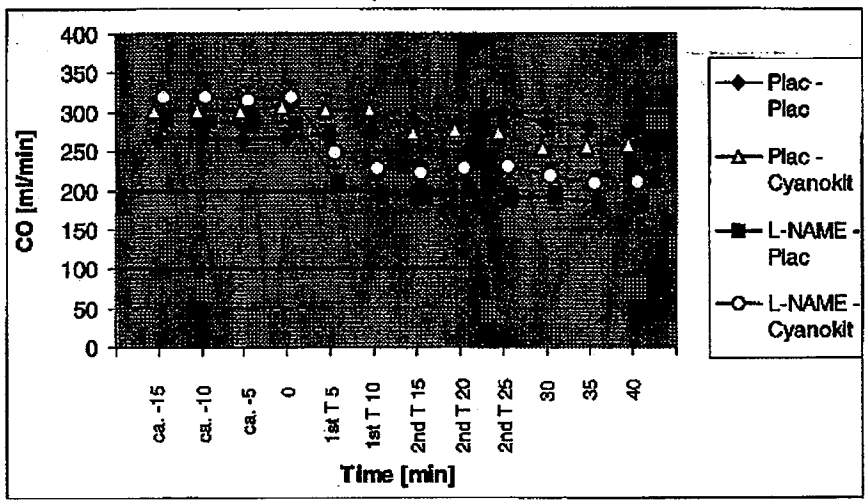
Infusion of L-NAME (30 ug/kg/min, IV) alone produced increased MAP (+26%) and TPR (+77%), decreased CO (-28%), SV (-24%) and HR (-6%). Pretreatment with L-NAME antagonized the effects of hydroxocobalamin.

Infusion of angiotensin II increased MAP (+23%) and TPR (+31%), decreased CO (-6%), decreased SV (-7%), but did not change HR. Angiotensin II did not inhibit the cardiovascular changes following hydroxocobalamin infusion.

The following figures were reproduced from the sponsor's submission:

Figure 1 **Haemodynamics in Anaesthetized Placebo Treated Rabbit (Plac – Plac) or in Rabbits Treated with L-NAME (L-NAME - Plac), Cyanokit® (Plac – Cyanokit) or L-NAME and Cyanokit® in Combination (L-NAME – Cyanokit)**





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