SCIENTIFIC DISCUSSION

1. Introduction

Problem statement

- **Cyanide Poisoning, toxicity**

The most toxic cyanide forms are hydrogen cyanide (HCN, hydrocyanic acid) and its sodium and potassium salts, as well as cyanogen or dicyanogen and its halides. Cyanogens are more of an irritant than cyanides.

The possible use of cyanide as an agent of terror is addressed in the EMEA/CPMP guidance document (EMEA/CPMP/1255/03) on the use of medicinal products for the treatment of patients exposed to terrorist attacks with chemical agents.

Cyanide poisoning can occur following inhalation, ingestion, or contact of cyanide with the skin or mucus membranes. The main exposure route is by inhalation of volatile cyanides, particularly threw fire smoke for which hydrogen cyanide significantly contributes to toxicity. Cyanide poisoning can also occur following industrial accidents. Regarding the two other routes, absorption through intact skin or mucous membranes following dermal or oral exposure is good and systemic toxicity can be expected.

Cyanide and cyanogens are cellular poisons binding to enzymes, mainly metallo-enzymes, with high affinity for Fe^{3+} and consequent cytochrome oxidase inhibition, thus blocking the aerobic respiration in the mitochondrial oxidative phosphorylation of the respiratory chain, and leading to lactic acid accumulation. Cyanide has also been shown to bind to N-methyl-D-aspartate receptors and nitric oxide synthase, resulting in increased production of the vascular relaxant, nitric oxide (NO), which likely contributes to the hypotension typically observed in victims of cyanide poisoning.

The major pathway of endogenous detoxification is conversion, by means of thiosulfate, into the less toxic compound thiocyanate which is then excreted in the urine. Minor routes of elimination are excretion of hydrogen cyanide through the lungs and binding to cysteine or hydroxocobalamin (vitamin B12). But they are insufficient at toxic amounts of cyanide.

- **Clinical symptoms**

Symptoms of cyanide poisoning appear within seconds to minutes after inhalation and a number of immediate fatalities may be expected. In these severe cases, clinical signs of hyperventilation, via direct stimulation of the respiratory centre and metabolic acidosis, are followed by loss of consciousness with convulsions and ultimately cardiovascular collapse and/or respiratory arrest. Other clinical signs include dizziness, weakness, palpitations, and anxiety, followed by dyspnoea, pulmonary oedema, confusion, ataxia, and paralysis. Cyanogens are also respiratory irritants, and may cause pulmonary effects if the victim survives asphyxia. After oral administration, symptoms may be delayed for about 30 minutes, sometimes more.

Cyanide concentrations less than 8 µmol/L (0.02 mg/L) are associated with no symptoms, flushing and tachycardia appear for concentrations from 20 to 40 µmol/L (0.5 to 1 mg/L). Poisoning has been reported with concentrations above 40 µmol/L, coma and respiratory depression are reported for concentrations from 100 to 120 µmol/L (2.5 to 3 mg/L), and fatal outcome for concentrations greater than 120 µmol/L (3 mg/L). Time for occurrence of symptoms and death decreases with increasing blood cyanide concentrations. However, a serious drawback to the use of plasma cyanide determinations in the assessment of poisoning is the pronounced instability of cyanide in plasma.
In case of fire smoke inhalation, patients present with soot around the mouth, nose, and/or oropharynx. Intoxicated patients also usually present with altered neurological status. Neurological signs are a

sensitive but not specific indicator of cyanide intoxication, but in combination with hypotension are highly suggestive of cyanide intoxication. It has to be noted that the clinical picture of this type of intoxication is highly polymorphous and cyanide intoxication is often associated with carbon monoxide intoxication, for which clinical symptoms are quite similar. According to animal data, cyanide and carbon monoxide could have additive and synergistic toxicity. In humans, the presence of other toxicants such as carbon monoxide may contribute to lethal outcomes in the presence of even smaller blood cyanide concentrations.

A plasma concentration of lactate ≥ 10 mmol/l can be used as a sensitive but non-specific marker of cyanide poisoning.

- **Treatment management for Cyanide Poisoning**

As there is no widely available, rapid cyanide blood assay, the presence and amount of cyanide are often initially unknown. Treatment decision must therefore be made on the basis of clinical history and/or signs and symptoms of cyanide intoxication. As cyanide poisoning is associated with early toxicity and possible death and neurological sequelae, treatment should be initiated as soon as possible. Conventional treatment of cyanide poisoning includes decontamination, supportive and specific treatment. Management of the intoxication is determined by its severity. In mild poisoning, oxygen, reassurance of the subject and bed rest without antidote, can be sufficient.

First aid measures is decontamination that should be adapted to the exposure route:
In case of inhalation, the patient must be removed from the contaminated environment/atmosphere; as dispersion of the gas is very rapid, decontamination is usually not necessary. In case of dermal exposure, careful removing of clothes and thorough washing of the skin with water and soap is necessary. In case of oral poisoning, if the patient is stabilised and in the rare cases when almost immediate measures can be applied, the administration of activated charcoal is recommended as a primary method for gastrointestinal decontamination. Decontamination should never postpone supportive treatment.

Protective measures such as masks and protective clothes are necessary for rescuers.

Symptomatic Treatments include:
- supportive care including oxygenation with 100 % O_2 that is the basic treatment of such intoxication, and if necessary, mechanical ventilation (mask or/and intubation);
- management of seizures by anticonvulsants according to standard protocols;
- arrhythmias management according to standard protocols;
- hypotension management by vasopressors as by standard protocols;
- metabolic acidosis management as by standard protocols.

Antidote treatments are available in Europe:
Nevertheless, non-clinical and clinical information on these compounds is rather limited, and medical practice is not standardized across European countries and is based on availability of a specific antidote and medical practice. Frequently, the simultaneous or successive administration of different antidotes has been recommended.

The European guidance document (EMEA/CPMP/1255/03) *"on the use of medicinal products for the treatment of patients exposed to terrorist attack with chemical agents"* summarizes properties of the different antidotes, which can be categorized into 3 groups:

- Complexation agents that act by direct binding of the cyanide ions. Two products are available:

- Dicobalt edetate which is an efficient complexation antidote to cyanide; but its use should be restricted to cases when the diagnosis of cyanide poisoning is certain and only for severe poisoning, since it has serious cardio-vascular adverse effects in the absence of cyanide.

- Hydroxocobalamin which is considered to be the best choice antidote, if available, as it is experimentally well documented with clear advantages in situations such as fires with concomitant exposure to agents that reduce oxygen transport, such as carbon monoxide.

- Sulfur supplying agents which act by promoting conversion of cyanide to thiocyanate, thus enhancing the physiological cyanide detoxification process:

- Thiosulphate is the main product in this class; it should be considered together with the other cyanide antidotes in sequential treatment (but should no be administered as a mixture at the same time as hydroxocobalamin), as it is a rather slow acting agent.

- Methaemoglobin inducers which act by complexation of cyanide to methaemoglobin:

- The agents belonging to this class include sodium nitrite, amyl nitrite and 4-dimethylaminophenol. Due to the risk of excessive methaemoglobinemia, they should be used in case of non-availability of hydroxocobalamin or dicobalt edetate.

About the product

Hydroxocobalamin is the biologically active form of Vitamin B_{12}. The mechanism of action is based upon its ability to very tightly bind cyanide ions. The resulting molecule, cyanocobalamin, is a physiologically stable, non-toxic compound that is excreted in the urine.

Hydroxocobalamin was first demonstrated to be effective as a cyanide antidote in 1952 (Mushett). In Europe, it has been authorized in France since May 1996.

Each vial of Cyanokit 2.5 g contains 2.5 g hydroxocobalamin lyophilizate is recommended to be reconstituted with 100 mL of sterile saline (0.9 % NaCl) solution, or other diluents as Lactated Ringers Solution, 5 % dextrose, which have also been found to be compatible with hydroxocobalamin.

The proposed therapeutic **indication** for Cyanokit 2.5 g was:

"Treatment of known or suspected cyanide poisoning."

Scientific Advice

No request for Scientific Advice was received at the EMEA.

2. Quality aspects

Introduction

Cyanokit is a sterile, lyophilised powder for solution for intravenous infusion and contains 2.5 g of hydroxocobalamin as active substance. Each vial of Cyanokit is recommended to be reconstituted with 100 mL of sterile saline (0.9% NaCl). Other diluents (i.e., Lactated Ringers Solution, 5% dextrose) have also been found to be compatible with hydroxocobalamin. The reconstitution diluent is not provided in the final marketed product.

The only excipient is Hydrochloric acid.

The primary container consists in a single use Type II glass vials closed with a bromobutyl rubber stopper and an aluminum cap with a plastic lid.

The proposed final marketed kit consists of:

- Two closed glass vials packed.
- Two sterile transfer spikes allowing the transfer of the diluent into the vial for reconstitution of the lyophilisate (sterile medical device with CE marking)

- One sterile IV infusion set (sterile medical device with CE marking)
- One sterile short catheter for administration to children (sterile medical device with CE marking)

Active Substance

Hydroxocobalamin, the hydroxylated active form of vitamin B_{12}, is a large molecule in which a cobalt ion is coordinated in 4 positions by a tetrapyrrole ring. It is a hygroscopic, odourless, dark red crystalline powder that is freely soluble in water and ethanol, and practically insoluble in acetone and diethyl ether.

Information on hydroxocobalamin has been supplied in the form of an ASMF.

- Manufacture

The manufacturing process is carried out in two stepsAdequate In-Process Controls are applied during the synthesis of the drug substance. Control methods for intermediate products, starting materials and reagents, have been presented.

Batch analysis data batches from the manufacturer are presented and confirm consistency and uniformity of the manufacturing process.

- Specification

The active substance specification includes tests for appearance, identification of the active substance (IR, HPLC), identification of cobalt, assay (HPLC, UV), related substances (HPLC), residual solvents (GC), acetates, chlorides, loss on drying (Ph Eur), pH, bacterial endotoxin (Ph Eur), microbial contamination (Ph Eur).
Batch analysis data of 3 commercial batches of active substance are provided. The results are within the specifications and consistent from batch to batch.

- Stability

Stability studies were performed on three batches of a satisfactory size according to ICH program).
Results from long term storage at 5ºC±3ºC for 24 months and accelerated storage, 6 months at 25ºC±2ºC 60%RH±5%, were performed. Loss on drying, assay by UV spectroscopy and related substances were tested on stability samples
The results support the proposed re-test and storage conditions.

Medicinal Product

- Pharmaceutical Development

The development of the manufacturing process was guided by the physicochemical properties of the active substance. Cyanokit is prepared by an aseptic process, as the stability of hydroxocobalamin is not compatible with a heat sterilisation process. The sterile solution is then freeze-dried, as the lyophilisate form significantly improves the stability of hydroxocobalamin compared with a liquid injectable preparation.
The manufacturing process development was focused on the critical steps of the process. Compatibility studies were performed in order to determine the compatibility of Cyanokit with different drugs and diluents for IV infusion available in Europe and/or on the US market.
All formulation excipients comply with Ph Eur specifications.
Cyanokit is packaged in a type II colourless (clear) glass vial closed by a gray bromobutyl rubber stopper and an aluminum cap with a plastic lid (flip-off). The containers/closures used are composed of materials compatible with the lyophilisate and compliant with European Pharmacopoeia requirements and certified suitable for food use. The suitability of the container-closure is based on

stability studies where no interactions between the product and the container-closure system were detected.
The Kit contains two sterile transfer spikes allowing the transfer of the diluent into the vial for reconstitution of the lyophilisate, one sterile IV infusion set, and one sterile short catheter for administration to children. They are covered by an EC marking Certificate and the reconstituted solution is compatible with the IV infusion set in the conditions of use.

The clinical formulation used in the clinical trials is identical with one proposed for marketing.

- Manufacture of the Product

Manufacturing consists of preparation of the bulk solution, followed by prefiltration, sterile filtration and filling, lyophilisation, capping and visual check. Validation studies have been carried out by a number of studies for the major steps of the manufacturing process in 6 commercial batches and is satisfactory. The in process controls are adequate for this pharmaceutical form.

- Product Specification

The drug product specifications include appropriate tests for description, identification (IR, HPLC), tests on lyohilizate (mean mass of vial contents, uniformity of dosage unit, residual humidity %, particulate contamination), tests on reconstitute solution (dissolution, pH), assay%, HPLC), residual substances (Ph Eur), sterility (Ph Eur), and bacterial endotoxin (Ph Eur).

Batch analysis results (n=6) confirm consistency and uniformity of manufacture and indicate that the process is under control. Impurity limits in the specification are justified by toxicology studies

- Stability of the Product

Initial stability studies conducted according to ICH recommendations were performed on 3 batches of Cyanokit. Supportive stability studies conducted according to ICH recommendations were also performed on 2 batches of Cyanokit used for clinical studies. Primary stability data of 3 batches stored according ICH conditions (25°C/60%RH and 30°C/65%RH) for 30 months were provided.

“In-use” stability studies data for 3 batches (performed on reconstituted solution) stored for up to 24 months at 5, 25 and 40°C were also provided.

The parameters tested during stability study are identical with the release specifications.

The influence of light was studied on batch 1 batch of Cyanokit in the primary packaging. The study was performed on the finished product and on reconstituted solution of sterile saline (0.9% NaCl). Vials were exposed to light in the photostability cabinet according to the recommendations of the ICH Guideline Q1B “Photostability testing of new active substances and medicinal products.”

Temperature, humidity, temperature and humidity, oxidation studies were performed with both the reconstituted and unreconstituted product.

Vials were exposed to 3 different thermal cycles simulating the variations of temperature during usual transport , transport in the desert and freezing/defrosting cycles.
As a conclusion from the stability studies, the results indicate satisfactory stability and support the shelf life and conditions of used stated in the SPC.

Discussion on chemical, pharmaceutical and biological aspects

Information on development, manufacture and control of the drug substance and drug product have been presented in a satisfactory manner. The results of tests carried out indicate satisfactory consistency and uniformity of important product quality characteristics, and these in turn lead to the conclusion that the product should have a satisfactory and uniform performance in the clinic. At the

time of the CHMP opinion, there were a number of minor unresolved quality issues having no impact on the Benefit/Risk ratio of the product. The applicant gave a Letter of Undertaking and committed to resolve these as Follow Up Measures after the opinion, within an agreed timeframe.

3. Non-clinical aspects

Introduction

The non-clinical testing strategy was designed to supplement historical non-clinical and clinical data that were published in the literature. New toxicity studies comprised pivotal IV studies in the dog, a standard battery of genotoxicity studies, an *in vitro* phototoxicity study, studies in rats to investigate the toxicity of batches with high levels of impurities and a 14-day repeated dose study in dogs with the detoxification product, cyanocobalamin.

The non-clinical studies specifically conducted in support of this application were carried out in accordance with current guidance and in compliance with the principles of GLP.

However it has been reported that the laboratory used for the validation of the bioanalytical methods for measurement of 'total' and 'free' cobalamins and cyanocobalamin in plasma,'total' cobalamins in human urine, was not fully compliant with GLP principles during the period the studies were conducted. The non-compliance involved inappropriate integration of chromatographic data. The EMEA and all European member states were advised of this inspection conclusion.
Concerned studies were dog efficacy, single dose rat and dog toxicity studies. The review of the bio-analytical data concluded on the absence of impact on the safety factors originally calculated from these studies.

Pharmacology

- Primary pharmacodynamics

In vitro
The rationale for administering hydroxocobalamin as an antidote to cyanide poisoning is based on the high affinity of the cyanide ion for cobalt compounds. Hydroxocobalamin (OHCo) is a natural form of Vitamin B_{12}, each molecule containing a trivalent cobalt ion carrying a hydroxo ligand. It stoichiometrically binds cyanide ions by substituting the hydroxo ligand to form cyanocobalamin. This reaction is supposed to occur primarily in blood.

$$OHCo + HCN \rightleftharpoons CNCo + H_2O$$

One study performed *in vitro* on human cyanide-loaded fibroblasts showed that OHCo can penetrate in the cells and complex cyanide to form CNCo. Cyanocobalamin is very stable and is excreted in the urine.

In vivo
A primary pharmacodynamic study in cyanide-poisoned dogs was conducted at 75 mg/kg and 150 mg/kg to support the efficacy of hydroxocobalamin in the proposed indication. The selection of the animal model for the efficacy study was based on pharmacodynamic and pharmacokinetic similarities between dog and man as well as some practical considerations such as blood volume to be withdrawn to accommodate all relevant pharmacodynamic and PK endpoints.
Cyanide poisoning may result from exposure to smoke from closed space fires, inhalation, ingestion or dermal exposure. In the clinical setting, particularly for the treatment of individuals exposed to smoke, exposure to cyanide occurs mainly via inhalation. However in the dog efficacy study, cyanide was administered intravenously.
The choice of route was justified based on the observation that cyanide poisoning by all routes results in similar signs and symptoms, as well as a similar distribution pattern, although the rate of onset of poisoning differs and is also dose-dependent. The intravenous route allowed the most controlled administration to produce defined apnoea and continue infusion for a further 3 minutes to ensure

potentially lethal exposure. Oral absorption is variable and would allow less control of the study, and administration by inhalation presents even more practical difficulties.
Mortality data showed that the survival of Cyanokit-treated animals 4 hours and 15 days after cyanide-poisoning was increased compared to vehicle-treated ones. Survival at day 15 was superior in the high-dose group (100%) compared to the low-dose group (79%). Furthermore, Cyanokit treatment allowed a more rapid recovery of cardiovascular parameters and respiratory function. A protective effect against cyanide-induced brain lesions in terms of incidence and severity was demonstrated at 150 mg/kg, and partially shown at 75 mg/kg.
No lesions were interpreted to be related to hydroxocobalamin administration at the histopathological examination.
Kinetic data support the proposed mechanism of action for hydroxocobalamin, *i.e.* cyanide trapping. In Cyanokit-treated animals, the initial decrease in cyanide concentration paralleled the increase in cyanocobalamin concentration.
Overall, both the 75 mg/kg and 150 mg/kg dose levels were effective in the treatment of cyanide poisoning in dogs. The therapeutic dose proposed in humans (5.0 g, equivalent to 75 mg/kg) is in the range of the effective doses tested in dogs.

- Secondary pharmacodynamics

Hydroxocobalamin was shown to possess a NO-scavenging property in anesthetized rabbits treated by IV route. This resulted in increased mean arterial blood pressure and total peripheral resistance, together with decreased cardiac output. An increase in blood pressure has also been observed in treated patients (see clinical safety). Cyanocobalamin, the reaction product of hydroxocobalamin with cyanide, did not possess this NO-scavenging property.

- Safety pharmacology programme

No special safety pharmacology study was performed. However, the effect of hydroxocobalamin on various parameters related to cardiac function was evaluated in repeated-dose toxicity studies conducted in dogs. The results showed that ECG-parameters such as heart rate, PQ-, QRS- and QT-interval (including QT-correction according to Van de Water) were not influenced by hydroxocobalamin treatment. Considering the CNS and the respiratory system, specific safety pharmacology studies were neither conducted. However, in the efficacy study in cyanide-poisoned dogs, the recovery of cardiovascular parameters and respiratory function was more rapid in Cyanokit-treated animals than in control ones. Additionally, a protective effect against cyanide-induced brain lesions in terms of incidence and severity was shown in Cyanokit-treated dogs.

- Pharmacodynamic drug interactions

No studies have been conducted

Pharmacokinetics

Methods of analysis
Hydroxocobalamin reacts with plasma constituents to form various cobalamin-(III) complexes. A rapid ligand exchange was shown with coordinative amino acid residues such as histidine and cysteine to form high molecular non-filterable plasma protein complexes, and with low molecular ligands such as thiols, histidine and thiocyanate forming so-called “free cobalamins-(III)”. Such coordinative ligand exchanges are known to be equilibrium reactions, which proceed much more slowly than the usual non-covalent protein binding of drugs. The HPLC-UV method developed by the applicant to determine free cobalamins-(III) and total cobalamins-(III) levels is considered properly validated. It is based on the property of cobalamin complexes to form cyanocobalamin quantitatively in the presence of excess cyanide. Additionally, the HPLC-UV method developed to determine cyanocobalamin levels after the administration of cyanocobalamin or its formation in vivo (pharmacological study in cyanide-poisoned dogs) is also considered validated.

Absorption

No studies were conducted and are not needed as Cyanokit is administered intravenously.

Distribution
The distribution of hydroxocobalamin is influenced by coordinative binding to proteins, with the hydroxo ligand being replaced by histidine and thiol groups of proteins to form cobalamin-(III) complexes. Equilibrium is reached within 1 to 2 hours after infusion of hydroxocobalamin. The free fraction of cobalamin-(III) varies between species (5% in humans, 16% in dogs and 24% in rats). Although tissue distribution was not specifically studied, indirect evidence suggests that distribution follows the physiological organs of storage of vitamin B12 which are the liver, kidneys and nervous system.

Metabolism
The binding of hydroxocobalamin to proteins may be regarded as reversible metabolism. Hydroxocobalamin also reacts with cyanide thereby forming cyanocobalamin. This complex is highly stable and is therefore regarded as a physiological end product of hydroxocobalamin especially during cyanide intoxication.

Excretion
Both hydroxocobalamin and cyanocobalamin are excreted mainly via the kidneys in dogs. In human volunteers, hydroxocobalamin was also excreted mainly via the renal route (58-74% of total clearance). The mean half-lives for 'free' cobalamins were 3h in the rat, 6h in the dog and 28h in humans. For cyanocobalamin, the half-lives were 0.3 to 0.5h in rats, 0.8 to 1h in dogs and 9.3 ± 3.2 hours in humans.

PK drug interactions
No studies were conducted. Due to the high molecular weight of hydroxocobalamin, interaction with drug metabolising enzymes such as CYP P450 is unlikely to occur. In addition, since hydroxocobalamin is intended for single administration, induction studies were also considered unnecessary.

Toxicology

- Single dose toxicity

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

- Repeat dose toxicity (with toxicokinetics)

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

Single cell necroses, affecting mainly macrophages, were observed at all dose levels in the bone marrow. However, they did not impact either the cellularity of the bone marrow, or the bone marrow functioning. Moreover, histopathological examination in the 4-week toxicity study showed a trend towards recovery in terms of incidence and severity.
Adverse effects were observed in other organs/tissues in 3-day and 4-week studies. In the 3-day toxicity study, heart alterations occurring at the top dose of 1200 mg/kg/day and spleen alterations observed at 600 and 1200 mg/kg/day were attributed to redistribution of plasma water. In the 4-week study, heart and spleen findings were also observed and were considered to result from a non specific inflammatory reaction and from the overload phenomenon, respectively.

Taking into consideration the nature and reversibility of the effects reported in the single dose study, a single dose of 300 mg/kg is considered as well tolerated in dogs.
With the exception of liver fibrosis reported at 300 mg/kg in the 4-week study, all the treatment-related findings observed in repeat-dose studies were either fully reversible or showed a trend to recovery after 8 weeks of treatment-free period. Liver fibrosis likely results from the inflammatory reaction reported after 4-week treatment and as a sequel of the observed sinusoid edema related to redistribution of plasma water and overload phenomenon. Therefore, the risk of hepatic fibrosis resulting from the therapeutic use of Cyanokit seems unlikely. In view of the nature of the adverse effects reported at ≤150 mg/kg/day, of their full or on-going reversibility after a 8-week recovey period, and of the toxicological concerns in patients arising from cyanide-poisoning itself, the dose of 150 mg/kg/day can be considered as a NOAEL.

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

- Genotoxicity

Hydroxocobalamin was non-genotoxic in a standard battery of *in vitro* (Ames tests, $TK^{+/-}$ mouse lymphoma assays) and *in vivo* (rat micronucleus) tests.

- Carcinogenicity

Carcinogenicity studies have not been performed. This is considered acceptable in the absence of genotoxic potential and because Cyanokit will be administered once (or at most twice) as an IV infusion over 15 minutes.

- Reproduction Toxicity

Only limited embryo-foetal toxicity studies are available in rats and rabbits, and do not allow to draw any clear conclusion on the embryotoxic potential of hydroxocobalamin. However, it should be noted that no teratogenic effect was reported in both species, which is adequately reported in the SPC section 5.3. In order to complete the knowledge in this area, the applicant committed to perform GLP embryo-fetal toxicity studies in rats and rabbits according to current standards as a follow-up measure.
No studies on fertility and on peri/postnatal development were performed, which is clearly reported in the SPC sections 4.6 and 5.3. Offspring's studies (dosing of juvenile animals) have not been conducted either.

- Toxicokinetic data

Toxicokinetics of hydroxocobalamin were studied in rats and in dogs after single administration. In dogs, the AUCs of free cobalamins-(III) and total cobalamins-(III) increased proportionally to the dose. Mean Cmax measured for free- and total cobalamins-(III) were 1 to 5 fold higher than those measured in humans treated with 5.0 and 10.0 g hydroxocobalamin. Terminal half-lives reached

approximately 6 and 8 hours for free and total cobalamins-(III), respectively in dogs. Corresponding figures in rats amounted to 3 and 5 hours. In dogs, the clearance of total cobalamins-(III) (0.064 to 0.083 L/h/kg) was 6-7 fold lower than clearance of free cobalamins-(III).

In rats and dogs administered cyanocobalamin intravenously, TK data show that AUC increased in a dose-related manner, but less than dose proportionally. This is partly due to the increased clearance at higher doses. Cyanocobalamin did not accumulate after repeated administrations in both species. This is in agreement with the short half lives calculated in rats and in dogs for cyanocobalamin.

- Local tolerance

Local tolerance was evaluated in dog toxicity studies. No clinical or histopathological treatment-related effect was noted at the injection sites.

- Other toxicity studies

Studies on impurities
Batches of drug product containing impurities at a level up to 10.2% (after storage in stressed conditions) were no more toxic in an acute IP study in female rats than a batch with 2.3% impurities, with similar findings and LD_{50} values. These batches were also negative in an Ames test. The results of further qualification studies will be submitted in post-authorisation (follow-up measure). In addition, as all impurities are cobalamin-related products, there is no concern for the clinical use of the therapeutic batches.

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

Ecotoxicity/environmental risk assessment

As a vitamin, hydroxocobalamin is unlikely to result in a significant risk to the environment and an environmental risk assessment is not necessary. Nevertheless the Applicant provided a calculation of the predicted environmental concentration (PEC) for hydroxocobalamin in the aquatic compartment based on a refinement of the marketing penetration factor resulting in a refined PEC value below the 0.01µg/l threshold. However, it should be mentioned that any PEC refinement at this stage is not acceptable unless it is based on published data, e.g. epidemiological studies or medicine statistics.

Discussion on the non-clinical aspects

In the dog efficacy model, Cyanokit significantly improved survival following cyanide administration, and produced a more rapid recovery of cardiovascular and respiratory parameters compared with controls.
Hydroxocobalamin binds coordinatively to plasma proteins and also reacts with cyanide forming cyanocobalamin which is a highly stable complex. In dogs and in humans, both hydroxocobalamin and cyanocobalamin are mainly excreted in the urine.
Hydroxocobalamin has a low acute toxicity. The main target organs of toxicity in repeat-dose studies were the liver, kidney and bone marrow, with findings suggestive of tissue overload with high quantities of hydroxocobalamin. Similar findings were seen in a repeated dose study with cyanocobalamin.
No genotoxic potential have been identified in a standard tests battery. The carcinogenic potential was not studied which is acceptable in view of the toxicological profile and the indication.

Data on the reproductive potential are limited and this is reflected adequately in the SPC. In order to complete the knowledge in this area, embryo-foetal studies (rats and rabbits) will be performed as a post-authorisation commitment (follow-up measure).
No significant risk to the environment is expected.

4. Clinical aspects

Introduction

Because of ethical considerations, no placebo-controlled clinical study of the efficacy of hydroxocobalamin in acute cyanide poisoning was performed. To overcome this lack, a primary pharmacodynamics study was performed in cyanide-exposed dogs to support the efficacy of hydroxocobalamin in the proposed indication. (See section non-clinical aspects)

The clinical dossier comprises 1 safety study with pharmacokinetics (PK) evaluation as secondary objective, and 4 non comparative efficacy studies (one prospective study and three retrospective reports) in the contexts of suspected cyanide-poisoning due to fire smoke inhalation and of known cyanide-poisoning.

Overall, 347 subjects were exposed to hydroxocobalamin in submitted clinical studies; 102 were healthy volunteers who received doses comprised between 2.5 and 10 g; and 245 were subjects with suspected or known cyanide-poisoning.

GCP

The Clinical trials were performed in accordance with GCP as claimed by the applicant.
The 3 retrospective reports did neither require submission to an Ethics Committee nor signed informed consent from subjects.

Pharmacokinetics

Hydroxocobalamin is intended for the treatment of cyanide intoxication (known or suspected) in emergency situation. The IV route is therefore the most appropriate as it allows a rapid disposition of the drug.
The sponsor has conducted a single-ascending dose study in healthy volunteers (study EML 015722-H101) and has provided sparse data in patients from published literature.

Analytical methods
An HPLC-UV method was employed and the non-protein-bound cobalamin-(III) is referred to as free-cobalamins-(III). Total cobalamin-(III) referred to the sum of free and protein-bound fraction. The analytical technique is adequately documented and a full validation report is provided. However, comparison of data obtained in healthy volunteers from the study performed by the applicant with published data from literature should be made cautiously.

Study EML 015722-H101

Methods

This study investigated the PK behaviour of hydroxocobalamin after administration by IV route in adult healthy volunteers This was a double blind, placebo controlled, single-ascending-dose study, with a 4-week follow-up, of the safety, tolerability and PK of 4 intravenous doses (2.5, 5, 7.5 and 10 g) of hydroxocobalamin in healthy volunteers. The primary objective was to test the safety and tolerability of the four single intravenous doses and the second objective was the determination of the pharmacokinetics of the free and total cobalamins-(III) in plasma and the total-cobalamin-(III) in the urine in a subgroup of 9 subjects (for the 2.5, 5 and 7.5 g doses) and 12 subjects (10 g dose). For all doses the infusion rate of hydroxocobalamin was 1g/3 minutes. Plasma concentrations were

adequately monitored over a 96 h time period (19 time points). Urine samples were collected over a 72 h time period post dosing.

Results

- Distribution

Following IV infusion of 2.5, 5, 7.5 and 10 g of hydroxocobalamin at a constant rate of approximately 1 g/3 minutes, free cobalamin-(III) reached maximum concentrations generally at the end of the infusion. Fast complexation of hydroxocobalamin with plasma proteins is suggested by the findings of the study as Tmax observed for the free-cobalamins-(III) is very close to that observed with total-cobalamins-(III).

The volume of distribution at steady-state (Vss) for both free and total cobalamins-(III) is not dependant upon the administrated dose. Vss ranged from 280.7 up to 349.5 L for the free cobalamins-(III) and from 21.8 up to 25.6 L for total cobalamins-(III). This could be explained by the rapid distribution of free-cobalamins-(III) into tissues.

- Elimination

Cobalamins (free and total) are slowly eliminated from plasma as the apparent plasma elimination half-live is approximately 30 h. The Cmax and AUC of both free and total cobalamins evolve proportionally to the dose over the studied range (2.5 up to 10 g). Cmax values ranged from 73.1 up to 197.2 µg eq/ml for free-cobalamins-(III) and from 287.6 up to 995.3 µg eq/ml for total cobalamins-(III). AUC0-t values ranged from 188.4 up to 762.5 µg eq/ml*h for free cobalamins-(III) and 3566 up to 14271.5 µg eq/ml*h. Estimated from AUC ratios, the systemic exposure to free cobalamins-(III) is approximately 5 % that of total cobalamins-(III).

- Excretion

Kidney is a major route of elimination as up to 74 % of the administered dose is recovered in the urine. The total body clearance (CL) of free cobalamins-(III) ranged from 12.5 up to 13.2 L/h , which exceeds the normal glomerular filtration rate (approximately 4.8 up to 7.9 L/h).. This high clearance may be due to the extensive binding of free cobalamins to plasma proteins. Clearance of total cobalamins-(III) ranged from 0.566 to 0.645 L/h across all doses.

Overall, the PK profile obtained is well characterized with a linear PK behaviour of hydroxocobalamin over the range of the tested doses (2.5 to 10g).

However, the PK behaviour in cyanide-intoxicated patient may be quite divergent from that observed in healthy volunteers as hydroxocobalamin reacts with cyanide. The lack of PK investigation in the patients is considered acceptable with views of ethical and feasibility constraints.

Additional sparse data from literature are reported in the application documentation. Among them studies performed by Houeto et al. (1995), Houeto et al. (1996) and Astier and Baud (1995) are of interest. These studies evidenced the formation of cyanocobalamin formation in patients exposed to cyanide. A correlation between blood cyanide levels and cyanocobalamin is suggested by these studies. The concentrations of cyanocobalamin in humans, following high cyanide exposure and subsequent IV administration of 5 g of hydroxocobalamin, were in the range of 212.4 ± 30.9 µmol/L (282±41 µg eq/mL) in Houeto et al. (1996) study and 227 ± 95 µmol/L (302±126 µg eq/mL) in Houeto et al. (1995) study.

- Special populations

There are no known differences in the PK of hydroxocobalamin in older *versus* younger subjects. The basis for paediatric recommendations is discussed further. (See further sub-section “special populations”).

- Pharmacokinetic interaction studies

No interaction studies with other medicinal products have been performed.

Pharmacodynamics

- Mechanism of action

Hydroxocobalamin is a natural form of Vitamin B_{12} that was first demonstrated to be effective as a cyanide antidote in 1952, and that has been used in clinical practice for more than 30 years.
Hydroxocobalamin is a complexation agent that acts by direct binding of the cyanide ions, resulting in cyanocobalamin which is a highly stable, nontoxic compound that is excreted in the urine.
In addition, increased blood pressure observed in some healthy subjects of the phase I clinical study and results of a non-clinical study performed in anesthetized rabbits suggest an interference of hydroxocobalamin with the NO system.

- Primary and Secondary pharmacology

Pharmacodynamic properties have been studied in non-clinical models. For ethical reasons, a controlled clinical efficacy study on the antidotal properties of hydroxocobalamin against cyanide-poisoning could not be performed.
Therefore, the applicant conducted a primary pharmacodynamic study in cyanide-poisoned dogs to support the efficacy (see section "Non-clinical aspects").

Clinical efficacy

In support of efficacy of hydroxocobalamin in cyanide poisoning, 4 non-comparative clinical studies were submitted as shown in the next table. These studies include:

- 1 prospective efficacy study with additional retrospective safety data collection (Baud study 1), and 2 supportive retrospective studies (Baud study 2 and Fortin study) in situation of suspected cyanide-poisoning consecutive to fire smoke inhalation;
- 1 retrospective study (Baud Study 3) in cyanide exposure from sources other than fire smoke.

Due to ethical aspects linked to potential seriousness of cyanide poisoning, no clinical placebo-controlled study was performed. To overcome this lack, a non clinical study placebo-controlled was performed in dogs as primary evidence of efficacy (see section "Non-clinical aspects").

Table of Clinical studies

Study	Number of Subjects Source of Cyanide Exposure	Study Design	Hydroxocobalamin Dose (g) Median (range)
Prospective study – Main study in fire smoke inhalation			
Baud Study 1	69 (36 ♀/33 ♂) age: 49.6 ± 20 years (adults only) Fire smoke	Phase III, prospective, open-label with subsequent retrospective collection of additional safety data	5.0 (4.0 - 15.0)
Retrospective studies – Fire smoke inhalation (supportive studies)			
Baud Study 2	61 (31 ♀/30 ♂) age: 54.3 ± 18.3 years (adults only) Fire smoke	Retrospective observational	5.0 (2.5 – 15.0)
Fortin Study	101 (48 ♀/53 ♂) age: 47.1 ± 20.7 (range: 2 - 88 years) Fire smoke	Retrospective observational, prehospital use	5.0 (1.0 – 10.0)
Retrospective study – Main study in cyanide ingestion or inhalation			
Baud Study 3	14 (2 ♀/12 ♂) age: 36.3 ± 13.2 (1 15-years old patient, other: adults) Ingestion or inhalation (other than fire smoke)	Retrospective observational	10.0 (5.0 – 20.0)

- Dose response studies

The initial 5.0 g dose of hydroxocobalamin used in the clinical studies was based on *in vitro* data, non-clinical data in cyanide-poisoned dogs and on clinical experience.

- *In vitro* data (Kaczka *et al.*, 1950; Marques HM *et al.*, 1988) showed stoichiometric binding of hydroxocobalamin to cyanide, which would predict that 5.0 g could neutralize a single lethal dose of cyanide. However, a PK study in cyanide-poisoned victims (*Houeto, 1995*) suggested that larger doses of hydroxocobalamin may be required due to less than stoichiometric binding *in vivo*. Additional clinical experience suggested a benefit of a second dose in more serious poisonings.
- The preclinical controlled efficacy study N106342 evaluated 2 doses of hydroxocobalamin, 75 and 150 mg/kg in dogs. The observed increase in survival at 150 mg/kg compared with 75 mg/kg, further supported the assumption of less than stoichiometric binding *in vivo* as the 75 mg/kg would have been expected to neutralize the entire delivered dose of potassium cyanide. The absence of complete stoichiometric binding is probably due to the competitive binding of hydroxocobalamin to (plasma) proteins and other small molecular ligands.
- The safety/tolerance study EML015722-H101 performed in healthy subjects evaluated pharmacokinetics of 4 doses of hydroxocobalamin (2.5, 5, 7.5 and 10 g) as secondary objective. This study revealed the linear PK behaviour of hydroxocobalamin over the range of tested doses, and suggested no major differences in the plasma PK parameters of free and total cobalamins-(III) between men and women.

Regarding dose per body weight or C_{max} of total cobalamins-(III), the 75 and 150 mg/kg doses studied in dogs are approximately equivalent to 5.0 and 10.0 g in humans respectively (see table below). Based on these considerations, a comparable extent of cyanide detoxification as observed in the dog efficacy study is expected in humans.

Table. Comparison of Total Cobalamins-(III) Cmax in Dogs and Humans

Dog Efficacy Study (IV) N106342		Human Safety Study (IV) EML 015722-H101	
Dose, gender	**C_{EOI} [1] (µg eq/mL)**	**C_{max} (µg eq/mL)**	**Dose**
75 mg/kg, Male	542 ±104	579.0 ±112.6	5.0 g
75 mg/kg, Female	592 ±95		
150 mg/kg, Male	1139 ±175	995.3 ±149.1	10.0 g
150 mg/kg, Female	1263 ±145		

[1] C_{EOI} taken instead of C_{max} due to high variability of the latter one

Therefore, based on experimental data and clinical practice, a 5.0 g dose (approximately 70 mg/kg) of hydroxocobalamin appears to be an appropriate starting dose for the treatment of cyanide poisoning in humans. A 2nd dose can be administered, based on poisoning severity and on clinical response.

- Main studies

Two main studies in 2 different situations were provided:
- one prospective study (Baud Study 1) in suspected cyanide-poisoning consecutive to fire smoke inhalation;
- one retrospective study (Baud Study 3) in cyanide-poisoning following ingestion or inhalation of cyanide or cyanogenic compounds.

METHODS

Prospective study in suspected cyanide intoxication due to fire smoke (Baud Study 1)

Objectives
Baud Study 1 was a prospective, uncontrolled, open-label study to evaluate efficacy of hydroxocobalamin in subjects with suspected cyanide exposure due to fire smoke inhalation. Additional cardiovascular safety data were retrospectively collected. The study was conducted over approximately 7 years between 1987 and 1994, in the Fernand Widal Hospital in Paris, France, known as a French reference treatment centre for poisoning cases.

Study participants
Subjects were eligible for the study if they were over 15 years of age, suspected of cyanide intoxication due to smoke inhalation, with soot in the mouth and expectoration, and had disturbances of higher function such as confusion or slowness of thought, or an altered neurological status defined as either impaired consciousness as evidenced by coma, drowsiness, restlessness or transient loss of consciousness when found by the rescue workers.

Cyanide intoxication was confirmed *a posteriori* by assay of the blood sample taken at the fire scene. The thresholds for toxic blood cyanide (BCN) concentration and potentially lethal BCN concentration were predefined as ≥ 39 µmol/L and ≥ 100 µmol/L, respectively.

Subjects with multiple traumas, with second or third degree burns over at least 20 % of the body surface area, or with severe burns on the face and neck were excluded from the study. Women who were pregnant were also excluded from the study.

Treatments
Subjects were treated with a solution of 5.0 g of hydroxocobalamin in 100 mL of water for injection (approximately 70 mg/kg) by intravenous (IV) infusion, over 15 to 30 minutes at the accident scene or in the ICU at hospital admission. Depending on the clinical response, the infusion could be repeated once or twice. The maximum total dose could not exceed 15.0 g of hydroxocobalamin. Symptomatic treatments, mainly oxygen via a mask or after intubation were administered to all patients. Supportive care were performed based on the patient's condition. As smoke inhalation involves intoxication with a variety of toxicants in addition to cyanide, including carbon monoxide, the latter was treated with normobaric and hyperbaric oxygen therapy.

Outcomes/endpoints
Subjects were evaluated at the accident scene before receiving hydroxocobalamin, at the end of the hydroxocobalamin infusion, upon arrival in the hospital, and on days 1, 2, and 3 post-intoxication. Efficacy parameters included: survival, systolic blood pressure (SBP) and diastolic blood pressure (DBP), heart rate (HR), and neurological status. Biological parameters included blood concentrations of cyanide and carbon monoxide, and plasma lactate concentrations.

Statistical methods
Since this was a non-comparative study only descriptive statistics were generated and sample size was no predefined.
The analysis was performed in all included patients (Intention To Treat); and in subpopulations of patients defined according to initial BCN concentration, and to presence or absence of initial cardiac arrest.

Retrospective study in cyanide intoxication from sources other than fire smoke (Baud Study 3)

Objectives
The objective of this retrospective study was to assess the efficacy and safety of hydroxocobalamin in patients with "pure" cyanide poisoning, not involving fire smoke inhalation.

Study participants
Database of admissions to the toxicological ICU of 2 French hospitals (Fernand Widal and Lariboisière Hospitals) was reviewed to identify patients with a diagnosis of cyanide poisoning by ingestion or inhalation and treated with hydroxocobalamin since the first use of hydroxocobalamin in these hospitals in 1988 and until 2003. Cases involving smoke inhalation were excluded.

Treatments
Patients received hydroxocobalamin as soon as possible, in addition to standard supportive therapy. The initial dose was 5 g (approximately 70 mg/kg) by IV infusion with a maximum dose of 20g.

Outcomes/endpoints
The observation period included the prehospital medical intervention at the scene of the intoxication and hospitalization period in ICU or other departments. Retrospective collection of data was based on the review of medical files from ICU. Efficacy variables included survival, changes in neurological status and vital signs.

Statistical methods
Because of the retrospective non-comparative design, all results were presented descriptively without any statistical hypothesis testing.

RESULTS

Baud Study 1

Participants flow
Approximately 18% (15/84) of patients were not analysed. These patients received hydroxocobalamin on the scene but were not admitted to the Fernand Widal Hospital and most of them met predefined exclusion criteria, mainly age below 15 years or severe extensive burns which could interfere with the assessment of the antidote efficacy. Since these patients were not included in the study, related data are rather limited and do not allow further analysis.

Baseline data
Demographic and other baseline characteristics are summarized in the table below:

Table. Demographic and Other Baseline Characteristics

Characteristic	All Patients N = 69 n (%)

Gender	
Female	36 (52.2)
Male	33 (47.8)
Age (year)	
Median (range)	44 (20-94)
Mean ±SD	49.6 ±20
Patients 15 < age <65 years old	54 (78.3)
Patients ≥ 65 years old	15 (21.7)
Patients ≥ 75 years old (subset of ≥ 65 years old)	10 (14.5)
Patients with at least 1 relevant past medical history element	**15**
Cardiac Disorders	**3**
Atrioventricular block	1
Coronary artery disease	3
Congenital, Familial and Genetic Disorders	**1**
Neurofibromatosis	1
Nervous System Disorders	**4**
Stroke	1
Convulsion	2
Dementia	1
Multiple sclerosis	1
Psychiatric Disorders	**1**
Respiratory, Thoracic and Mediastinal Disorders	**1**
Chronic obstructive airways disease	1
Social circumstances (bedridden)	1
Surgical and Medical Procedures	**2**
Cardiac pacemaker insertion	1
Mitral valve replacement	1
Vascular Disorders	**8**
Hypertension	7
Thrombophlebitis	1
Burns	26/69
Cyanide intoxication (BCN ≥ 39 µmol/L)	42/63
Carbon monoxide intoxication (BCO ≥ 1mmol/L)	57/69
Altered neurological status	66/69

Overall, 69 adult patients (36 women and 33 men) were included, median age 44 (20-94) years old, of whom 15 were 65 years old or older. Initial cardiac arrest was reported in 15 patients and altered neurological status in 66 patients, of whom 39 in coma.

Blood cyanide concentration (BCN) was assayed in 63 patients; 42 (67%) of them had BCN ≥ 39 µmol/L. Therefore, suspicion of cyanide intoxication based on clinical history and/or signs and symptoms of cyanide intoxication such as presence of soot in the mouth or expectoration and altered neurological status was justified in at least 67% cases. The highest BCN concentrations were observed in patients with initial cardiac arrest.

Assessment of carbon monoxide concentrations showed that most subjects had mixed intoxications, as 57 patients (82.6%) had carbon monoxide intoxication.

The total hydroxocobalamin median dose used in this study was 5 g (range from 4.0 to 15.0 g) and varied according to the clinical state and course of the patient. The highest doses were given to the patients in the most critical condition.

Outcomes and estimation
Clinical outcome values after hydroxocobalamin treatment in the various groups are summarized in the table below:

Table. Clinical Outcome in all patients and in subpopulations according to initial blood cyanide concentration and presence of initial cardiac arrest

Clinical Outcome	All patients	Initial BCN		Initial Cardiac Arrest		
		≥ 39μmol/L	< 39μmol/L	Yes	No	
						BCN≥39μmol/L
	N = 69	N = 42	N = 21	N = 15	N = 54	N = 31
Gender						
Female	36	22	9	9	27	16
Male	33	20	12	6	27	15
Initial Cardiac Arrest	15	11	2	15	0	0
Neurological Impairment						
Initial Neurological Impairment	**66**	**41**	**19**	**15**	**51/54**	**30/31**
Resolution of neurological symptoms	38	21	13	2	36	19
Neuropsychiatric sequelae at discharge	9	6	3	0	9	6
Death	19	14	3	13	6	5
Outcome:						
Non-fatal	50	28	18	13	48	26
Fatal	19	14	3	2	6	5
Leading Causes of Death:						
Decerebration	13/19	9/14	2/3	13/13	0/6	0/5
Septic shock	5/19	4/14	1/3	0/13	5/6	4/5
Hypoxemic pneumonia	1/19	1/14	0/3	0/13	1/6	1/5

BCN: Blood Cyanide Concentrations

A total of 19 deaths (27%) occurred in the study; 13 of these 19 patients were found in cardiac arrest at the accident scene. Among the 15 patients who had initial cardiac arrest, 13 deaths (87%) occurred, whereas 6 deaths (11%) occurred among the 54 patients who did not have initial cardiac arrest. Among the 42 patients with initial BCN concentrations ≥ 39 μmol/L (*median*: 96.1 μmol/L, *range*: 40 to 250 μmol/L) 14 died (33.3%) and percentage of deaths increased with BCN, whereas 3 subjects died (14%) among the 21 non-intoxicated subjects (*median* BCN: 8.1 μmol/L, *range*: 0 to 27 μmol/L).
Most patients had a favourable neurological outcome, apart from decerebration in patients with initial cardiac arrest. Neurological symptoms initially present in 66 patients improved with no sequelae in 38 cases. Nine patients had neuropsychiatric sequelae at discharge as psychomotor retardation, memory impairment, confusion, intellectual deterioration, dementia, aphasia, or cerebellar syndrome.

In the intoxicated subjects, the median SBP generally started to increase before the end of the hydroxocobalamin infusion and remained at the same level 1 hour thereafter. In non-cyanide-poisoned subjects, hemodynamic status was generally stable.

In smoke inhalation victims, baseline plasma lactate concentration ≥ 10 mmol/L appears to be a sensitive marker of cyanide intoxication, as the highest lactate concentrations were observed in patients with confirmed cyanide intoxication and in those with initial cardiac arrest.
These results suggest that survival outcome is strongly correlated with cardiac arrest, and severity of neurological impairments and of the intoxication.

Overall, the response to hydroxocobalamin was assessed as positive by the investigator in 31 patients (44.9%), partial in 15 patients (21.7%), and absent in 10 patients (14.5%). The response was assessed as unknown in 13 patients, when the documentation available in the prehospital or ICU patient

records was insufficient for the assessment of the response. The positive response rate was higher in patients with severe states (initial BCN ≥ 39 µmol/L and/or initial cardiac arrest).

The results are presented in the table below:

Table. Response to Hydroxocobalamin Treatment

Response to Hydroxocobalamin	Total Population n=69	BCN ≥ 39 µmol/L n=42	BCN < 39 µmol/L n=21	Initial Cardiac Arrest n=15	Without Initial Cardiac Arrest n=54
Positive	31	25	4	8	23
Partial	15	8	6	2	13
Negative	10	3	6	2	8
Unknown	13	6	5	3	10

Baud Study 3

Demographic and baseline data
Medical files of 14 subjects, 12 males and 2 females, with known cyanide poisoning treated with hydroxocobalamin as first line antidote were reviewed. The mean age was 36.3 ± 13.2 years with a range from 14.8 to 64.0 years.

Age, gender, type of cyanide ingested or inhaled, circumstances of cyanide poisoning are summarized in the table below:

Table. Type of Cyanide, Age, Gender and Circumstances of Poisoning

Patient Number	Age (years)	Gender	Type of Cyanide	Circumstances of Poisoning
136	25	Male	KCN	Ingestion in suicide attempt
137	28.3	Female	KCN	Ingestion in suicide attempt
138	50.9	Male	Suspected KCN	Suspected ingestion (unknown circumstances)
139	26.9	Male	KCN	Ingestion in suicide attempt
141	32	Male	KCN	Ingestion in suicide attempt
142	51.6	Male	KCN	Ingestion in suicide attempt
143	38.7	Male	KCN	Ingestion in suicide attempt
144	31.7	Female	KCN	Ingestion in suicide attempt
145	64	Male	KCN	Ingestion in suicide attempt
146	38.3	Male	Cyanogen bromide	Inhalation in an occupational accident
147	14.8	Male	Hg CN	Ingestion in suicide attempt
148	43.7	Male	KCN	Ingestion in suicide attempt
152	39.9	Male	Acetonitrile	Ingestion in suicide attempt
153	22.3	Male	KCN	Ingestion in suicide attempt

Twelve cases were reported as suicide attempts by ingestion of cyanide.

Initial blood cyanide concentration, systolic and diastolic blood pressure, heart rate, respiratory rate and GCS (Glasgow Coma Score) are presented below:

Table. Initial Blood Cyanide Concentration, Systolic and Diastolic Blood Pressure, Heart Rate, Respiratory Rate and GCS

Patient Number	Blood Cyanide Concentration (µmol/L) N=12	SBP (mmHg) N=14	DBP (mmHg) N=11	HR (bpm) N=14	RR (breaths per minute) N=9	GCS N=14
136	124.6	150	90	100	-	15
137	153.8	110	60	120	8	12
138	103	0	0	0	0	3
139	150	95	50	110	3	3
141	125	65	-	80	-	15
142	158	200	120	110	25	15
143	238	120	70	90	14	12[a]
144	196	0	0	0	0	3
145	260	50	0	30	-	3
146	12.7	130	80	72	18	15
147	217	100	-	120	-	15
148	-	80	-	120	0	3
152	170	90	60	80	-	15
153	-	115	80	140	20	15
Mean (SD)	*159.0 (66.3)*	*93.2 (53.9)*	*55.5 (40.1)*	*83.7 (44.6)*	*9.8 (9.7)*	*10 (6)*
Range	*12.7 - 260*	*0 - 200*	*0-120*	*0 - 140*	*0 - 25*	*3 - 15*

[a] Patient 143 was described as having GCS of 5 on hospital arrival, prior to receiving hydroxocobalamin

Among determined blood cyanide concentrations, 11 out of 12 exceeded 100 µmol/L which is usually defined as the lethal threshold, and reached values above 200 µmol/L in 3 cases. One case was reported as mild poisoning resulted from inhalation of cyanogen bromide in an occupational accident. Circumstances of poisoning of the last case were unknown, the subject was a 50 years old man with history of depression, found with altered consciousness and ventricular fibrillation, and toxicological analysis during hopitalization revealed blood cyanide concentration of 103 µmol/L. Overall, clinical features revealed serious poisoning as 2 patients were found in cardiac arrest, 4 were found in shock, and 5 patients had GCS of 3.

Conduct of the study

Patients received hydroxocobalamin as soon as possible, in addition to standard supportive therapy. The initial dose was 5 g (approximately 70 mg/kg) by IV infusion. The dose could be repeated in case of incomplete or transient response. Hydroxocobalamin was the only cyanide antidote used in 9 of the 14 cases; it was associated with sodium thiosulphate in 4 cases, and with sodium thiosulphate and dicobalt edetate in 1 case. The median total dose of hydroxocobalamin administered at the scene plus the hospital ICU was 10 g with a range from 5 to 20 g.

Individual patient data on hydroxocobalamin treatment and associated antidotes are presented in the table below.

Table. Antidotal Therapy

Patient Number	Hydroxocobalamin Total Dose (g)	Other Antidotes	Time Between Ingestion or Inhalation and Hydroxocobalamin Administration (h)
136	5	-	-
137	10	Sodium thiosulphate	0.25
138	10	-	12
139	20	-	0.5
141	10	-	3
142	5	-	5.5
143	10	Sodium thiosulphate	2.17
144	15	Sodium thiosulphate, dicobalt edetate	-
145	10	-	4 *
146	5	-	1.58
147	5	Dimercaprol, dimethylsuccinic acid	2 *
148	9	Sodium thiosulphate	1.5
152	10	Sodium thiosulphate	4

153	5	-	1

*: estimated time

One patient received dimercaprol (British anti-Lewisite) 200 mg and dimethylsuccinic acid 400 mg for associated mercury poisoning. Four patients received sodium thiosulphate and 1 patient received sodium thiosulphate and dicobalt edetate, in addition to hydroxocobalamin.

Based on clinical evidence of need for repeated dose, multiple doses of hydroxocobalamin were frequently required in these primarily suicidal ingestions. Only 5 subjects received a single 5 g dose, 7 subjects required a total dose of 9-10 g, 1 subject required 15 g and 1 received 20 g hydroxocobalamin. All patients with cyanide ingestion had stomach pumped out. Most patients required oxygenation. Two patients found in cardiac arrest underwent cardiopulmonary resuscitation. Six patients received a catecholamine at the scene of poisoning or on hospital admission. Two patients required catecholamine treatment within the 2 first days following hospital admission. The most concomitant medications were plasma substitutes and crystalloid infusion solutions, antipsychotics, anesthetics, and cardiac therapy.

Outcomes and estimation

The lack of a control group and the retrospective design of this study limit to descriptive analysis of the results and do not allow quantitative analysis of the effect of hydroxocobalamin.

Survival outcome, blood cyanide concentration, vital signs and time duration between poisoning and hydroxocobalamin treatment are presented in the following table:

Table: Survival Outcome

Patient number	Initial Blood Cyanide Concentration (µmol/l) N=12	Initial GCS N=14	Initial Respiratory Rate (breaths per minute) N=10	Time Between Ingestion or Inhalation and Hydroxocobalamin Administration (h)	Outcome
136	124.6	15	-	-	Survival
137	153.8	12	8	0.25	Survival
138	103	3	0	12	Survival
139	*150*	*3*	*3*	*0.5*	*Death - 5 days - Coma and hemodynamic failure*
141	125	15	-	3	Survival
142	158	15	25	5.5	Survival
143	*238*	*12*[a]	*14*	*2.17*	*Death - 4 days - Decerebration*
144	*196*	*3*	*0*	-	*Death - 4 days - Refractory shock, coma*
145	*260*	*3*	-	*4*[b]	*Death - 12 days - Decerebration*
146	12.7	15	18	1.58	Survival
147	217	15	-	2[b]	Survival
148	-	3	0	1.5	Survival
152	170	15	-	4	Survival
153	-	15	20	1	Survival

[a] Patient 143 was described as having GCS of 5 on hospital arrival, prior to receiving hydroxocobalamin
[b] Estimated time

Ten of the 14 subjects survived, including 1 subject who was in cardiac arrest on initial evaluation. Nine subjects were treated with hydroxocobalamin between 0.25 and 5.5 hours after poisoning and survived without sequelae. The last patient who survived with anoxic encephalopathy and memory impairment was in cardiac arrest and received late antidote treatment as he was treated with hydroxocobalamin 12 hours after his discovery. The 4 patients who died had high blood cyanide concentrations (150 to 260 µmol/L) and were all in cardiac or respiratory arrest prior to receiving hydroxocobalamin.

In conclusion, hydroxocobalamin appeared to be of interest in critically poisoned patients with supralethal blood cyanide concentrations, particularly before the onset of cardiac arrest that lead to anoxic brain damage. Indeed, efficacy of the antidote treatment appeared to be determined by several factors including time between cyanide exposure and hydroxocobalamin treatment, severity of poisoning defined by blood cyanide concentration and critical clinical events preceding antidote therapy, quantitative relationship between antidote and cyanide, and adequacy of symptomatic treatment. These results underline the necessity for immediate antidote treatment in case of suspicion of cyanide poisoning, preferably at the scene of the incident and without awaiting confirmatory tests.

- Clinical studies in special populations

No specific clinical studies in special population have been conducted.

Paediatric patients Documentation on efficacy is available for 54 paediatric patients. The mean age of the paediatric patients was about six years and the mean dose of hydroxocobalamin was about 120 mg/kg body weight. The survival rate of 41% depended very much on the clinical situation. Out of the 20 paediatric patients without initial cardiac arrest, 18 (90%) survived, of whom 4 with sequelae. In general, the effectiveness of hydroxocobalamin in paediatric patients was similar to that of adults.

The clinical outcome for the 54 documented paediatric cases is summarised in the table below.

Table: Outcome of 54 pediatric patients:

	Cardiac Arrest			
	Total	Yes	No	Unknown
	N=54	N=32	N=20	N=2
Outcome				
Alive	22 (41%)	3 (9%)	18 (90%)	1 (50%)
Dead	31 (57%)	29 (91%)	1 (5%)	1 (50%)
Unknown	1 (2%)	0	1 (5%)	0
Total	54 (100)	32 (100)	20 (100%)	2 (100%)

The proposed posology, initial dose of 70 mg/kg which can be repeated without exceeding 10 g, is considered to be adequate and should provide maximum efficacy with acceptable tolerance. This is reflected in the SPC.

Hepatic and renal impairment
Although the safety and efficacy of hydroxocobalamin have not been studied in these populations, no dosage adjustment is required in these patients. This is reflected in the SPC.

- Supportive studies

Two supportive retrospective reports in situation of suspected cyanide intoxication due to fire smoke inhalation were provided.

Baud Study 2 and Fortin Study are both retrospective, uncontrolled, open-label studies, aimed to describe French clinical experience in hydroxocobalamin treatment for suspected cyanide-poisoning in smoke inhalation victims, at the scene of fires and/or after hospital admission.

Baud Study 2 collected data from files of the Department of Medical and Toxicological Critical Care of 2 Paris hospitals of patients treated with hydroxocobalamin between 1988 and 2004. Fortin Study collected data from medical intervention reports of the Paris Fire Brigade (PFB) for patients treated with hydroxocobalamin between 1995 and 2003.

Although the 2 studies periods overlap and take place in the same city, there was no duplication of patients enrolled in both studies.

Retrospective study in suspected cyanide intoxication due to fire smoke (Baud Study 2)

Methods

Baud Study 2 is a retrospective, uncontrolled, open-label study. Data were collected from files of the Department of Medical and Toxicological Critical Care of 2 French hospitals (Fernand Widal and Lariboisière Hospitals) of patients treated with hydroxocobalamin between 1988 and 2004. The files included firemen reports, medical ambulatory emergency and first aid unit reports, and hospital records.
Patients included were adults, suspected of acute cyanide poisoning caused by smoke inhalation, received hydroxocobalamin and had available reports from the intensive care units (ICU). Paediatric patients were transported to other hospitals and were not included.

Efficacy variables included survival, neurological status including Glasgow Coma Score (GCS), hemodynamic status and restoration of spontaneous circulation in patients with initial cardiac arrest. Toxicology parameters (blood cyanide and lactates concentrations) were measured in some subjects. Blood cyanide concentrations were categorized as ≥ 40 µmol/L (intoxication) or < 40 µmol/L (no intoxication). Lactates concentrations were categorized as ≤ 10 mmol/L (no poisoning) *versus* > 10 mmol/L (poisoning).

Due to the retrospective non-comparative design, all results were presented descriptively without any statistical testing.

The main analysis describes the data for the whole study population. Analyses were also performed in subpopulations, according to presence or absence of initial cardiac arrest, and to BCN concentration measured before any antidote treatment.

Results

A total of 61 smoke inhalation victims (31 women and 30 men), of mean age 54.3 ± 18.3 years (range from 19.7 to 91.9 years), were treated with hydroxocobalamin and had corresponding hospital records between 1988 and 2004. However, initial blood cyanide concentrations were only available for 25 patients, mean value was 45.1 ± 51.3 µmol/L; and 9 of these 25 patients (36.0 %) had blood cyanide concentrations ≥ 40 µmol/L.

Soot deposits were present in 53 patients (86.9%); out of them 36 had soot in their lower airways. Altered neurological status was reported in 51 patients (83.6%), 34 had an initial loss of consciousness, 35 were in a coma, and 15 were diagnosed as having psycho-organic syndrome. Mean GCS, evaluated in 60 patients, was 9 ± 5. Initial cardiac arrest at the fire scene was reported in 17 (27.9%) patients. None of the 8 patients who were > 75 years old experienced cardiac arrest at the fire scene. Burns were more prevalent in patients who experienced cardiac arrest (58.8%) compared with patients who did not experience cardiac arrest (29.5%).

The median total dose of hydroxocobalamin administered at the fire scene plus the hospital ICU was 5 g with a range from 2.5 to 15.0 g. No other antidote, such as thiosulphate, was associated with hydroxocobalamin. Other treatments were: epinephrine, administered to 15 of the 17 patients who experienced cardiac arrest; sedation administered to 31 patients (50.8%) were, IV fluids to 48 patients (78.7%), isobaric oxygen therapy to 60 patients (98.4%), hyperbaric oxygen therapy to 43 patients (70.5%), and mechanical ventilation in 43 patients (70.5%).

Thirty-four patients (55.7%) survived and were discharged, and 24 patients died either in the ICU or other hospital departments. For the remaining 3 patients (4.9%), the hospital outcome was missing.

Change in Neurological Status were not evaluable in most patients due sedation. Among the 20 evaluable patients, 3 patients shown improvement, 15 patients were stable, and 2 patients shown worsening.

Haemodynamic measurements at hospital admission were available in 14 of the 16 patients who were in cardiac arrest and received hydroxocobalamin at the fire scene: following treatment with hydroxocobalamin, SBP was 121.1 ± 54.8 mmHg (range: 36 to 219 mmHg), DBP was 69.6 ± 32.8 (range: 19 to 123 mmHg), and heart rate was 96.7 ± 17.0 bpm (range: 70 to 135 bpm).

GCS was 9 ± 5 in 60 patients evaluated at arrival to the fire scene, and 8 ± 5 in 30 patients evaluated at hospital admission.

Regarding toxicology parameters, at hospital admission, mean blood cyanide concentration assessed in 32 patients was 28.0 ± 38.7 µmol/L (range: 3.5 to 161.5 µmol/L), mean blood carbon monoxide concentration assessed in 52 patients at hospital admission was 1.4 ± 1.1 mmol/L (range: 0.0 to 4.6 mmol/L), and mean blood lactate concentration assessed in 55 patients was 8.8 ± 7.6 mmol/L (range: 1.6 to 42.4 mmol/L).

Retrospective study in suspected cyanide intoxication due to fire smoke (Fortin Study)

Methods

Contrary to previous studies which were hospital studies, the aim of Fortin Study was to describe Paris Fire Brigade (PFB) clinical experience in treating suspected cyanide-poisoning in smoke inhalation victims at the scene of fires with hydroxocobalamin. This is a retrospective, uncontrolled, open-label study. Data were collected from medical intervention reports of the PFB during their routine practice, and from a copy of the hospital discharge summary when available, for patients treated with hydroxocobalamin between 1995 and 2003. Contrary to previous studies which were hospital studies, this study was performed by the Paris Fire Brigade. Therefore, data after hospitalisation were not always available and can be incomplete.

This study included patients suspected of acute cyanide poisoning due to smoke inhalation and treated with hydroxocobalamin by the PFB, with available reports from PFB. Patients were categorized according to their conditions when the fire-fighters arrived at the scene of the fire, as follows: (i) Patients with cardiac arrest, irrespective of whether they exhibited other clinical signs; (ii) Patients without initial cardiac arrest were classified according to the other main symptoms of cyanide poisoning: shock or hemodynamic instability (SBP ≤ 90 mmHg), neurological impairment (GCS < 15, or loss of consciousness), or no predominant clinical signs.

Efficacy variables included survival, neurological status including GCS, hemodynamic status and restoration of spontaneous circulation in patients with initial cardiac arrest. No blood samples were taken for measurement of cyanide levels.

Due to the retrospective non-comparative design, all results were presented descriptively without any statistical testing.

Results

A total of 101 patients (48 female and 53 males) with smoke inhalation were treated with hydroxocobalamin by the PFB between 1995 and 2003. Among them, 38 had initial cardiac arrest, 46 altered neurological status and 5 were in shock.

The mean age of patients in the study was 47.1 ± 20.7 years with a range from 2 to 88 years. Most patients were adults; 8 patients were between 2 and 14 years old.

Soot was present in 71.3% of the patients, mostly in the throat. Thirty-six patients had carboxyhemoglobin (HbCO) measured at ICU arrival, and carbon monoxide intoxication (HbCO

≥ 10%) was found in 22 of those patients. Mean SBP and DBP were 131.0 ± 34.1 mmHg and 76.4 ± 15.7 mmHg. Overall, the initial mean GCS was 8.0 ± 5.1.

The median dose of hydroxocobalamin administered to the total population was 5.0 g (range: 1 to 10 g) and was comparable across subgroups. The median dose recorded in 7 of 8 children was 1.5 g (range: 1.0 to 3.5 g). No other antidote, such as thiosulphate, was administered together with hydroxocobalamin.

The outcome and reason for death in the total population and in each subpopulation are shown in the table below:

Table. Survival

	Total Population N=101	**Cardiac Arrest N=38**	**Shock N=5**	**Neurological Impairment N=46**	**No Predominant Sign N=12**
Died (n (%))	**42 (41.6)**	**34 (89.5)**	**1 (20.0)**	**6 (13.0)**	**1 (8.3)**
Died at fire scene	17 (16.8)	17 (44.7)	0	0	0
Died at ICU	25 (24.8)	17 (44.7)	1 (20.0)	6 (13.0)	1 (8.3)
Survived (n (%))	**30 (29.7)**	**2 (5.3)**	**3 (60.0)**	**20 (43.5)**	**5 (41.7)**
Hospital outcome missing (n (%))	29 (28.7)	2 (5.3)	1 (20.0)	20 (43.5)	6 (50.0)
Time to death (days)	**n=42**	**n=34**	**n=1**	**n=6**	**n=1**
Mean ± SD	4.0 ± 6.3	1.9 ± 1.8	3.0 ± 0.0	14.8 ± 11.2	9.0 ± 0.0
Median (range)	1 (1-30)	1	3	17	9
Reason for death n (%)	**n=42**	**n=34**	**n=1**	**n=6**	**n=1**
Cardiac arrest	18 (42.9)	18 (52.9)	-	-	-
Multiple organ failure	10 (23.8)	4 (11.8)	1 (100)	4 (66.7)	1 (100)
Cerebral anoxia	10 (23.8)	10 (29.4)	-	-	-
Other	4 (9.5)	2 (5.9)	-	2 (33.3)	-

Regarding survival before hospital admission, 84 of the 101 patients survived. The 17 patients who died at the fire scene were in the cardiac arrest subgroup. Hospital outcome was only available for 55 of the 84 hospital patients. Among them, 25 patients (24.8%) died in the ICU and 30 patients (29.7%) survived and were either discharged or transferred to another department. A higher percentage of patients died in the cardiac arrest subgroup than in other subgroups. Death occurred on average 4 days after the event, within 2-3 days in the "cardiac arrest" and "shock" subgroups and within 9 and 15 days in the "no predominant sign" and the "neurological impairment" subgroups.

Baud Study 2 was a hospital study that retrospectively collected data from adult hospital patients and as a result did not include patients who died at fire scene, whereas Fortin study was a Paris Fire Brigade's study that collected data from pre-hospitalization reports and from hospital discharge summaries when available. Therefore, the lower percentage of survival observed in Fortin study compared with Baud Study 2 can be explained by difference between populations included in the 2 studies, i.e. before admission to hospital for Fortin Study and after hospital admission for Baud study 2.

Of the 38 patients who had an initial cardiac arrest, 17 patients died on site, and 21 patients had a return of spontaneous circulation before hospitalization. Most of these patients died within 1 to 8 days after admission to the ICU. Of the 21 patients who were admitted to the ICU, 17 patients died, 2 patients survived, and 2 patients had hospital outcome missing.

Hemodynamic improvement was calculated for all patients with $0 < SBP \leq 90$ mmHg prior to administration of hydroxocobalamin, and was defined as at least 1 follow-up SBP measure > 90 mmHg. Of the 12 hemodynamically unstable patients, 9 patients recovered normal blood pressure, on average 30.6 minutes after start of hydroxocobalamin administration. The remaining 3 patients did not recover.

Change in mean GCS was calculated for 52 patients. It was not calculated due to missing final values in 8 patients and due to concomitant administration of sedative drugs in 41 patients. In the 52 patients

who were evaluated, the mean GCS increased from 7.9 ± 5.4 to 8.5 ± 5.7. Ten patients showed improvement, 41 patients were unchanged, and 1 patient worsened. In the 46 patients with initial neurological impairment, the GCS improved in 9 of 18 unsedated patients, was unchanged in 8 patients, and worsened in 1 patient.

- Analysis performed across trials (pooled analyses and meta-analysis)

There was a broad age range across all studies, from 2 to 94 years old. Fortin Study included 8 paediatric subjects aged between 2 and 14 years old, and Baud 3 Study included one almost 15 years old subject. Median age was somewhat younger in Baud Study 3 than in the other studies. Baud Study 3 included more men than women, whereas there were approximately equal numbers of males and females in the other 3 studies.

It has to be noted that blood sampling has been performed more or less rapidly after cyanide exposure; therefore measured BCN not always indicate actual maximal cyanide concentration. Among measured BCN, more severe cyanide intoxications were reported in Baud Study 3 with 79% subjects ≥ 100 µmol/L, and 42 of the 63 (67%) subjects that had BCN measurements in Baud Study 1 were above the toxic threshold of 39 µmol/L. Few patients had BCN determined in Baud Study 2, and no one in Fortin Study. Demographic characteristics of subjects with cyanide concentrations above the toxic threshold were generally similar to those of subjects with cyanide concentrations below the toxic threshold.

The median dose of hydroxocobalamin was 5.0 g in the 3 smoke inhalation studies and was 10.0 g in Baud Study 3. This is consistent with the higher degree of cyanide intoxication in Baud Study 3 than in the other 3 clinical studies.

Overall, despite a slightly greater percentage of males than females included in the studies, a higher percentage of females than males had initial cardiac arrest. The median age of subjects with initial cardiac arrest was generally slightly younger than the median age of the whole population.

Every subjects included in Baud Study 1, had plasma lactate concentrations measured at hospital admission. As expected, subjects with initial cardiac arrest had much higher levels (median: 24.6 mmol/L) than other subjects and subjects with BCN ≥ 39 µmol/L had higher plasma lactate levels (median: 11.5 mmol/L) than subjects with BCN < 39 µmol/L (median: 6.1 mmol/L). In Baud Study 2, plasma lactate levels were available for only 15 subjects, but as for Baud Study 1, plasma lactate concentrations > 10 mmol/L were consistent with initial cardiac arrest and cyanide intoxication.

In Baud Study 3, median plasma lactate concentration measured at the scene in 5 subjects was 17.6 mmol/L. Thirteen subjects had plasma lactate concentrations measured upon arrival at the hospital, median was 12.9 mmol/L (range: 2.1-53 mmol/L).

Among the 213 subjects with known survival outcome, 124 survived (58%). Among subjects who died, most had initial cardiac arrest. As expected, a higher percentage of subjects (68%) without initial cardiac arrest survived across all studies, compared with subjects with initial cardiac arrest (8%).

Although cyanide intoxication can result in markedly decreased blood pressure, this result was observed only in a few subjects in the absence of cardiac arrest. In these subjects, SBP generally improved following administration of hydroxocobalamin. In the Fortin Study, 5 of 7 subjects with SBP ≤ 90 mmHg demonstrated improvement to > 90 mmHg. In Baud Study 3, 2 subjects without initial cardiac arrest who had SBP ≤ 90 mmHg and who received hydroxocobalamin before admission to hospital, demonstrated cardiovascular improvement upon arrival to the ICU. However, vasopressors were frequently employed, which exerted an additive effect to hydroxocobalamin on blood pressure. As no control group was available one cannot attribute the blood pressure increasing effect solely to hydroxocobalamin.

In subjects with cardiac arrest and in whom spontaneous circulation was restored, a marked improvement of blood pressure, often to normal levels, was observed following administration of hydroxocobalamin. It should be noted that in many instances, hydroxocobalamin was administered concomitantly with other drugs such as vasopressors for hemodynamic improvement.

Cyanide intoxication can result in neurologic signs and symptoms ranging from confusion and disorientation to coma. Neurologic status before and after hydroxocobalamin administration was therefore evaluated; data presented in this section represent subjects without initial cardiac arrest. Patients who received sedating drugs were excluded from the analysis as disease related effects could not be distinguished from iatrogenic effects.

Neurologic improvement following administration of hydroxocobalamin was most pronounced in the prospective Baud Study 1, in which 51 of 54 subjects without initial cardiac arrest had initial neurologic impairment. Of these 54 subjects, 38 subjects (72%) were discharged alive with no sequelae. Neurologic improvement in the retrospective clinical studies was more difficult to assess, since many subjects were unevaluable due to sedation, and since the studies were not designed to specifically and consistently collect that information. Nevertheless, evidence of neurologic improvement was demonstrated in some subjects in the retrospective clinical studies. For example, 9 of the 10 subjects who survived in Baud Study 3 had no neurologic sequelae at hospital discharge.

In Baud Study 1, 66 of 69 subjects had initial neurological impairment. Among the 47 patients who survived, complete resolution was achieved in 38 subjects, and neuropsychiatric sequelae were observed in 9 subjects. Neuropsychiatric sequelae included psychomotor retardation (n=1), memory impairment (n=2), confusion (n=2), intellectual deterioration (n=1), dementia (n=1), aphasia (n=1), cerebellar syndrome (n=2), with some patients presenting more than 1 symptom.

In Baud Study 2, 20 subjects were evaluable for neurological status; 3 subjects demonstrated improvement, 15 subjects were stable and 2 subjects demonstrated worsening of neurologic status. Of the remaining 41 subjects, 33 were not evaluable due to sedation and data were missing for 8 subjects.

In Fortin Study, 52 of 101 subjects were evaluable for neurological status, 10 subjects demonstrated improvement, 41 subjects demonstrated no change, and 1 subject demonstrated worsening of neurologic status. Of the remaining 49 subjects, 41 were not evaluable due to sedation and data were missing for 8 subjects.

In Baud Study 3, 9 of 10 subjects survived without neurologic sequelae, and 1 subject was discharged with post-anoxic encephalopathy.

- Discussion on clinical efficacy

The efficacy of hydroxocobalamin as a cyanide antidote was studied in 5 studies of which one was a non clinical, placebo-controlled study performed in cyanide-intoxicated dogs, and 4 were non controlled, non comparative French clinical studies either in *suspected* cyanide-poisoning due to fire smoke inhalation: Baud Study 1 (prospective) and Baud 2 and Fortin Studies (retrospective), or in *known* cyanide exposure from sources other than fire smoke: Baud Study 3 (retrospective). Furthermore, a pharmacokinetic study has been performed in forty-one healthy volunteers to study the pharmacokinetic profile a 5 and 10 g doses. The main information brought by this study is the linear PK behaviour of hydroxocobalamin over the range of the tested doses (2.5 to 10 g).

The non clinical, placebo-controlled study in 54 cyanide-poisoned dogs was performed to overcome the lack of clinical placebo-controlled study due to ethical reasons. Day 14 survival was significantly greater in both hydroxocobalamin dose groups (75 mg/kg: 79% and 150 mg/kg: 100%) compared with placebo (18%). The results also suggested a dose related reduction in neurologic sequelae.

The 5.0 g starting dose (70 mg/kg in children), and the possibility to administer another 5.0 g dose if needed, is supported by clinical experience, by in vitro data, and by the non clinical study. Indeed, based on dose per body weight and on C_{max} of total cobalamins-(III), the 75 and 150 mg/kg doses

studied in dogs are approximately equivalent to respectively the 5.0 and 10.0 g doses in humans. Based on these considerations, a comparable extent of cyanide detoxification as observed in the dog efficacy study is expected in humans.

The literature research reveals that there are only few published comparative data, and that they mostly refer to animal studies. Clinical data mainly refer to case reports.

No comparative study to another antidote, or to oxygen therapy and symptomatic treatments alone in case of mild intoxication, was submitted by the applicant. In the situation of *suspected cyanide-poisoning*, according to the EMEA/CPMP guidance document (EMEA/CPMP/1255/03) on the "use of medicinal products for the treatment of patients exposed to terrorist attacks with chemical agents", hydroxocobalamin is the antidote of choice, in particular for safety reasons. Indeed, dicobalt edentate, the other complexation agent, is more potent on a molar basis; however it has serious cardio-vascular adverse effects in the absence of cyanide. Thiosulphate is both efficient and safe, but acts with delay. And methaemoglobin-forming agents are potent, but associated with a risk of excessive methaemoglobinemia with impaired tissue delivery of oxygen. Consequently, the lack of a comparator antidote-group in studies on victims of fire smoke inhalation, for whom cyanide-poisoning is mostly suspected rather than known, can be acceptable.

Over the *4 submitted clinical studies*, data from 245 subjects were analysed, of whom 72 subjects in cardiac arrest. Most subjects (231/245) were victims of fire smoke inhalation. Suspicion of cyanide-poisoning in these patients was based on clinical history and/or signs and symptoms of cyanide intoxication such as presence of soot in the mouth or expectoration and altered neurological status. Given the impossibility to rapidly assay blood cyanide at the fire scene, cyanide intoxication was later confirmed at hospital. Each subject received oxygenation that is the basic treatment of such intoxication and, if necessary, symptomatic treatments as mechanical ventilation, and management of seizures, arrhythmias, hypotension and metabolic acidosis. The *median dose* of hydroxocobalamin was 5.0 g in the 3 smoke inhalation studies and was 10.0 g in Baud Study 3. This is consistent with the higher degree of cyanide intoxication in Baud Study 3 than in the other 3 clinical studies.

Survival outcome is known for 213 subjects, of whom 124 (58%) survived. Most subjects (63/89) who died presented cardiac arrest before treatment. Initial cyanide concentrations were only assessed in 100 subjects. Among the 34 subjects with BCN above the lethal threshold of 100 µmol/L, 21 (62%) survived following treatment with hydroxocobalamin. Time between cyanide exposure and treatment was often not controlled, but results suggested a negative link between this time and survival outcome. There was a trend toward greater survival in males than females, but no conclusion on a potential gender effect can be drawn due to the limited sample size. Subjects younger than 65 years old tend to have more non fatal outcome than older subjects. Regarding *paediatric subjects*, only 9 patients younger than 19 years old were included in the clinical studies, with poor survival related to high frequency of initial cardiac arrest, but analysis of a total of 54 paediatric cases revealed a similar survival rate to that of adults.

Blood pressure generally improved following administration of hydroxocobalamin. However, vasopressors were frequently employed, which exerted an additive effect to hydroxocobalamin on blood pressure. As no control group was available, the blood pressure increasing effect cannot be only attributed to hydroxocobalamin.

Cyanide intoxication can result in *neurological signs* and symptoms ranging from confusion and disorientation to coma. Neurological status before and after hydroxocobalamin administration was therefore evaluated. Neurological improvement following administration of hydroxocobalamin was most pronounced in the prospective Baud Study 1, 72% subjects were discharged alive with no sequelae. Neurological improvement in the retrospective clinical studies was more difficult to assess, since many subjects were unevaluable due to sedation, and since the studies were not designed to specifically and consistently collect that information. Nevertheless, evidence of neurological improvement was demonstrated in some subjects.

Baseline plasma lactate concentration ≥ 10 mmol/L appeared to be a sensitive marker of cyanide intoxication, as the highest lactate concentrations were observed in patients with confirmed cyanide intoxication and in those with initial cardiac arrest.

According to the European guidance document EMEA/CPMP/1255/03, the simultaneous or successive administration of different antidotes has been frequently recommended. In the current dossier, only few data are available on the sequential combination of hydroxocobalamin and the sulphur supplying agent, sodium thiosulphate, and do not allow to conclude.

Clinical safety

The safety data from clinical trial populations are issued from 5 studies:

- Study EML 015722-H101, a prospective study of hydroxocobalamin in 136 healthy volunteers (including 34 placebo volunteers).
- Baud Study 1, a prospective study that examined the efficacy and safety of hydroxocobalamin in treating suspected cyanide poisoning in 69 victims of smoke inhalation. Additional data was collected retrospectively to provide a more complete dataset.
- Baud Study 2, a retrospective study that examined the safety and efficacy of hydroxocobalamin in treating suspected cyanide poisoning in 61 victims of smoke inhalation.
- Fortin Study, a retrospective study that examined the efficacy and safety of hydroxocobalamin in treating suspected cyanide poisoning in 101 victims of smoke inhalation.
- Baud Study 3, a retrospective study that examined the efficacy and safety of hydroxocobalamin in treating suspected cyanide poisoning in 14 victims of cyanide poisoning from sources other than fire smoke.

Due to the important differences between the clinical trials (prospective phase I study, prospective with a retrospective collection of data, retrospective data collection), it was considered as not appropriate to pool the data of these studies.

- Patient exposure

Safety data are available for 347 subjects who have been administered at least 1 dose of hydroxocobalamin, either as treatment for known or suspected cyanide poisoning or as part of a clinical trial to evaluate the safety of hydroxocobalamin in healthy volunteers. In addition, clinical studies have been conducted to evaluate the efficacy and safety of hydroxocobalamin in the treatment of cyanide poisoning in various settings: smoke inhalation, ingestion, and occupational accidents.

Of these 347 subjects, 245 subjects had suspected exposure to cyanide at the time of hydroxycobalamin administration. The remaining 102 subjects were healthy subjects who had not been exposed to cyanide at the time of hydroxocobalamin administration.

- Adverse events

Study EML 015722 –H101, conducted in healthy volunteers, showed a well tolerated product. No serious adverse event was reported, no death was reported. The occurrence of allergic reaction in 10 g dose group lead to discontinue the administration of hydroxocobalamin. The main AEs, considered by the Investigator to be possibly study-drug related, comprised the following events:

- Chromaturia: all subjects in each active dose group experienced chromaturia (red-coloured urine) after hydroxocobalamin infusion. This event occurred with the first urine sample, was graded as intense and resolved in two or four weeks according to the dose active groups.
- Erythema (skin redness): A total of 89 (87.3%) of all subjects treated with hydroxocobalamin, i.e., 62 in the 5.0-g dose group and all subjects at the two highest dose levels showed redness of the skin most often graded this event as moderate, but in some cases as intense. Skin reddening in the 5 – 10-g dose groups mainly occurred within 10 to 33 min after start of infusion, i.e. after infusion of

at least 3g hydroxocobalamin. In most cases the event resolved after approximately 7 days with a total range of 1 to 15 days. This effect can be a potential source of interference on the assessment of burn lesions. This is reflected in the product information.

- Pustular rash: Pustular rash was reported in 11 (16.7%) of 66 subjects, in the 5 g hydroxocobalamin dose group in 4 of 9 subjects in the 7.5g hydroxocobalamin group and 3 of 18 subjects of 10-g dose groups. The Investigator mainly considered the intensity as mild. The onset of these AEs ranged from 7 to 25 days post-dose and lasted for approximately 6 to 38 days across all dose groups. The pustules were most often located in the face and neck, but also once on the chest and once on the back. This AE was not observed in any of the subjects included in the lowest dose group and in the placebo groups.
- Headache: During the study, 22 subjects complained about headache ('headache' and 'head pressure'), 19 subjects (18.6%) after Hydroxocobalamin infusion and 3 subjects (8.8%) following placebo. Overall, an increase in the number of subjects with the AE 'headache' related to the increase in dose was found in the two highest dose groups (55.6% and 33.3% in the 7.5- and 10-g groups versus 22.2% and 9.1% in the 2.5- and 5-g dose groups). Incidences in the 2.5- and 5-g groups appeared to be similar, in particular in view of the differences in sample size. In 9 out of 24 subjects with clinically relevant increases in blood pressure, headache was observed starting immediately after start of infusion up to about 24 h after start of infusion. In the two lower dose groups, only 1 subject suffered from headache and increased blood pressure compared to 8 subjects of the higher dose groups. Three of the 9 headaches occurred within about 2.5 hours after start of infusion, 3 within about 12 hours and the remaining from about 16 to 24 hours after start of infusion. Therefore, a direct correlation between the rise in blood pressure and headache seemed to be unlikely.
- Increased diastolic blood pressure: besides redness and chromaturia, increase in blood pressure particularly diastolic blood pressure was reported in 12 patients (18.2%) in the 5 g dose group and in 5 patients (27.8%) in the 10 g dose group. Blood pressure (BP) increases from baseline amounting up to maxima of 22.9-27.0 mmHg for systolic blood pressure (SBP), 14.3-25.4 mmHg for diastolic blood pressure (DBP) and 17.1-25.8 mmHg for mean arterial BP The blood pressure started to increase within 2-5 min after start of Hydroxocobalamin infusion, reached a maximum around the end of infusion and returned to near baseline values within 4-8 hours post-dose.
- Decreased lymphocyte count and increased CRP.
- Erythema at the injection site. **:** At dose levels of 5.0 g, 7.5 g and 10.0 g hydroxocobalamin, injection-site reactions were reported in 14 subjects. The symptoms comprised oedema, pain, exanthema (local macula), reddening of the vein or arm at the infusion site. The symptoms redness of infusion vein or arm and local redness at infusion site in the 10-g active group were rated as mild or moderate by the Investigator, started between about 34 and 52 min after begin of infusion and persisted for about 2 to 48 hours. None of the subjects, who received placebo, reported any injection site reaction.
- In addition, 3 cutaneous allergic reactions (rash papular) out of 18 subjects occurred in the 10-g dose group. No such reactions have been observed in Baud 1 study, which could be explained by several factors (decreased identification due to burns or others skin lesions, underreporting in the emergency context, other medications with anti-allergic properties administered in life threatening conditions etc.)
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Baud Study 1 included 69 patients (36 females, 33 males). The median age of the patients was 44 years (range: 20 to 94 years). Fifteen of the 69 patients (22%) were ≥65 years of age and among these, 10 were ≥75 years of age. A total of 49 patients (71%) received 5g hydroxocobalamin, and 19 patients (27.5%) received 10 g and one patient received 15 g.

A total of 65 of 69 patients (94.2%) experience at least 1 AE. The most common AEs were respiratory disorders (n=48), infections (n=37) and metabolic disorders (n=35). These AEs are linked to the cyanide intoxication and considered not related to the hydroxocobalamin administration. A total of 19 of the 69 patients (27.5%) experienced at least one AE possibly related to hydroxocobalamin.

Table . Incidence of possibly Related Adverse Event (ADRs)

System Organ Class Preferred Term	Total Population n
Patients with at least 1 possibly related AE	**19**
Cardiac Disorders	**1**
Ventricular extrasystoles	1
Eye Disorders	**1**
Conjunctival hyperemia	1
General Disorders and Administration Site Conditions	**1**
Injection site extravasation	1
Investigations	**4**
Blood pressure decreased	1
Blood pressure increased	2
ECG repolarisation abnormality	1
Renal and Urinary Disorders	**6**
Chromaturia	6
Respiratory, Thoracic and Mediastinal Disorders	**1**
Pleural effusion	1
Skin and Subcutaneous Disorders	**6**
Erythema	2
Skin discoloration	4
Vascular Disorders	**3**
Hypertension	3

Fourteen of the 69 patients (20.3%) experienced complications during the course of hospitalisation, either linked to their past medical history or to the intoxication itself .

A review of cardiovascular safety was added in this study. Data collected concern the changes of blood pressure (BP), systolic blood pressure (SBP) and diastolic blood pressure (DBP) and heart rate (HR) before and after the first infusion of Hydroxocobalamin.

Systolic Blood Pressure : The initial median SBP on arrival of rescuers determined in 69 patients was 130.0 mmHg (range 0 to 220 mmHg). In patients in initial cardiac arrest, the median was 0.0mmHg while the median initial SBP in patients without initial cardiac arrest was 140.0 mmHg (range 80.0 to 220.0 mmHg). 25% of all patients had an initially elevated SBP. Eleven of the 69 patients had an elevated SBP at a level consistent with the Stage 2 Hypertension Classification of Joint National Committee(JNC) (S2SBP)VII.

After administration of OHCo including patients with elevated SBP prior to OHCo a total of 27 of 69 patients were noted to have at least one measurement of SBP >160.0 mmHg (S2SBP). As was the case for pre-infusion values, 25% of all patients had an elevated SBP after OHCo infusion between 150.0 and 180.0 mmHg.

Diastolic Blood Pressure: The initial median DBP on arrival of rescuers, determined in 50 patients was 80.0 mmHg (range 0.0 to 110mmHG). In patients with initial cardiac arrest, the median DBP was 0.0 mmHg while the median DBP in patients without cardiac arrest was 80.0 mmHg (range 50.0 to 110.0 mmHg). When one excludes the patients with no BP initially (initial cardiac arrest) the percentage of patients with S2DBP reaches 18. 9 %.

After administration of OHCo, including patients with elevated DBP prior to OHCo, 20 of 69 of patients (29%) were noted to have at least one measurement of DBP>100.0 mmHg (S2DBP) after administration of OHCo. The median difference in DBP after administration of OHCo was 7.5mm Hg

(range 30.0mmHG to 90.0 mmHg) in the total population. In patients with initial cardiac arrest, the median difference was 10.0 mmHg (0.0 to 9.0 mmHg compared with 5mmHg in patients no initially in cardiac arrest. The median difference in DBP pre- and post- OHCo was 5.0 mmHg in patients less than 65 years of age and 10.0 mmHg in patients more elderly.

The results of this review show there is not a clear dose-effect relationship between elevated systolic blood pressure and the dose of hydroxocobalamin administered.

Heart Rate (HR): The median HR prior to the first infusion of hydroxocobalamin, in patients not in cardiac arrest was: 100.0 bpm (range: 75-140 bpm) in patients not cyanide-poisoned, and 100.0 bpm (range: 59-140 bpm) in patients with initial BCN $\geq$ 39 μmol/L. In general there was a slight decline in HR after treatment with hydroxocobalamin.

Baud Study 2 included 61 patients (smoke inhalation victims). A total of 59 patients (96. 7%) received hydroxocobalamin alone and two received hydroxocobalamin plus sodium thiosulfate. 36 patients (61%) received a total of 5g of hydroxocobalamin 12 patients 20.3% received a total de 10g,and 6 patients (10.2%) received a total of 15 g. 54 of 61 patients (88.5%) experienced a least 1 AE. 16 of the 61 patients (26.2%) experienced AEs related to hydroxocobalamin: chromaturia: there were 12 reports of dark red urine, skin discoloration: there were 4 reports of red to pink skin, blood disorders: there were 2 reports of red colored plasma, eyelid oedema (n=1), generalized erythema (n=1), hypertension (n=1). The incidence of AEs did not appear to be related to the dose of administered hydroxocobalamin or to gender.

Fortin Study included 101 patients (13 ADR in 10 patients were reported): Chromaturia or skin discoloration: there were 5 reports of red or pink coloration of urine or skin, Allergic reaction, cutaneous rash (n=1), cardiac arrest (n=2), respiratory distress (n=1), gastric and cutaneous hemorrhages (n=1). These AE have been considered as not drug related.

A review of cardiovascular safety was added in this study: SBP and DBP measurements were collected retrospectively for 101 smoke inhalation victims from the medical intervention reports of the PFB during their routine practice. Time points for blood pressures collection included arrival at the scene, T0 (time of hydroxocobalamin administration), T+5, T+10, T+15, T+20, T+25, T+30, T+40, T+50, T+60, T+70 minutes. Data for all patients was not available for all time points. Elevations in SBP and DBP were examined in all patients and in the subgroups listed below. BP thresholds were selected according to the definition of Stage 2 hypertension.

The mean SBP upon arrival at the scene of the fire for patients not in initial cardiac arrest was 131.0±34.1 mmHg (range 70.0-300.0, n=61). After administration of OHCo, in 25/101 (24.8%) patients there was at least 1 SBP value ≥160 mmHg between T0 and T+70 minutes. The peak mean SBP of 144.3±43.5 mmHg (range 80.0-250.0, n=23) was reached at T+30. At T+70, the mean SBP had decreased to 142.9±33.1 mmHg (range 80.0-210.0, n=17).

The mean DBP upon arrival at the scene of the fire for patients not in initial cardiac arrest was 76.4±15.7 mmHg (range 50.0-118.0, n=54). After of administration of OHCo the peak mean DBP of 89.5±22.6 mmHg (range 60.0-150.0, n=19) was reached at T+30. At T+70, the DBP mean had decreased to 87.9±15.7 mmHg (range 67.0-110.0, n=12).

At least 1 isolated elevation in SBP and DBP exceeded the threshold value for Stage 2 hypertension (JNC VII) in 25/101 (24.8%) and in 20/101 (19.8%) of patients respectively, between T0 and T+70.

The mean SBP and DBP began to increase within 5 minutes of the start of OHCo infusion. Most patients had a peak mean increase at 20 and return to baseline levels within 70mn. None of the BP elevations have had any harmful consequences. As expected patients with a history of cardiovascular disease or the elderly had higher mean averages for initial, final, and peak BP measures.

Baud study 3 included 14 patients with a diagnosis of cyanide poisoning by ingestion or inhalation. 8 (57%) of the 14 patients experienced an ADR. The main drug related ADR are the following:

chromaturia (dark red urine) (n=5), skin discoloration (red to pink skin) (n=3), circulatory collapse (n=2) and hypertension (n=1).

- Serious adverse event/deaths/other significant events

In the phase I study EML 015722 –H101 no serious adverse events and no deaths were reported.

In studies Baud 1, Baud 2 Baud 3 and Fortin: no serious adverse events and 89 (36%) deaths were reported but none was considered possibly related to administration of hydroxocobalamin.

- Laboratory findings

Interference of hydroxocobalamin with laboratory tests:

Because of its deep red colour, hydroxocobalamin has the potential to interfere with determination of blood chemistry, haematology, coagulation, and urine parameters. Interferences were studied *in vitro* and in healthy volunteers. Since the pharmacokinetic profile of hydroxocobalamin may be modified in the presence of cyanide, the extent and duration of interference in cyanide-poisoned patients may differ from healthy volunteers. Data suggested that laboratory results may vary considerably from one analyser to another, and depend on hydroxocobalamin concentration and partially on the time between sampling and measurement. Therefore, caution is required when reporting and interpreting laboratory results. This is reflected in the SPC and the labelling (educational sticker for medical and healthcare professionals).

- Safety in special populations

The study protocol of the clinical study EML 015722 was amended in adding blood sample to the study to be used for genotyping experiments. The aim of this additional investigation was to evaluate a possible correlation between genotype of the $^{-786}$C/T polymorphism of the human *nos-3 gene* and cardiovascular diseases. From the 90 blood samples, only 53 samples following administration of active treatment were evaluable and a failure of analysis has been observed for about 22%.

Indeed a potential association was reported between C/T *nos-3* gene polymorphisms and an increased risk of coronary heart disease. It was demonstrated that patients with a single nucleotide polymorphism at position-786 in the promotor of the *nos-3* gene had a decreased capacity to produce nitric oxide (NO) in the endothelium.. Nitric oxide playing a role in regulation vascular tone and in maintenance of properties of vessel wall, the presence of the genotype CC may predispose patients to or accelerate coronary heart disease.

No correlation was found with any of the 3 genotypes, CT, CC, TT for either SBP, DBP, MAP, HR.

- Safety related to drug-drug interactions and other interactions

No interaction studies have been performed, which is considered acceptable in regards to the indication.

- Discontinuation due to adverse events

One subject in clinical study EML 015722 was discontinued in 10 g dose group of hydroxocobalamin due to allergic reaction. This reaction occurred 12 min after the start of administration of hydroxocobalamin while the patient had received 3g.

- Post marketing experience

Cyanokit has marketing authorisations in France (1996), Hong Kong (1999) and USA (2006). Cyanokit has been distributed, based on the French marketing authorisation, in 37 other countries (18 EU member states and 19 countries outside Europe).

However, the experience with Cyanokit outside France is very limited. The few data from literature seem to report similar use of hydroxocobalamin outside France as in France, with similar efficacy and tolerance.

- Discussion on clinical safety

The IV administration of hydroxocobalamin (5.0 or 10.0 g) appears safe and well tolerated, even when administered in the prehospital setting. In general, there was little difference in the incidence of AEs across age categories or between genders. The safety of hydroxocobalamin has been evaluated in study EML 015722-H101, a prospective study in 136 healthy volunteers, and in 4 French clinical studies of subjects with known or suspected cyanide poisoning. A number of ADRs, most of which were transient and non-serious, have been associated with the use of hydroxocobalamin:

Chromaturia and erythema are the primary side effects of treatment with hydroxocobalamin and cannot be avoided. These effects can be a potential source of interference on the assessment of burn lesions (erythema) and of a number of laboratory parameters. This is reflected in the SPC and the labelling (educational sticker for medical and healthcare professionals). However, there are no other known medical consequences of these effects.

• Chromaturia was detected in most subjects treated with hydroxocobalamin. Chromaturia appeared promptly after administration of hydroxocobalamin and resolved without treatment or sequelae.

• Erythema was detected in most subjects treated with 5.0 g or more of hydroxocobalamin. Erythema appeared during the administration of hydroxocobalamin and resolved without treatment or sequelae.

• Elevated blood pressure was detected in some healthy volunteers treated with 2.5 g or more of hydroxocobalamin. A return to near pre-dose mean blood pressure values was generally observed within 4 to 8 hours post-dose.

• In some smoke inhalation victims, hydroxocobalamin was associated with reversible and transient increases in blood pressure and, more rarely, with tachycardia. The observed alterations in cardiovascular status rarely, if ever, appear to pose a risk to patients. The risk of these potential side effects must be weighed against the very real lethality induced by exposure to cyanide.

• Allergic reactions have been reported infrequently in healthy volunteers as well as in post-marketing experience. Similar reactions have been reported with chronic use of low-dose hydroxocobalamin in the treatment of Vitamin B12 deficiency.

• Pustular or papular rash was detected in a small number of subjects treated with 5 g or more of hydroxocobalamin. The rash was most often located on the face and neck, and resolved spontaneously without treatment.

• The results of haematological, coagulation, chemistry, and urinary analyses may well be altered by hydroxocobalamin. Interpretation of such values should be performed with caution.

• No clinically relevant ECG alterations, respiratory alterations, or blood gas alterations have been attributed to the use of hydroxocobalamin.

5. Pharmacovigilance

Detailed description of the Pharmacovigilance system

The CHMP considered that the Pharmacovigilance system as described by the applicant fulfils the legislative requirements.

Risk Management Plan

The MAA submitted a risk management plan.

Table Summary of the risk management activities

Safety concern	Proposed Pharmacovigilance activities	Proposed risk minimization activities
Erythema and Chromaturia	Routine Pharmacovigilance activities.	These risks are adequately described in the product information (see section 4.8, and non-promotional educational sticker)
Burn assessment interference	Routine Pharmacovigilance activities.	This risk is adequately described in the product information (see section 4.4, and non-promotional educational sticker).
Clinical laboratory analysis interference	Routine Pharmacovigilance activities.	This risk is adequately described in the product information (see section 4.4, and non-promotional educational sticker).
Allergic reaction	Routine Pharmacovigilance activities.	This risk is adequately described in the product information (see section 4.8).
Increase in blood pressure	Routine Pharmacovigilance activities.	This risk is adequately described in the product information (see section 4.8).

The CHMP, having considered the data submitted in the application, is of the opinion that no additional risk minimisation activities are required beyond those included in the product information. Health care professionals will be warned on potential interference of Cyanokit with burn lesions assessment or laboratory tests via a non-promotional educational sticker that will be included in each Cyanokit pack and that could be attached to the patient's medical record

6. Overall conclusions, risk/benefit assessment and recommendation

Quality

The quality of this product is considered to be acceptable when used in accordance with the conditions defined in the SPC. Physicochemical and biological aspects relevant to the uniform clinical performance of the product have been investigated and are controlled in a satisfactory way. At the time of the CHMP opinion, there were a number of minor unresolved quality issues having no impact on the Benefit/Risk ratio of the product. The applicant gave a Letter of Undertaking and committed to resolve these as Follow Up Measures after the opinion, within an agreed timeframe.

Non-clinical pharmacology and toxicology

In the dog efficacy model, Cyanokit significantly improved survival following cyanide administration, and produced a more rapid recovery of cardiovascular and respiratory parameters compared with controls.
Hydroxocobalamin binds coordinatively to plasma proteins and also reacts with cyanide forming cyanocobalamin which is a highly stable complex. In dogs and in humans, both hydroxocobalamin and cyanocobalamin are mainly excreted in the urine.
Hydroxocobalamin has a low acute toxicity. The main target organs of toxicity in repeat-dose studies were the liver, kidney and bone marrow, with findings suggestive of tissue overload with high quantities of hydroxocobalamin. Similar findings were seen in a repeated dose study with cyanocobalamin.
No genotoxic potential have been identified in a standard tests battery. The carcinogenic potential was not studied which is acceptable in view of the toxicological profile and the indication.
Data on the reproductive potential are limited and this is reflected adequately in the SPC. In order to complete the knowledge in this area, embryo-foetal studies (rats and rabbits) will be performed as a post-authorisation commitment (follow-up measure).
No significant risk to the environment is expected.

Efficacy

Based on the provided data, the efficacy of Cyanokit is established. The efficacy of the antidote treatment appeared to be determined by several factors including time between cyanide exposure and hydroxocobalamin treatment, severity of poisoning defined by blood cyanide concentration and critical clinical events preceding antidote therapy (mainly cardiac arrest), quantitative relationship between antidote and cyanide, and adequacy of symptomatic treatment. These results underline the necessity for immediate antidote treatment in case of suspected or known cyanide poisoning, preferably at the scene of the incident and without awaiting confirmatory tests.

Despite the lack of comparative study between different cyanide antidotes, conclusions of the guidance document EMEA/CPMP/1255/03 "*on the Use of Medicinal Products for the Treatment of Patients Exposed to Terrorist Attacks with Chemical Agents*" are still valid. Hydroxocobalamin can be considered as the best choice antidote if available, as it is experimentally well documented with clear advantages in situations such as fires with concomitant exposure to agents that reduce oxygen transport, such as carbon monoxide.

Safety

The safety database was collected from five studies: 3 retrospectives studies, one phase I study in healthy volunteers and one phase III prospective study. Overall, this safety population included 347 subjects/patients who received at least 1 dose of hydroxocobalamin.

Based on the provided data, the intravenous administration of hydroxocobalamin (5.0 or 10.0 g) appears safe and well tolerated, even when administered in the pre-hospital setting. In general, there was little difference in the incidence of AEs across age categories or between genders. The safety of hydroxocobalamin has been evaluated in the phase I study (EML 015722-H101) in 136 healthy volunteers, and in 4 French clinical studies of subjects with known or suspected cyanide poisoning.

Most of the adverse drug reactions (ADR) were transient and considered as non serious. Chromaturia was observed in most subjects/patients, as well as erythema which may interfere with the clinical assessment of burns. Both reactions resolved without treatment. Allergic reactions were rare. Other cutaneous reactions such as pustular or papular rash were observed in approximately a third of the healthy volunteers of study EML 015722-H101 but not in the Baud Study 1. Increases in blood pressure were observed in 18.2% of healthy volunteers treated with a 5.0 g dose and 27.8% of those treated with a 10 g dose. More rarely, tachycardia was associated. Increases started 2-5 minutes after start of the infusion, reached a maximum at the end of the infusion and returned to near baseline values 4-8 hours post-dose. Similar finding were observed in the Baud Study 2.

Results of haematological, coagulation, chemistry, and urinary analyses may be altered by hydroxocobalamin. Interpretation of values of these laboratory parameters should be performed with caution. Lastly, no clinically relevant ECG, respiratory or blood gas alterations have been attributed to the use of hydroxocobalamin.

Overall, despite the small number of patients studied, hydroxocobalamin was well tolerated in subjects in case of non cyanide poisoning. The intra venous administration was not associated with a deleterious safety profile. Section 4.8. of the proposed SmPC adequately reflects the safety profile of Cyanokit. Specific warnings and precautions for use are also adequately reflected in section 4.4. of the SmPC.

From the safety database all the adverse reactions reported in clinical trials have been included in the Summary of Product Characteristics

Having considered the safety concerns, the CHMP considered that the proposed activities described in the risk management plan adequately addressed these

- User consultation

The study was performed according to an acceptable methodology.
The report shows clearly that all respondents were able to answer correctly and without difficulty.
Therefore, the test is considered to be satisfactory.

Risk-benefit assessment

Hydroxocobalamin is a complexation agent that acts by direct binding of the cyanide ions. Each hydroxocobalamin molecule can theoretically bind 1 cyanide ion by substituting the hydroxo ligand that is linked to the central trivalent cobalt ion. The resulting molecule cyanocobalamin is a highly stable, nontoxic compound that is excreted in the urine.

In the current dossier, evidence for efficacy is based on 5 studies of hydroxocobalamin as a cyanide antidote:

- One non clinical study placebo-controlled performed in 54 cyanide-poisoned dogs showed the beneficial effect of hydroxocobalamin on survival and neurological sequelae compared to placebo in the setting of acute cyanide poisoning.

- Four non-controlled, non comparative clinical studies either in suspected cyanide-poisoning due to fire smoke inhalation or in known cyanide exposure from sources other than fire smoke, provided evidence of hydroxocobalamin efficacy despite the lack of controlled data.

Based on provided data, hydroxocobalamin appears to be safe and well tolerated. Neither serious adverse event nor death related to the drug was reported, even in healthy volunteers.

In conclusion, hydroxocobalamin, at initial dose of 5 g which can be repeated once if needed, appears to be well tolerated and efficient as a cyanide antidote on survival outcome and prevention of neurological sequelae. As no percentage of success is available with other antidotes or with supportive treatment, no comparative analysis is possible. However, compared to other available antidotes, due to its favourable safety profile even in non-intoxicated subjects, hydroxocobalamin presents advantages in situations were cyanide-intoxication is only suspected, such as fire smoke inhalation.

A risk management plan was submitted. The CHMP, having considered the data submitted, was of the opinion that:

- Routine pharmacovigilance was adequate to monitor the safety of the product. Health care professionals will be warned on potential interference of Cyanokit with burn lesions assessment or laboratory tests via a non-promotional educational sticker (part of the product

information) that will be included in each Cyanokit pack and that could be attached to the patient's medical record.
- No additional risk minimisation activities were required beyond those included in the product information.

Recommendation

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considered by consensus, that the risk-benefit balance of Cyanokit (hydroxocobalamin) was favourable for the following indication: "Treatment of known or suspected cyanide poisoning. Cyanokit is to be administered together with appropriate decontamination and supportive measures." Therefore, the CHMP recommended the granting of the marketing authorisation.