Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Methylthioninium chloride Proveblue

International Nonproprietary Name: methylthioninium chloride

Procedure No.: EMEA/H/C/002108

Note

assessment report as adopted by the CHMP with all information of a commercially confidential nature deleted.

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List of abbreviations

BIS	Bispectral index
BLMB	Benzoyl leuco methylene blue
BP	British Pharmacopeia
CHMP	Committee for Medicinal Products for Human Use
CNS	Central nervous system
DRF	Dose-ranging finding
EMA	European Medicines Agency
ERA	Environmental risk assessment
GC	Gas Chromatography
G6PD	glucose-6-phosphate dehydrogenase
GLP	Good Laboratory Practice
HPLC	High-performance liquid chromatography
ICH	International Conference on Harmonisation
ICP-MS	Inductively Coupled Plasma Mass Spectroscopy
IR	Infrared Spectroscopy
IV	Intravenous
LMB	Leucomethylene blue
LOD	Limit of detection
LOQ	Limit of quantification
MB	Methylene blue
MetHB	Methaemoglobin
NADH	nicotinamide adenine dinucleotide
NADPH	nicotinamide adenine dinucleotide phosphate
NOAEL	No-observed adverse effect level
NTP	National Toxicology Program
Ph Eur	European Pharmacopoeia
RMP	Risk management plan
SmPC	Summary of product characteristics
USP	United States Pharmacopeia

1. Background information on the procedure

1.1. Submission of the dossier

The applicant Provepharm S.A.S. submitted on 30 December 2009 an application for Marketing Authorisation to the European Medicines Agency (EMA) for Methylthioninium chloride Proveblue, through the centralised procedure under Article 3(2)(b) of Regulation (EC) No 726/2004. The eligibility to the centralised procedure was agreed upon by the EMA/CHMP on 25 June 2009. The eligibility to the centralised procedure under Article 3(2)(b) of Regulation (EC) No 726/2004 was based on demonstration of significant technical innovation.

The legal basis for this application refers to Article 10(3) of Directive 2001/83/EC, as amended – Hybrid application.

The chosen reference product is:

Reference medicinal product which is or has been authorised in accordance with Community provisions in force for not less than 6/10 years in the EEA:

- Product name, strength, pharmaceutical form: Methylthioninium Chloride Injection USP 1%, solution for injection, 10 mg/ml
- Marketing authorisation holder: Martindale Pharmaceuticals Limited
- Date of authorisation: 3 November 1987
- Marketing authorisation granted by: United Kingdom
- Marketing authorisation number: PL 00156/0039

Reference medicinal product authorised in the Community/Member State where the application is made:

- Product name, strength, pharmaceutical form: Methylthioninium Chloride Injection USP 1%, solution for injection, 10 mg/ml
- Marketing authorisation holder: Martindale Pharmaceuticals Limited
- Date of authorisation: 3 November 1987
- Marketing authorisation granted by: United Kingdom
- Marketing authorisation number: PL 00156/0039

Differences compared to the reference medicinal product: change in therapeutic indication (to extend the indication to the treatment of chemical products-induced methaemoglobinaemia and to the use in children) and change in strength (quantitative change to the active substance).

The application submitted is composed of administrative information, complete quality data, non-clinical and clinical data based on bibliographic literature substituting all non-clinical tests and clinical studies.

The applicant applied for the following indication: Treatment of medicinal and chemical products-induced methaemoglobinaemia. Methylthioninium chloride Proveblue is indicated in adults and children above the age of 3 months.

Information on Paediatric requirements

Not applicable

Scientific Advice:

The applicant received Scientific Advice from the CHMP on 25 June 2009. The Scientific Advice pertained to non-clinical and clinical aspects of the dossier.

Licensing status

The product was not licensed in any country at the time of submission of the application.

1.2. Steps taken for the assessment of the product

The Rapporteur and Co-Rapporteur appointed by the CHMP and the evaluation teams were:

Rapporteur: **Kristina Dunder**

Co-Rapporteur: **Patrick Salmon**

- The application was received by the EMA on 30 December 2009.
- The procedure started on 20 January 2010.
- The Rapporteur's first Assessment Report was circulated to all CHMP members on 9 April 2010. The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on 9 April 2010.
- During the meeting on 17-20 May 2010, the CHMP agreed on the consolidated List of Questions to be sent to the applicant. The final consolidated List of Questions was sent to the applicant on 21 May 2010.
- The applicant submitted the responses to the CHMP consolidated List of Questions on 13 October 2010.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of Questions to all CHMP members on 26 November 2010.
- During the CHMP meeting on 13-16 December 2010, the CHMP agreed on a List of Outstanding Issues to be addressed in writing by the applicant.
- The applicant submitted the responses to the CHMP List of Outstanding Issues on 13 January 2011.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of Outstanding Issues to all CHMP members on 31 January 2011.
- During the meeting on 14-17 February 2011, the CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a Marketing Authorisation to Methylthioninium chloride Proveblue on 17 February 2011.
- The CHMP opinions were forwarded in all official languages of the European Union, to the European Commission, which adopted the corresponding Decision on 6 May 2011.

2. Scientific discussion

2.1. Introduction

Methaemoglobinaemia is characterised by a reduced ability of the blood to carry oxygen because of reduced levels of normal haemoglobin. Therefore, this condition manifests as tissue hypoxia and ischemia. Methaemoglobinaemia can be either inherited or acquired following the exposure to toxic agents, the latter being more common. Acquired methaemoglobinaemia occurs after exposure to drugs, toxins, or their metabolites or may arise from dietary aetiologies. These may include chlorate and bromate local anaesthetics, antibiotics (*e.g.* trimethoprim, sulfonamides and dapsone), metoclopramide, zopliclone, nitrates and nitrites from food, water chemical and medicines, aniline products and pesticides.

Acquired methaemoglobinaemia is a rare condition with an approximate estimate of 75 patients suffering from acquired methaemoglobinaemia per year in Europe. Infants are more likely to develop methaemoglobinaemia due to several factors related to their young age. The redox protective system is not mature before the age of 3 months and the gastric pH in infants encourages the conversion of nitrates into nitrites. Furthermore, the frequency of poisonings is two to four times higher for children under four years than for adults. The elderly are subject to methaemoglobinaemia when suffering from anaemia or cardiopulmonary disorders, especially since Diaphorase I (NADH) is less responsive.

Acute methaemoglobinaemia may be life-threatening and requires an emergency treatment. Treatment of methaemoglobinaemia relies on rapid identification of the condition, the removal of the offending drug or toxin and the administration of substances that rapidly convert the ferric iron back to its ferrous state. Currently, a consensus opinion exists among the medical community using methylthioninium chloride in case of methaemoglobinaemia.

The eligibility to the centralised procedure based on demonstration of significant technical innovation was granted as at present there is no commercial source of methylthioninium chloride active substance in compliance with Ph Eur. Methylthioninium chloride has a strong affinity for metals, and it is difficult to manufacture a substance that complies with the Ph Eur monograph. The applicant utilises a manufacturing process which allows obtaining an active substance which is metal-free and contains very low levels of organic impurities.

Methylthioninium chloride acts as an electron donor for the non-enzymatic reduction of methaemoglobinaemia. A distinct enzyme, NADPH methaemoglobin reductase, converts methylthioninium chloride (the oxidized form) to leucomethylene blue (LMB, *i.e.* the reduced form), using NADPH. The reduced form then chemically reduces methaemoglobin ($Hgb(Fe^{3+})$) to haemoglobin ($Hgb(Fe^{2+})$).

Methylthioninium chloride Proveblue is provided as a solution for injection, 5 mg/ml, and is intended for intravenous usage administered by healthcare professionals only. The usual dose in adults and children is 1 to 2 mg (0.2-0.4 ml) per kg body weight over a period of 5 minutes. In infants ≤ 3 months the recommended dose is 0.3 to 0.5 mg (0.06 to 0.1 ml) per kg body weight over a period of 5 minutes. A repeat dose may be given after one hour if required (see SmPC). The maximum recommended total dose in adults and children is 7 mg/kg. There are no dose adjustments recommended in organ impairment, but caution is recommended in moderate and severe renal impairment.

Since Methylthioninium chloride Proveblue is hypotonic the SmPC suggest that it may be diluted in glucose 5% solution for injection. It should be noted that dilution with sodium chloride solution for injection should be avoided because chloride reduces the solubility of methylthioninium chloride.

2.2. Quality aspects

2.2.1. Introduction

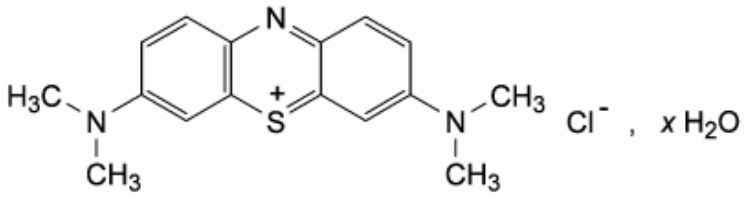
Methylthioninium chloride Proveblue is presented as solution for injection, containing 50 mg of methylthioninium chloride (active substance) in 10 ml (5 mg/ml). The only excipient is water for injections.

The solution is clear, deep blue with a pH value between 3.0 and 4.5 and osmolality between 10 and 15 mOsm/kg.

The product is supplied in 10 ml type I glass ampoules.

2.2.2. Active Substance

Methylthioninium chloride (INN), also known as methylene blue (USAN name), is chemically designated as 3,7-bis(dimethylamino)-phenazathionium chloride as has the following structure:



Methylthioninium chloride is a dark blue, crystalline powder with a copper-coloured sheen or green crystals with a bronze-coloured sheen.

The solubility of methylthioninium chloride has been determined in water at different temperatures. The substance is slightly soluble in water and very slightly soluble in DMSO and ethanol.

In addition, the potential for precipitation of methylthioninium chloride diluted in NaCl 0.9% w/v has been investigated. The presence of NaCl could lead to precipitation of the active substance. Solubility measurements have been undertaken in pure water and in water at different pH where the ionic strength was adjusted by KCl. Significant differences were observed. This difference is attributed to a common ion effect with the chloride ions. Chloride ions decrease the solubility by a factor of 50.

The molecule of methylthioninium chloride is highly charged and the pKa is in the range 0-1. The log P value (octanol:phosphate pH 7 buffer) is -0.9, and the log D value is 0.06.

Several polymorphic forms of methylthioninium chloride exist. As the finished product is a solution of the active substance, the polymorphic form is not considered relevant for the finished product in this case.

Manufacture

A technical innovation in the manufacturing process leads to a substance which is essentially free from heavy metal impurities. This is based on a metals-free synthesis route, where no purification step to remove metals is included. The process has been patented by the applicant.

The aim of the development was to obtain methylthioninium chloride compliant with the Ph Eur monograph, in particular with respect to metal levels and related substances suitable for intravenous use.

The manufacturing process utilised allows obtaining an active substance which is metal-free and contains very low levels of organic impurities. Methylthioninium chloride has a strong affinity for metals, and it is difficult to manufacture a substance that complies with the Ph Eur monograph.

The manufacturing process of the active substance has been described in sufficient detail. The critical steps and intermediates have been satisfactorily described and the process controls have been justified. The process has been prospectively validated on three production scale batches. Results confirm that the process can yield a consistent and reproducible substance which complies with the acceptance criteria, and which has a low metal content.

Confirmation of the chemical structure of methylthioninum chloride was provided by the route of synthesis and it has been verified by IR and UV spectroscopy. This is normally not considered satisfactory, but since this is a well-known compendial substance and comparison with the Ph Eur methylthioninium chloride CRS has been performed, this was regarded sufficient for the general structure elucidation.

Potential impurities have been well discussed in relation to their origin (raw material, manufacturing process and degradation products) and potential carry-over into the final drug substance.

Specification

The active substance specification is in compliance with the Ph Eur monograph for methylthioninium chloride, except for solubility in water and ethanol. This difference may arise from the purification process in this case. The specification includes tests for identity (UV/VIS, TLC, colour reaction, reaction of chlorides), appearance, solubility (water and ethanol), assay, related substances, metals (Al, Cd, Cr, Cu, Sn, Fe, Mn, Hg, Mo, Ni, Pb, Zn), loss on drying, sulphated ash, methanol-insoluble substances, residual solvents (GC and HPLC), pH, impurities from synthesis, microbial purity and endotoxins.

The active substance is tested with methods according to the Ph Eur monograph for methylthioninium chloride for appearance, solubility, assay, identification, methanol-insoluble substances, related substances (Azure B, each other, sum of impurities other than Azure B), loss on drying and sulphated ash. The trace metals are determined by in-house ICP-MS method which is similar to the one prescribed in the Ph Eur monograph. Residual solvents are determined by GC and HPLC methods. Impurities from synthesis are quantified by a HPLC method with UV detection and compendial methods are used for the microbiological tests.

Non-compendial methods have been validated (trace elements by ICP-MS, residual solvents by GC and HPLC, synthesis impurities by HPLC).

The ICP-MS method has been validated for the respective metals for linearity, accuracy and precision in appropriate ranges. The limit of detection (LOD) and limit of quantification (LOQ) have also been estimated and are considered sufficient to allow accurate and precise determination at the respective specification limits. The specificity of the method was not addressed in the validation, but the ICP-MS method can be considered inherently specific for the respective elements.

The residual solvents method has been satisfactorily validated in suitable ranges for specificity, linearity, accuracy and precision. The LOD and LOQ have been estimated and are considered sufficient for the determination of the solvents. The stability of solutions has also been evaluated.

The HPLC method for residual solvent has been satisfactorily validated for linearity, accuracy and precision in suitable ranges. The stability of solutions has also been investigated. Specificity has been verified by chromatograms.

The HPLC method for synthesis impurities has been suitably validated for each of the synthesis impurities as regards linearity, accuracy and precision. LOD and LOQ have been estimated and verified. Specificity has been demonstrated by relevant chromatograms. The stability of solutions and the robustness of the method have also been evaluated.

Batch analysis data were provided for three production scale batches manufactured by the proposed commercial manufacturer according to the process. Results complied with the proposed specifications. The residual solvents and trace metal contents were below LOQ. It can be concluded that the batch analysis results indicate that the process is under control and the substance of acceptable purity is consistently produced.

Stability

Stability studies according to ICH guidelines have been initiated on three production scale batches of the drug substance. The batches are stored at accelerated (40°C/75% RH) and long term conditions (25°C/60% RH, 30°C/65% RH) and kept in the proposed container closure system. The testing is performed against the specification and with the proposed methods.

Supportive stability data from three pilot scale batches were also provided.

In addition forced degradation studies have been conducted on one pilot batch of the drug substance. Methylthioninium chloride in solution was treated with HCl, NaOH or hydrogen peroxide. The drug substance in the solid state has also been subjected to testing upon exposure to light (500 $W/h/m^2$) and elevated temperature. The untreated sample was used as reference. Obtained results showed that methylthioninium chloride is sensitive to light, alkaline and acid conditions.

2.2.3 Finished Medicinal Product

Pharmaceutical Development

The products found on the EU market, both registered and unregistered products, as well as in the US were described as 1% solutions of methylthioninium chloride. The BP only gives the content as methylthioninium chloride trihydrate 95.0-105.0% of the label claim without specifying any particular strength of the drug product.

The objective of the formulation development has been to obtain a solution for injection of methylthioninium chloride complying with the general monographs of the Ph Eur and also with the BP monograph *Methylthioninium Injection* for use in the treatment of medicinal and chemical products-induced methaemoglobinaemia.

Methylthioninium chloride manufactured by the applicant is very low in both organic and inorganic impurities and fulfils the strict criteria for metal contents of the Ph Eur. The applicant has found a strong common ion effect (Cl^-) on solubility, and dilution of the product with saline should be avoided. Because of the lower solubility of the substance as compared to other less pure grades of methylthioninium chloride, it has not been considered suitable to manufacture a finished product with the same strength as the reference product, 10 mg/ml. Instead, the 5 mg/ml strength was proposed to avoid precipitation. As the solubility is also very temperature dependent the product should not be refrigerated or frozen to avoid precipitation.

The USP and BP monographs for methylthioninium chloride injection require that the pH value is of 3.0-4.5. This criterion was adopted for the proposed formulation, especially because at higher pHs, degradation was observed upon autoclave sterilisation.

Methylthioninium chloride is sensitive to light and because of this the primary packaging suitable would be brown glass. However, methylthioninium chloride absorbs metals from the brown glass material both in solution and in the solid state, and thus clear glass ampoules were chosen as the primary packaging. A storage precaution to protect the product from light by keeping the ampoules in the outer carton was included in the product information. Chloride ions significantly reduce the solubility methylthioninium chloride and the finished product is not compatible with 0.9% saline due to risk for precipitation. Compatibility with 5% glucose solution has been demonstrated.

Manufacturing process development has been well documented. It has been shown that the manufacturing process is robust and its choice was considered justified.

The formulation development was generally described satisfactorily. The key critical parameters were identified and successfully evaluated.

Adventitious agents

None of the substances used in the manufacture of Methylthoninium chloride Proveblue is of human or animal origin.

Manufacture of the product

The manufacturing is standard and involves dissolution, filtration and terminal sterilisation. The critical steps in the process have been identified and described and the process has been sufficiently validated with three consecutive production scale batches. A bulk holding time was considered acceptable. The bulk solution is filtrated through 0.2 µm before filling and the product is terminally sterilised according to Ph Eur criteria (121°C for 20 minutes). The sterilisation step has been demonstrated to not have effect on the appearance, pH and assay. The validation batches passed the sterilisation test and also complied in all instances with the finished product specifications. The filter used in the process has been adequately validated. The process is able to produce a product of consistent and adequate quality.

Product specification

The product specifications include tests for appearance, pH, extractable volume, uniformity of dosage units, identification (TLC and HPLC), assay (and identification), related substances (HPLC), particulate contamination, sterility, bacterial endotoxins.

Analytical methods have been sufficiently described, some of them are compendial methods described in the Ph Eur. Adequate validation data have been provided for non-compendial methods.

Validation information has been provided for the HPLC methods for assay of the active and the two methods for impurities. The remaining methods are compendial. For sterility and endotoxin tests the applicant demonstrated these methods were suitable for the methylthioninium chloride containing product.

Batch analysis results on three commercial scale batches demonstrated compliance with the proposed specifications and confirmed consistency and uniformity of the product. The results were consistent from batch to batch and proved that the product can be manufactured reproducibly according to the agreed specifications.

Stability of the product

Stability studies according to ICH guidelines are on-going for three commercial scale and two pilot scale batches of finished product in its marketing package.

The studies comprise accelerated (40°C/75% RH) and long term storage (25°C/60% RH, 30°C/65% RH) according to ICH guidelines.

A photo-stability study has also been carried out on one batch of finished product. The study was performed according to ICH Q1B. The following samples were subjected to light: Naked ampoules, ampoules in blister without paper cover, ampoules in blister and with paper cover. An ampoule not subjected to light served as reference. No other forced degradation studies have been conducted on the finished product as it is only an aqueous solution of the drug substance.

It is stated in the Ph Eur monograph that methylthioninium chloride should be stored protected from light and this was confirmed in the forced degradation studies on the drug substance. Although the photodegradation was not observed during the testing it is proposed that the product is stored in the outer carton on order to protect from light.

The results generated during the stability studies and statistical analyses support the proposed shelf life and storage conditions as defined in the SmPC.

2.2.4 Discussion on chemical, pharmaceutical and biological aspects

Information about the active substance, methylthioninium chloride, has been provided. The active substance is a known substance with a monograph in the Ph Eur. The applicant utilises a manufacturing process which allows obtaining an active substance in a form which has a low metal content and contains low levels of organic impurities, and is therefore suitable for intravenous use.

The impurity profile of methylthioninium chloride has been satisfactorily addressed.

The control tests and specifications for drug substance product are satisfactory and in accordance with the Ph Eur monograph for this substance.

A retest period was supported by satisfactory stability studies.

The finished product is a simple aqueous solution of methylthioninium chloride in water for injections filled in a glass ampoule and terminally sterilised in an autoclave.

The method of manufacture is considered standard and has been satisfactorily described, including hold times and in-process tests. The scale of manufacture is supported by batch data. The data shows consistent manufacture and is considered sufficient for this standard manufacturing process.

The product is controlled according to acceptable specifications. The batch data demonstrate consistent manufacture.

The stability programme is considered satisfactory. The batches placed on stability are considered representative of the product to be marketed. The results generated during the stability studies support the proposed shelf life and storage conditions as defined in the SmPC.

2.2.5 Conclusions on the chemical, pharmaceutical and biological aspects

The drug substance and the drug product have been appropriately characterised and generally satisfactory documentation has been provided. The results indicate that the active substance and the finished product can be reproducibly manufactured and therefore the product is suitable for intravenous use and should have a satisfactory and uniform performance in the clinic.

2.3. Non-clinical aspects

2.3.1. Introduction

The non clinical development program of Methylthioninium chloride Proveblue is mainly based on bibliographical data.

Some complementary studies have been performed to bridge to the reference product (Methylthioninium Chloride Injection USP 1% w/v, marketing authorisation holder: Martindale Pharmaceuticals Limited authorised in UK).

Two of the studies submitted by the applicant were GLP compliant, the Ames test and the 1-month repeat-dose toxicity study in dogs.

Methylthioninium chloride is also known as methylene blue (MB).

2.3.2. Pharmacology

Methaemoglobinaemia is characterised by a reduced ability of the blood to carry oxygen because of reduced levels of normal haemoglobin. Therefore, this condition manifests as tissue hypoxia and ischemia.

Primary pharmacodynamic studies

In human and veterinary medicine, methylthioninium chloride is primarily prescribed for acquired methemoglobinemia arising from the ingestion of nitrites, quinines, aniline, nitrobenzenes, nitrotoluenes, and sulfonamides – compounds which oxidize hemoglobin (Fe2+) to methemoglobin (Fe3+). The presence of methemoglobin decreases the oxygen-carrying capacity of the blood and shifts the oxygen dissociation curve to the left. In the erythrocyte, methemoglobin is chiefly reduced to hemoglobin via methemoglobin reductase which utilises reduced NAD as the electron donor. A second pathway uses reduced NADPH derived from the metabolism of glucose via glucose-6-phosphate dehydrogenase (G6PD). In the case of poisoning, the intrinsic ability of the red blood cell to reduce methemoglobin is rapidly overwhelmed. In these cases, an exogenous electron donor, usually methylthioninium chloride is administered. In the erythrocyte, methylthioninium chloride is reduced to the colourless leucomethylene blue only by NADPH. Leucomethylene blue then reduces methemoglobin nonenzymatically, resulting in hemoglobin and methylthioninium chloride. The leuco form of the dye is regenerated by NADPH completing the cycle (see Figure 1). Methylthioninium chloride is administered either iv or orally for the treatment of acute and idiopathic methemoglobinemia.

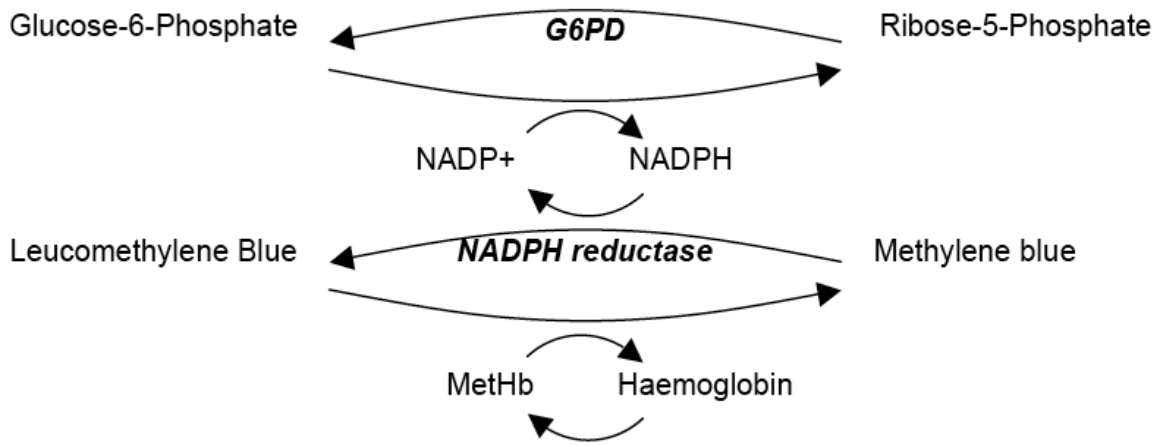


Figure 1: Reduction of methaemoglobin to haemoglobin by NADPH reductase.

No primary pharmacodynamic studies were submitted. The applicant submitted bibliographic data to support the application (data not shown).

Secondary pharmacodynamic studies

No secondary pharmacodynamic studies were submitted. The applicant submitted bibliographic data to support the application (data not shown).

Safety pharmacology programme

MB acts as a cofactor accelerating the conversion of methaemoglobin to haemoglobin in erythrocytes.

However, at higher concentrations, MB can cause some conversion of haemoglobin back to methaemoglobin and may cause methaemoglobinaemia. Haemolysis has also been noted; however, this effect is more evident in *in vitro* studies and may be minimal in intact erythrocytes.

Cardiovascular system

Following bolus intravenous injections of MB in rats (5 and 50 mg/kg) blood pressure and heart rate remained within the normal physiologic ranges (Vutskits 2008).

However, an increase in blood pressure followed by hypotension was observed following administration of MB at 10 and 20 mg/kg IV (Oktay 1993). The effects on blood pressure appeared to be transient. Clinical experience with MB indicates that at therapeutic dose (1mg/kg) there are no significant cardiovascular effects of MB. However, at 7 mg/kg iv (the maximum daily recommended dose), electrocardiogram changes (T wave flattening or inversion) have been reported in humans without methaemoglobinaemia, resolving within 2–12 hours of the injection (Bradberry 2003).

Respiratory Function

Direct effects of MB on the respiratory system are expected to be limited compared to the impact of methaemoglobinaemia, since reduced levels of haemoglobin are expected to result in a compensatory increase in respiratory parameters such as rate and volume.

Central Nervous system

Neurotoxic effects after administration of MB in vitro and in vivo have been reported in the literature. The incubation of slices of young rat cerebellum incubated for one hour with MB (10 to 100 µM), caused a progressive destruction of the differentiating cells (Garthwaite 1988). Similarly, a suppression of evoked excitatory field potentials in hippocampal slices at 1h following incubation with 10 µM MB has been reported; this was associated with an increase in dying cells at doses of 10 µM MB (Vutskits 2008). Intrathecal administration of MB in cats resulted in neuronal damage and inflammation and associated paraplegia. In humans, doses of 5-10 mg/kg iv during parathyroidectomy have been associated with prolonged post operative disorientation and were associated with serotonin toxicity.

Pharmacodynamic drug interactions

No significant nonclinical data on pharmacodynamic drug interactions have been reported in association with MB.

2.3.3. Pharmacokinetics

Rats receiving a single IV administration of non-radiolabelled methylthioninium chloride at 2, 5, 7.5, 10, 15 and 25 mg/kg and were examined at 3 min post dose. Four tissues (kidney>heart>liver>lung) concentrated 25-36% of the dose, indicating very rapid uptake into tissues (Disanto 1972/III).

Tissue distribution was also measured in rats 1 h after receiving a single intraduodenal or IV administration of non-radiolabelled methylthioninium chloride. Following IV administration, the highest concentrations were measured in bile>brain>liver>blood, with no methylthioninium chloride in intestinal wall. Following intraduodenal administration, the highest concentrations were in bile>intestinal wall>liver>brain, with no methylthioninium chloride measured in blood. Following both routes of administration, levels in tissues were up to hundred times that measured in blood. The high concentration in bile after both routes of administration indicates that this was a major route of elimination (Peter 2000). Methylthioninium chloride radiolabelled with ^{123}I and ^{221}At was shown to be strongly bound to pigmented tissues. There was no obvious retention of radio-iodinated methylthioninium chloride in the brain over the observation period and in the eyes for at least the first 14 h (Link 1989; Link 1996).

In rabbits, the level of plasma protein binding was measured *in vitro* at 71-77%, increasing with concentration. Binding to BSA was of the same order (64-71%) but had no relationship with concentration (Kozaki 1981).

In the rat, following intra-duodenal and intravenous administration of MB, the bile was indicated as a major route of elimination (Peter 2000). In the rabbit, MB was excreted into urine and bile mostly as leucomethylene blue (Watanabe 1977a). In male dogs receiving a single oral administration of non-radio-labelled MB, 3.9% was recovered in the urine and 44.3% in the faeces Watanabe (1977b).

2.3.4. Toxicology

The following five studies were submitted:

- Zebrafish study carried out with three different sources of MB active substances.
- A mitochondrial study on living human fibroblasts to compare the toxicity of Methylthioninium chloride Proveblue and USP reference standard MB.
- An Ames test on three different sources of methylene blue active substances.
- A dose-ranging finding study in Beagle dogs to select the dose levels for the 1-month repeat dose study. This study was performed with Methylthioninium chloride Proveblue.
- A 1-month IV repeat-dose toxicity study in Beagle dogs to evaluate the potential toxicity of Methylthioninium chloride Proveblue 5 mg/ml solution for injection and compare the toxicological profile to the reference medicinal product.

Single dose toxicity

Single dose toxicity data from the literature were submitted as part of the application. No studies were conducted by the applicant.

In a study in sheep, a toxicological assessment was conducted using LD50 determination, MetHb production and haematological effects as evaluation parameters. From these data, it appears that as conditions may warrant, the dosage of methylthioninium chloride may be safely increased up to at least 15 mg/kg in sheep in therapy of severe methaemoglobinaemias (Burrows 1984).

Repeat dose toxicity

Reports from the US National Toxicology Program (NTP) investigating the effects of MB trihydrate on male and female rats and mice, in compliance with Good Laboratory Practice (GLP) Regulations were

submitted. The applicant performed a two-phase dose-range finding study and a pivotal 1-month repeat-dose toxicity study in Beagle dogs (see table 1).

Table 1: Repeat-dose toxicity studies on Methylthioninium chloride

Reference	Duration	Species	Study Design	Results/Conclusions
NTP TR540 2008	1 month	Mouse Rat	Groups of 10 male and 10 female mice ($B6C3F_1$) and rats (F344/N) Doses: 0, 125, 250, 500, 1000, 2000 mg/kg body weight/day; 5 days per week for 5 weeks; by oral gavage Observations of organs, haematology and chemistry tests, complete histopathology performed	- **Mice**: none of the mice in the 500, 1000 or 2000 mg/kg/d groups survived to the end of the study. Survival in the 250 mg/kg/d group was also reduced relative to control animals. Lesions in spleen, liver and forestomach associated with MB treatment. - **Rats**: at doses of 500 mg/kg and above, effects on the haematopoietic system and early deaths were observed. Toxicity observed: methaemoglobinaemia followed by anaemia, haemosiderin pigment deposition in the liver and kidney and subsequent hepato- and nephro-toxicity. Spleen and liver lesions associated with MB treatment.
NTP TR540 2008	3 months	Mouse Rat	Groups of 10 male and 10 female mice ($B6C3F_1$) and rats (F344/N) Doses: 0, 25, 50, 100 or 200 mg/kg/day; 5 days per week for 14 weeks; by oral gavage Observations of organs, haematology and chemistry tests, complete histopathology performed	- **Mice**: methaemoglobinaemia and regenerative Heinz body anaemia. Dose-dependent increase in spleen, heart and kidney weights and reduction in thymus weights. Decreased sperm motility and increased epididymal sperm counts in male at 200 mg/kg. - **Rats**: methaemoglobinaemia and regenerative Heinz body anaemia, significant increase in spleen weights, decrease in thymus and lung weights. Spleen lesions in dosed rats. Incidence of bone marrow hyperplasia significantly increased in groups administered 50 mg/kg MB or greater. No consistent effects of MB on reproductive system measures.
Study N° 36109 TSC (Provepharm)	14 days	Dog	Two-phase dose range-finding study for the 1-month study Slow IV infusion of Proveblue® (constant concentration of 5 mg/mL) in 4 beagle dogs Dose levels tested: - Phase I (1 male and 1 female): 7.5 mg/kg/day for 4 days and 15 mg/kg/day for 1 day - Phase II (1 male): 1.5 mg/kg/day during 14 days - Phase III (1 female): 0.3 mg/kg/day during 14 days	**Phase I**: Treatment stopped from day 6 because of important clinical signs of toxicity (cyanosis, hypoactivity) after one injection at 15 mg/kg/day. Both animals prematurely sacrificed on day 7. Observation of marked anaemia and an important inhibition of neutrophilic peroxydase activity. Increase in absolute and relative spleen weights attributed to treatment **Phase II**: marked regenerative anaemia associated with increased platelet count, fibrinogen and total bilirubin. Observation of high absolute and relative spleen weights attributed to treatment. Increase in liver weights noted. Relationship to treatment could not be excluded **Phase III**: no clinical observation and no treatment-related laboratory findings. Treatment related high absolute and relative spleen weights observed. No treatment-related macroscopic findings noted.
Study N° 36110 TSC (Provepharm)	1 month	Dog	4-week toxicity study by slow intravenous infusion to beagle dogs. 5 groups of 3 males and 3 females: - 1 control group - 3 groups with	No relevant findings were noted with respect to unscheduled deaths, clinical signs, bodyweight, food consumption, ophthalmology or electrocardiography at the end of the treatment period. On day 14, males and females receiving the test item at 1.00 mg/kg/day showed moderate regenerative, hypochromic and macrocytic anaemia associated with increased

Reference	Duration	Species	Study Design	Results/Conclusions
			Methylthioninium chloride Proveblue (doses: 0.25, 0.5 and 1 mg/kg/day) - 1 group with reference medicinal product (1 mg/kg/day) This study is compliant with EMEA guideline "Note for guidance on repeated dose toxicity" (ref. CPMP/SWP/1042/99 corr.).	mean platelet count and fibrinogen level. At the end of the treatment period, a dose-related decrease in mean red blood cell count, haemoglobin, packed cell volume and mean cell haemoglobin concentration associated with a dose-related increased mean reticulocyte and platelet counts were observed at all dose-levels. Few Heinz bodies and few erythroblasts were observed at 1.00 mg/kg/day (not seen in controls). Mean fibrinogen levels were higher in both males and females at 1.00 mg/kg/day. A minimal increase in mean total blood bilirubin values were noted at 0.50 mg/kg/day and higher in males and at 0.25 mg/kg/day and higher in females treated with the test item. Moderate bilirubin levels were observed at a higher incidence in urines in all treated groups in comparison to controls. Dose-related increases in mean spleen weights were correlated with increased haemopoiesis (from 0.25 mg/kg/day) and congestion (at 0.25 mg/kg/day and higher in females, and at 0.50 mg/kg/day and higher in males) in the spleen at microscopic examination of animals treated with the test item. These findings were compensatory to the anaemia. Green/brown pigment consistent with haemosiderosis in the spleen and liver at 0.25 mg/kg/day and above, kidneys at 1.00 mg/kg/day (secondary to the haemolysis and increased catabolism of haemoglobin) were also noted at microscopy. In addition, increased cellularity of the bone marrow was noted in all high-dose animals. A trend towards an increased incidence of thickened subcutaneous tissue at necropsy and incidence and/or severity of inflammatory findings at the injection sites in groups treated at 0.50 or 1.00 mg/kg/day which indicated a minimal irritant effect of the test item. Similar laboratory and pathology findings were reported in animals receiving the reference item at the same dose-level of 1.00 mg/kg/day (effects on haematological parameters on day 14 and spleen weights at necropsy were slightly more marked compared to the test item). Regenerative anaemia (decreased mean red blood cell count, haemoglobin and packed cell volume, increased mean reticulocyte count, presence of Heinz bodies and erythroblasts)was observed with treatment and the degree was associated with dose (0.25 (minimal), 0.5 (slight), 1.00 (moderate)). At each dose level regenerative anaemia was associated with an observed increase in platelets and an increase in bilirubin. Increased spleen weights were also associated with the anaemia. At each dose level gree/ brown pigments were noted in the liver at all doses, in the spleen at the mid and high dose and in the kidneys only at the top dose and is considered to be secondary to haemolysis and increased catabolism of haemoglobin. At the high dose, increased cellularity of the bone marrow was noted in all dogs and was considered related to the regenerative anaemia. Inflammatory findings at the injection sites (mid and high dose) indicated a minimal irritant effect of the test item, which was also observed with the test item. Overall no no-observed adverse effect level could be identified, however based on the finding at the high dose methylthioninium chloride

Reference	Duration	Species	Study Design	Results/Conclusions
				Proveblue (5 mg/ml) is considered to have a similar toxicological and toxicokinetic profile at Methylene Blue injection USP 1% w/v following daily injection for 4 –weeks.

Genotoxicity

GLP compliant Ames test in Salmonella typhimurium was performed on the strains: TA1535, TA1537, TA98, TA100 (3.9, 7.8, 15.6, 31.3, 62.5, 125 µg/plate) and on the TA102 strain (15.6, 31.3, 62.5, 125, 250, 500 µg/plate) in presence or absence of a rat liver metabolising system (Aroclor 1254-induced rat liver S9 mix) with Methylthioninium chloride Proveblue and two other MB products.

All the three tested MB products were mutagenic in presence and absence of rat metabolising system, which confirms what is already known regarding MB.

Methylthioninium chloride was mutagenic in gene mutation assays in mouse lymphoma cells but not in vivo mouse micronucleus assay when administered intravenously at 62 mg/kg.

Carcinogenicity

In 2 year study conducted by NTP, a carcinogenic potential was demonstrated in male mice (dosed up to 25 mg/kg/day) and in male rats (dosed up to 50 mg/kg/day). An equivocal evidence of carcinogenic activity was observed in female mice. No evidence of carcinogenic activity was observed in female rats (NTP report TR540).

No carcinogenicity studies were performed by the applicant. This is acceptable since Methylthioninium chloride Proveblue will be used for short durations in emergency situations only.

Reproduction Toxicity

According to the scientific advice (EMEA/H/SA/1211/1/2009/SME/ III) given by the CHMP on 25 June 2009, no reproductive toxicity studies were deemed necessary, but relevant reproductive toxicity data could be derived from the literature. The applicant presented publicly available data which showed that Methylthioninium chloride Proveblue is to be considered a reproductive toxicant (data not shown).

In vitro, Methylthioninium chloride Proveblue has been shown to reduce motility of human sperm in a dose dependant manner. It has also been shown to inhibit the growth of cultured two-cell mouse embryos and the production of progesterone in cultured human luteal cells.

In rats and rabbits, teratogenic effects have been reported, with foetal and maternal toxicity. In rats, increased resorption rates have been observed. The excretion of Methylthioninium chloride Proveblue in milk has not been studied in animals.

Toxicokinetic data

Toxicokinetic data have been submitted for the pivotal repeat dose toxicity study in Beagle dogs.

Following single and repeat dosing, females appear to have a higher exposure (AUC) in comparison to males at the higher doses (0.50 and 1.0 mg/kg) and this sex difference is also reflected in the reference compound. At equivalent doses, exposures (Cmax and AUC) appear to be higher in animals treated with the reference compound and this difference is more pronounced in males than females (see table 2).

Table 2: Toxicokinetic Data

Daily Dose (mg/kg)	0.25 (Test item)		0.50 (test item)		1.00 (Test item)		1.00 Reference	
	M	F	M	F	M	F	M	F
Day 1								
Cmax (ng/ml)	25.5	19.8	47.6	56.4	116	114	150	124
AUC (ng.hr/ml)	22.0	16.3	32.2	65.6	134	172	150	201
Tmax (h)	0.5	0.083	0.083	0.083	0.083	0.083	0.083	0.083
Day 28								
Cmax (ng/ml)	Nc	27.9	36.1	57.1	69.1	91.5	108	112
AUC (ng.hr/ml)	Nc	27.3	40.5	74.8	63.9	129	121	134
Tmax (h)	Nc	0.083	0.083	0.083	0.083	0.083	0.083	0.083

Note: No quantifiable plasma levels of test or reference item were detected in control animals.

The toxicokinetic profile for Methylthioninium chloride Proveblue can be considered comparable to the reference product in dogs following 4-week IV injection.

Local Tolerance

Local tolerance at the site of administration was addressed within the dose-ranging finding study and in the 1-month repeat-dose toxicity study in dog. In the DRF study, there was a local reaction characterised by red discoloration and a thickened subcutaneous tissue in the two dogs treated at the highest dose at 7.5 mg/kg however this study did not include control animals. Based on the results of the 1-month repeat dose toxicity study, it is considered that there is a slight irritant effect of both the test item and the reference item at both the mid and high dose.

Other toxicity studies

No studies on antigenicity, immunotoxicity, dependence, metabolites or impurities have been conducted which is considered acceptable.

2.3.5. Ecotoxicity/environmental risk assessment

An ERA was submitted as part of this application. Since Methylthioninium chloride Proveblue is intended for use as a treatment of medicinal and chemical product-induced methaemoglobinaemia, and this treatment is not administered on more than one occasion during the lifetime of a patient, the dose was annualised, which is accepted by the assessor. The PEC surface water calculated was below the PEC action limit of <0.01 µg/l and no further environmental assessment is required.

The log P value, determined experimentally, was -0.9. The log D for Methylthioninium chloride Proveblue was 0.06, a value well below 4.5 and no PBT (Persistent, bioaccumulative and toxic) assessment is required.

2.3.6. Discussion and conclusion on non-clinical aspects

The non clinical data submitted as part of this application are mainly from bibliographic sources.

The way in which methylthioninium chloride is able to reduce methaemoglobin to haemoglobin is well known and understood and no studies on primary pharmacodynamics are necessary. Methylthioninium chloride has been investigated and used in several medical conditions as described in the literature, and no studies on secondary pharmacodynamics are deemed necessary.

No Pharmacodynamic drug interactions data have been submitted and this is considered acceptable.PK studies indicated that bile was the major route of elimination.

Single dose toxicity data comes from the literature which is considered acceptable.

A two-phase dose-range finding study in Beagle dogs dosed up to 7.5 mg/kg was conducted. The main effects were blood related; decreased red blood cell count, haemoglobin and packed cell volume, increased reticulocytes and increased platelet count, fibrinogen and total bilirubin.

In addition, the applicant performed a pivotal 1-month intravenous repeat-dose toxicity study (36110TSC) in Beagle dogs. No no-observed adverse effect level (NOAEL) could be identified. The toxicity and toxicokinetic data for Methylthioninium chloride Proveblue and the reference product were comparable and no new toxicities were identified in this study (regenerative anaemia and associated findings) with respect to the studies previously reported. The observed findings are also considered to be in line with those observed within the literature for Methylthioninium chloride. A slight local irritant effect of both the test item and the reference item was seen at both the mid and high dose.

MB is mutagenic, carcinogenic and is considered a reproductive toxicant. These risks are outweighed by the fact that MB is an antidote that could be life-saving in cases of methaemoglobinaemia.

2.4. Clinical aspects

2.4.1. Introduction

Methylthioninium chloride Proveblue 5 mg/ml solution for injection is intended to be used as an antidote in the treatment of medicinal and chemical products-induced methaemoglobinaemia and the product belongs to the ATC code V03AB17.

The indication applied for initially was the following:

- Treatment of medicinal and chemical products- induced methaemoglobinaemia Methylthioninium chloride Proveblue is indicated in adults and children above the age of 3 months.

The finally approved indication is as follows:

- Acute symptomatic treatment of medicinal and chemical products- induced methaemoglobinaemia. Methylthioninium chloride Proveblue is indicated in adults, children and adolescents (aged 0 to 17 years old).

A scientific advice given by the CHMP on 25 June 2009 (EMEA/CHMP/SAWP/357282/2009, procedure number EMEA/H/SA/1211/1/2009/SME/III) concluded that a hybrid application was acceptable and the clinical part could consist of bibliographical data. Furthermore, the CHMP recommended that the applicant should demonstrate that the change of concentration/solubility of the active substance will not impact pharmacokinetics compared to the reference product.

No clinical studies have been performed with Methylthioninium chloride Proveblue but 2 in vitro studies were conducted by Provepharm to show similarity to the reference medicinal product:

1. plasma protein binding study with Methylthioninium chloride Proveblue and the reference product (study 17174) and
2. Cytochrome P450 (CYP) inhibition assays and CYP induction assays with Methylthioninium chloride Proveblue and the reference product (study bd00196).

In addition a literature search was performed to provide support for the three changes from the reference product:

- the change of the concentration (5 mg/ml instead of 10 mg/ml);
- the addition of the indication "treatment of chemical products-induced methaemoglobinaemia";
- and the use in children.

2.4.2. Pharmacokinetics

Absorption

Methylthioninium chloride Proveblue is administered intravenously injected very slowly over a period of 5 minutes and information on absorption is therefore not required.

Bioequivalence:

The Note for guidance on the investigation of bioavailability and bioequivalence (CPMP/EWP/QWP/1401/98) states that the applicant is not required to submit a bioequivalence study if the product is to be administered as an aqueous intravenous solution containing the same active substance in the same concentration as the reference product.

For this product, there is a difference in the strength compared with the reference product. Furthermore, there appears to be some other difference as well with respect to the solubility of the active drug substance. This was the background to the recommendation by the CHMP, i.e. that the applicant should demonstrate that the change of concentration/solubility of the active substance will not impact pharmacokinetics compared to the reference product. The applicant did not perform an in vivo bioequivalence study but provided two in vitro studies in conjunction with the published pharmacokinetic data to demonstrate that there was no difference between the products with respect to the pharmacokinetic features.

Distribution

The *in vitro* protein binding in human plasma of Methylthioninium chloride Proveblue and Methylthioninium chloride USP was estimated at a test concentration of 10 µM employing equilibrium dialysis (18±2 hours at 37°C, 12-14 K MWCO dialysis membrane 0.05 M phosphate buffer, pH 7.5). An HPLC-MS/MS method was applied to measured drug substance. The results are shown in table 3.

Table 3: Results of protein binding study

Compound	Test Concentration (M)	% Protein Bound			% Recovery		
		1st	2nd	Mean	1st	2nd	Mean
Methylthioninium chloride Proveblue 5 mg/mL Solution for Injection	1.0 E-05	91.8	95.4	93.6	47.2	52.2	49.7
Methylthioninium chloride Injection USP 1% w/v	1.0 E-05	97.5	97.4	97.4	70.6	56.8	63.7

Elimination

There are few publications describing the pharmacokinetic fate *in vivo* in humans. Following intravenous and oral administration, blood concentrations exhibit a multi-phasic time course with a terminal half-life around 5 hours but other results have also been reported (half-life in plasma and blood of approximately 18 hours and 14-15 hours, respectively). Differences are probably due to varying analytical methods and/or sampling periods and the longer half-lives were observed in the more recent studies with long sampling periods (up to 24 hours post-dose), adequate analytical methods which therefore appear more reliable.

Renal elimination of MB and LMB appears to be a major elimination pathway, but varying figures of the extent have been reported: 29% and 74% of the dose, respectively, was reported to be excreted in the urine as MB and LMB in two different publications. It is uncertain if additional elimination pathways for MB are present.

Pharmacokinetic interaction studies

The inhibition and induction of the CYP450 was studied by the applicant. The objective of this study was to assess whether there would be a difference in the *in vitro* drug-drug interaction potential between Methylthionium chloride Proveblue and Methylthionium chloride (MTC).

CYP450 Inhibition

The purpose of this study was to determine the relative inhibition potential (IC50 values) for two compounds, Proveblue and MTC, against the human cytochrome P450 (CYP) isoforms 1A2, 2B6, 2C9, 2C19, 2D6, and 3A4. Each CYP isoform was tested with appropriate known positive controls, as well as negative controls. In positive controls with known inhibitors, measured IC50 values were all within expected ranges. Overall, both compounds had similar inhibitory effects on the six isoforms tested. Both compounds were potent inhibitors of CYP 1A2, 2B6, 2C9 and 2C19.

CYP450 Induction

Cryopreserved plateable human hepatocytes were used to evaluate Proveblue and MTC to determine their induction potential for CYP1A2 and 3A4. CYP1A2 and 3A4 enzyme activities were measured using known substrates for these enzymes and the results are presented as a fold induction compared to the vehicle control. Both compounds behave in a similar fashion in this assay but in all experiments the positive controls result in very little effect. Both compounds elicited cytotoxic effects at the 100 µM concentration resulting in loss of cell viability.

2.4.3. Pharmacodynamics

Mechanism of action

A distinct enzyme, NADPH methemoglobin reductase, converts methylthioninium chloride (the oxidized form of the dye) to leucomethylene blue (LMB) (the reduced form), using NADPH. The reduced form then chemically reduces methemoglobin (Hgb(Fe3+)) to haemoglobin (Hgb(Fe2+)).

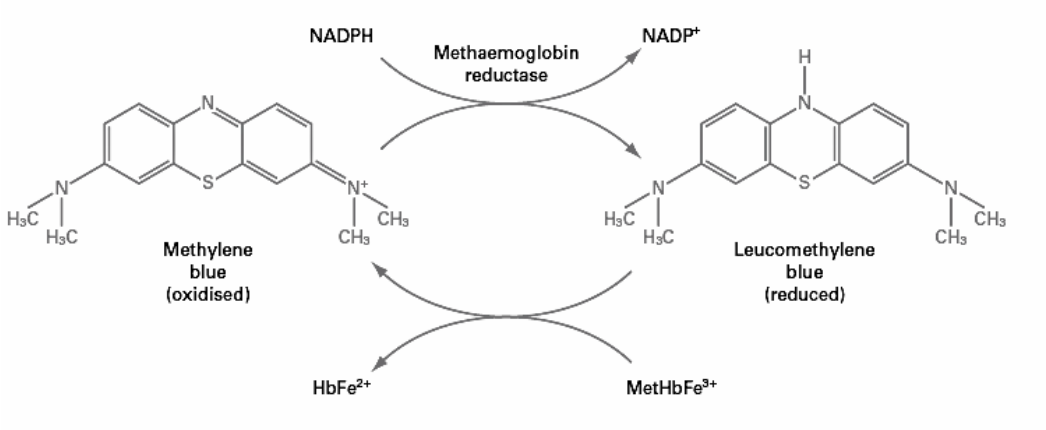


Figure 2: from Bradberry SM, Aw TC, Williams NR, Vale JA. Occupational methaemoglobinaemia. Occup Environ Med. 2001 Sep:58(9):611-5

The mechanisms that cause methaemoglobinaemia are well understood. Likewise, the way methylthioninium chloride function when used to treat methaemoglobinaemia is uncontroversial.

Similar redox carriers, such as toluidine blue or thionine have never gained such popularity, and only few attempts have been made to compare their efficacy.

2.4.4. Discussion on clinical pharmacology

The applicant provided two in vitro studies conducted by Provepharm to show that the products are similar. The protein binding study was very limited and only one concentration level was studied. A difference between the formulations tested in the recovery was indicated (50% versus 64%), which may imply a difference of the two products concerning adsorption to the equipment (filters and/or plastic material) used in the protein binding study and thus also to utensils used for dosing of the medicinal product. However, the possible difference is considered small and would not result in a clinically relevant difference in the administered dose, between the two formulations. Furthermore, there are no signs that adsorption would be an issue for methylthioninium chloride. The in vitro inhibition study did not reveal any differences between the products and the induction study was not conclusive due to little response from positive controls.

The Note for guidance on the investigation of bioavailability and bioequivalence (CPMP/EWP/QWP/1401/98) states that a bioequivalence study is not required if the product is to be administered as an aqueous intravenous solution containing the same active substance in the same concentration as the reference product. For this product, there is a difference in the strength compared with the reference product due to the difference with respect to the solubility of the active drug substance. The documentation provided by the applicant contributes little to conclusion whether there is a difference in the drug substances that would impact pharmacokinetics, and thereby the efficacy of the drug. However, given that the solubility difference is minor (1.1 to 1.3 m/v% versus 1.6 m/v%) and that this is as an aqueous solution for intravenous administration, a difference in the systemic exposure compared with the reference product is not expected. A bioequivalence study is therefore not required.

Regardless of elimination pathway, it is known that organ systems mature for some time after birth. Roughly, glomerular filtration as well as metabolic enzyme activity reaches adult capacity around 1-2 years of age, and thereafter depends on body size. Thus, if the purpose is to achieve similar systemic exposure in children and adults, the proposed dose recommendation in children may not be optimal. However, the dose proposed is in agreement with the commonly recommended by EU Poison centres.

2.4.5. Conclusions on clinical pharmacology

A slightly reduced solubility is not considered to have an effect on the pharmacokinetic features for a formulation where the drug substance is an aqueous solution to be administered intravenously. The clinical pharmacology documentation is considered sufficient.

2.5. Clinical efficacy

As considered lifesaving for many intoxicated patients, placebo-controlled randomised studies have not been performed. The Scientific advice from EMA concluded that proof of efficacy could be based on published data, mainly case reports.

The applicant has not performed any clinical studies with this product but has performed a literature search to provide support for the three changes from the reference product:

- the change of the concentration (5 mg/ml instead of 10 mg/ml)
- the addition of the indication "treatment of chemical products-induced methaemoglobinaemia"
- and the use in children.

EU Poison Centres data

A request form concerning drug-induced methaemoglobinaemia cases was sent by the Applicant to Poison Centres in European Union Member States and in Liechtenstein, Norway and Iceland, at the end of March 2009. The aim of this request was to obtain information about the number of poisoning cases leading to methaemoglobinaemia, the responsible compounds and the treatment used (see table 4).

Table 4: Cases of methaemoglobinaemia as reported by Poison Centres in European Union, Liechtenstein, Iceland and Norway

Member State	Total number of cases	Cause of Poisoning (number of cases)	Antidote used (manufacturer)	Protocol of MB treatment
Austria	5 cases since 2003	- Sodium Chlorate (3) - Gyromitra esculenta (1) - Hydrogene Sulfide (5)	Methylthioninium chloride 1% (magistral formulation)	1-2 mg/kg slowly IV
Belgium	69 cases since 1972: 40 adults 20 children (and 9 animals)	- Phenazopyridine (12) - Nitrites (11) - Chlorate (5) - Paracetamol (5) - Dapsone (4) - Loperamide (3) - Nitrates (3) - Substral (3) - Nitrobenzen (2) - Pyrimethamine (2) - Aniline (1) - Calorimeter (1) - Cocaïne (1) - Dextromethorphan (1) - Chervil soup (1) - Linuron (1) - Metamizole (1) - Methylthioninium chloride (1) - Organic nitrite (1) - Oxyphenbutazone (1) - Phenylsulfone (1) - Beetroot (1) - Toluidin (1) - Tributylnitrite (1) - unknown (5)	Methylthioninium chloride 10mg/ml (Sterop)	Adults and children: 1-2 mg/kg IV over 5 to 10 min. Dose may be repeated after 1 hr if needed.

Member State	Total number of cases	Cause of Poisoning (number of cases)	Antidote used (manufacturer)	Protocol of MB treatment
Czech Republic	32 cases since 2004	- Gentian violet (22)	Ascorbic acid only	Adults and children: 1-2 mg/kg IV (1% solution) over 5 min. Repeat dose in 30-60 min if no response.
		- Aniline (5)	1 case: Toluidin blue (unknown) 4 cases: no treatment	
		- Dapsone (2)	Not specified	
		- Ortho-toluidine (1)	Not specified	
		- Isobutylnitrite (Poppers) (2)	1 case: Toluidin blue (unknown)	
Estonia	No cases since Sept. 2008	Not applicable	Methylthioninium chloride (unknown)	1-2 mg/kg IV. Dose may be repeated if needed.
Finland	Not available	Not available	Methylthioninium chloride (unknown, compassionate use)	Not specified
France	A mean of 1 case per year	- Poppers (amyl nitrite) - Chlorate - some anesthetics	Methylthioninium chloride (unknown)	1-2 mg/kg IV Maximal dose: 5-7 mg/kg.
Hungary	No information in the database			
Liechtenstein/ Switzerland	13 cases since 1997	- Amyl nitrite (2) - Dapsone (2) - Isopropyl nitrite (1) - Isobutyl nitrite (1) - Nitrite compound (1) - Combustion (1) - Kohlrabi (1) - Chloroaniline (1) - 4-bromo-2-fluoroaniline (1) - primaquine (1) - Unknown (1)	7 cases: Methylthioninium chloride for 7 cases (unknown) 6 cases: no treatment	1-2 mg/kg given slowly IV, may be repeated up to a maximum of 7 mg/kg. Note: no report of adverse effect or serious adverse event caused by MB
Norway	2 adult cases since 16-feb-2004	- unknown product containing nitrites (1) - unknown (1)	Metytionin injektionsvätska inj. 10 mg/mL (Swedish injection product)	Adults and children: 1-2 mg/kg slowly IV over 5 min. Dose may be repeated after 1-4 hrs if required. Maximal total dose: 5-7 mg/kg.
Slovak Republic	2 cases since 2004	- nitrates in water (1) - aniline (1)	Toluidinblau (Dr. F. Kohler Chemie)	No MB available in the country
Spain	12 cases since May 2005	- linuron - naphtalene - phenazopyridine - pendimethalin (dinitroaniline herbicide)	Methylthioninium chloride 1% in 5% glucose solution (galenic formula made by pharmacists)	1-2 mg/kg IV over 5 min. Dose may be repeated after 1hr. Maximal dose: 7 mg/kg
Sweden	Less than 10 cases per year	Not specified	Methylthionine manufactured for National Use	Not specified

Member State	Total number of cases	Cause of Poisoning (number of cases)	Antidote used (manufacturer)	Protocol of MB treatment
The Netherlands	14 cases between 01-jan-2007 and 31-dec-2008	- cigarette with powder from firework (1) - aniline and P-aminoazo-benzene (1) - resorcinol (1) - dapsone (2) - ammonium nitrates (1) - Hydrogen peroxide 3% and phenacetin (1) - Isosorbide mononitrate (1) - nitrites and beer (1) - prednisolon and plaquenil (1) - nitrogen phosphate and potassium (1) - nitrites and cocaine (1) - Methylthioninium chloride (2)	- Methylthioninium chloride (10mg/mL) Alternative to MB: - Tolonium - Vitamin C (but less effective than MB)	Adults and children: 1-2 mg/kg IV during 5 min. Dose can be repeated. Maximal dose: 7 mg/kg.

Current medical practice in EU Poison Centres shows that methylthioninium chloride is the first-line treatment in children and adults cases of both medicinal and chemical products induced methaemoglobinaemia. In some Member States, methylthioninium chloride is not used because it is not available.

Dose-related data

Based on the answers received from EU poison centre, the usual dose recommended is 1 to 2 mg per kg body weight, i.e. 0.2-0.4 ml per kg body weight, given intravenously over a period of 5 minutes.

It must not be administered by subcutaneous or intrathecal injection.

A repeat dose (1 to 2 mg/kg body weight, i.e. 0.2-0.4 ml/kg body weight) may be given one hour after the first dose in cases of persistent or recurrent symptoms or if methaemoglobin levels remain significantly higher than the normal clinical range.

Treatment does not usually exceed one day.

Based on available literature data, the maximum recommended cumulative dose for the course of treatment is 7 mg/kg and should not be exceeded, since Methylthioninium chloride Proveblue administered above the maximum dose may cause methaemoglobinaemia in susceptible patients.

In the case of aniline- or dapsone-induced methaemaglobinaemia, the maximum recommended cumulative dose for the course of treatment is 4 mg/kg.

Too limited data are available to support a continuous infusion dose recommendation.

Methylthioninium chloride Proveblue is hypotonic and may be diluted in 50 ml glucose 50 mg/ml (5%) solution for injection to avoid local pain, in particular in paediatric population.

Medicinal-product induced methaemoglobinaemia – Case reports

- *Adults*

Most cases of acquired methaemoglobinaemia reported in the adult population are related to topical anaesthetics, particularly benzocaine spray applied during surgery, the use of the antibiotic dapsone, intoxication by aniline products and nitrites or nitrates (from food, water, chemicals and medicines).

In most case reports, patients with acquired methaemoglobinaemia were treated with IV infusion of methylthioninium chloride as a 10 mg/ml solution with a dose of 1 to 2 mg/kg over 5 to 15 min.

Table 5 and Table 6 list the medicinal product induced- methaemoglobinaemia case reports found in public domain:

Table 5: Case reports of efficacy in adults with medicinal product induced-methaemoglobinaemia

Reference	Cause	Patient	MB Treatment	Route	Results
Cause: Chlorate and bromate local anaesthetics					
Young 2008	Benzocaine oral spray	27-year-old man	65 mg (1-2 mg/kg)	IV	Complete recovery
Jiminez 2007	Hurricane spray (Benzocaine)	56-year-old woman	60 mg (1 mg/kg) over 5 min	IV	Cyanosis completely resolved within minutes of receiving MB
Kane 2007	Benzocaine	18 patients (mean age: 62.8 ± 16.0 years)	Mean dose ± SD: 1.3 ± 0.4 mg/kg body weight (range 0.7-2 mg/kg) 2 patients required a 2nd dose (after initial dose of 50 mg)	IV	Resolution of signs and symptoms within 1h (frequently within 20 to 30 min)
Birchem 2005	Benzocaine	69-year-old woman	2 mg/kg	IV	1h later, MetHb level decreased to 18.4% with improvement of cyanosis. 2h later, MetHb level = 4%
Ash-Bernal 2004	Benzocaine	52-year-old man	Not specified	IV	Rapid reverse of methaemoglobinaemia
Sachdeva 2003	Benzocaine	Case 1: 72-year-old man Case 2: 65-year-old man	Case 1: 2 mg/kg Case 2: 1 mg/kg	IV	Case 1: Resolution of cyanosis and decrease of MetHb level within 2h Case 2: Resolution of cyanosis within 15 min
Nguyen 2000	Benzocaine	71-year-old woman	1 mg/kg (1% solution) for 5 min	IV	Decrease of MetHb level from 22.5% to 2.4% 1h after administration. Full recovery.
Jaffery 2008	Topical benzocaine	44-year-old woman	2 mg/kg	IV	Resolution of symptoms
Lin 2007	Topical benzocaine	29-year-old woman	50 mg over 10 min (1% solution)	IV	Decrease of MetHb level
Saha 2006	Topical benzocaine	71-year-old man	1 mg/kg over 5 min	IV	Decrease of MetHb level within 4 hours
Bayard 2004	Topical benzocaine	26-year-old woman	90 mg (2 mg/kg) over 5 min	IV	3h later: MetHb level near zero
Rodriguez 1994	Benzocaine	83-year-old man	100 mg (approx. 1.5 mg/kg) 2nd dose 3h later: 80mg (1.1 mg/kg) 3rd dose 20h later: 80mg	IV	Decrease in MetHb concentration (from 54.1% to 4.3 % after 2nd dose). After 3rd dose: decrease of MetHb level from 30% to 4.2% within 4h
Bolyston 2002	Probably benzocaine	73-year-old woman	200 mg in 100 ml of isotonic sodium chloride solution infused during 30 min	IV	30 min after infusion: metHb level 9% 90 min after infusion: metHb level 2% Methaemoglobinaemia rapidly resolved
Wolak 2005	Multiple exposures to oxidizing agents (ie, mafenide acetate and benzocaine	21-year-old man	2 mg/kg over 5 minRepeat 1 mg/kg dose	IV	Recovery

	spray)				
Adams 2007	Prilocaine	45-year-old woman	43 mg	IV	Quick improvement
Wilburn-Goo 1999	Prilocaine	Case 1: 22-year-old woman Case 2: 33-year-old woman Case 3: 19-year-old woman	Case 1: 100 mg Case 2: dose not specified, treatment during 5 min Case 3: 1.5 mg/kg	IV	Case 1: full recovery Case 2: MetHb level decreased to 2% within 5 min Case 3: Recovery
Lunenfeld 2004	Topical anesthetics (Cetacaine)	52-year-old man	1st dose: 1 mg/kg 2nd dose: 1 mg/kg, 5min after the 1st dose	IV	Complete recovery
Douglas 1977	Topical anesthetic spray (Cetacaine)	Case 1: 77-year-old man Case2: 80-year-old woman	Case 1: 60 mg (6 mL of 1% solution) with 250 mg of ascorbic acid Case 2: 50 mg (5 mL of 1% solution)	IV	Case 1: Cyanosis resolved within 10 min Case 2: Cyanosis resolved within a few min
Cause: Antibiotics					
Arrivabene Caruy 2007	Dapsone	52-year-old man	1 mg/kg	IV	Clinical improvement 15 min after administration
Matisoff 2006	Dapsone	71-year-old woman	1st dose: 5 mL (1% solution) Then 5 mL (1% solution) increments up to 20 mL total	IV	Decrease of MetHb to 1.9%
Salamat 2003	Dapsone	66-year-old man	1 mg/kg over 10 min	IV	Prompt symptomatic improvement
Cause: Amyl nitrites					
Modarai 2002	Amyl nitrite	Case 1: 32-year-old woman Case 2: 28-year-old man	Case 1: 1.5 mg/kg over 5 min Case 2: 2 mg/kg over 5 min	IV	Case 1: Improvement within 40 min Case 2: Improvement within 10 min
Stambach 1997	Volatile amyl nitrites	Early twenties woman	1st dose: 2 mg/kg 2nd dose: 1 mg/kg	IV	Full recovery
Other causes					
Fung 2008	Zopiclone ingestion	43-year-old woman	1st dose: 80 mg (1 mg/kg) 2nd dose 90 min later: 80 mg (1 mg/kg)	IV	Decrease of MetHb from 23.8% to 3.6% 1h after 2nd dose Complete resolution of cyanosis
Mary 2000	Metoclopramide	88-year-old man	100 mg in 100 cc of normal saline given over 3 min	IV	Clinical improvement, MetHb level returned to normal within 24h

Published data support the proposed indication (medicinal product induced- methaemoglobinaemia) and posology.

The response to methylthioninium chloride treatment is so rapid, with marked lowering or normalization of methaemoglobin (metHb) levels within an hour or two that no other treatment is usually needed, but the patient should be observed carefully, because continued absorption of a toxic substance from the gastrointestinal tract may cause recurrence of methaemoglobinaemia (Beutler 2005).

Half-life of metHb is 55 minutes. After MB administration, cyanosis resolves within 15 to 30 minutes. Marked reduction in the metHb concentration, usually by 50%, is seen within 30 to 60 minutes. MB

itself has oxidizing properties at higher doses, with toxic effects appearing in doses > 7 mg/kg (Gupta 2000).

A second dose may need to be administered in very severe cases, such as if there is evidence of continuing chemical absorption or prolonged methaemoglobin formation. The latter is associated particularly with methaemoglobinaemia caused by poisons that require metabolic activation, such as aniline. A long half life and enterohepatic circulation of the parent compound and its metabolites may also contribute to prolonged methaemoglobin formation. If a second dose of methylthioninium chloride is administered, the clinical situation should be re-evaluated before any further dose is given, since higher or repeated doses of methylthioninium chloride may exacerbate methaemoglobin formation and cause intravascular haemolysis in these cases (Bradberry 2001).

Usually, methylthioninium chloride therapy should be continued until methemoglobin level is below 10% (Prasad 2008). The same dose may be repeated every 60 min as required up to a total dose of 7 mg/kg (Guay 2009).

Consequently, the SmPC of Methylthioninium chloride Proveblue, mentions that: "A second dose may be given one hour after the first dose in cases of persistent or recurrent symptoms or if methaemoglobinaemia levels remain higher than normal".

However, in case of aniline- or dapsone-induced methaemoglobinaemia, it is recommended not to exceed a cumulative dose of 4 mg/kg because of the risk of haemolytic anaemia. The SmPC has been updated accordingly.

In children, as for adults, the dose may be repeated after one hour in cases of persistence or recurrence of symptoms (Moodambail 2005). However, in infants below 3 months of age, it is recommended to administer a dose of 0.3 to 0.5 mg/kg for the initial dose as well as any repeat dose.

- *Children*

Published literature shows that methylthioninium chloride can successfully be used in children with medicinal product induced-methaemoglobinaemia (table 5).

The immature enzyme function in the newborn makes them more disposed for developing adverse events by the substance methylthioninium chloride itself. NADPH-methaemoglobin reductase in the newborn is approximately 60% of that in adults and reaches the normal adult concentration around 3 months of age. Also, infants younger than 3 months of age have higher levels of foetal haemoglobin, which has a structure that is easily oxidised into methaemoglobin. However methylthioninium chloride treatment option should be open also for this group, especially as they can easily develop methaemoglobinaemia, caused e.g. by nitrate in water, see case reports in next section.

In these patients, the recommended dose is 0.3-0.5 mg/kg body weight given over a period of 5 minutes (Guay 2009, Rauber-Luthy 2009). This dose may be as effective as the usual adult dose of 1.0 mg/kg which has sometimes induced haemolysis in this subpopulation (Guay 2009). The choice of the initial dose is confirmed by a study conducted on premature neonates

A repeat dose (0.3 to 0.5 mg/kg body weight, i.e. 0.06-0.1 ml/kg body weight) may be given one hour after the first dose in cases of persistent or recurrent of symptoms or if methaemoglobin levels remain higher than normal.

Extreme caution should be exercised when administering to newborns and infants below the age of 3 months due to lower concentrations of NADPH-methaemoglobin reductase necessary for reducing methaemoglobin to haemoglobin, making these infants more susceptible to methaemoglobinaemia produced by high doses of methylthioninium chloride.

Table 6: Case reports of efficacy in children with medicinal product induced-methaemoglobinaemia

Reference	Cause	Patient	MB Treatment	Route	Results
Cause : Chlorate and bromate anaesthetics					
Autret 1989	Nestosyl	2-year-old girl	1 mg/kg (MB 1%)	IV over 5 min	Cyanosis was cleared and O2 saturation and PaO2 were normalized 15 min after administration of MB.
Dahshan 2006	Topical use benzocaine spray	3-year-old toddler	1 mg/kg	IV infusion	MB promptly cleared the central cyanosis, restored normal oxygenation, and improved the arterial blood gas
Hersch 2004	Benzocaine exposure	Case 1: 2 ½ year old Case 2: 5 ½ year old	Case 1 : 10 mg Case 2: 20 mg	IV	Case 1: Within 8 min, cyanosis visibly faded with MetHb levels reduced to 4.4% within 6h Case 2: Cyanosis resolved within 15 min
Tush 1996	Benzocaine and resorcinol (Vagisil) cream (OTC medication)	6-day-old neonate	3 mg (1 mg/kg)	IV	Skin color returned to normal 45 min after the dose
Fullerton 2002	5% Mafenide Acetate Solution and Topical Lidocaine Spray	12-year-old boy	0.1 mL/kg (1% solution) over 5 min	IV	Respiratory status and cyanosis improved shortly after infusion of MB. Follow-up MetHb levels at 2h and 12h after treatment were 4.1% and 2.5% respectively.
Guay 2009	Benzocaine	Review on 242 cases (adults and children)	MB (n=155) or MB plus ascorbic acid (n=14) First dose MB between 0.5 and 5.5 mg/kg Cumulative MB dose: between 0.6 and 9.4 mg/kg	IV	In patients who received MB +/- ascorbic acid: - Time to metHb level of ≤2.0% varied from 0.33 to 36.2h. - Time to disappearance of clinical cyanosis: from 0.25 to 9h In 1-day-old newborn who received a single dose of 1.0 mg/kg MB: haemolysis attributed to MB administration
Ozdogan 2010	Prilocaine	40-day-old neonate	1 mg/kg	IV	Cyanosis resolved in 60 min. MetHb fraction regressed to 4% in 60 min and to 1.5% in 24h. Cyanosis disappeared completely after 120 min.
Bouziri 2010	Cutaneous application of a pomade containing benzocaine, resorcin, and oxyquinoline (Nestosyl)	16-month-old boy	Loading dose: 2 mg/kg of a MB 1% solution followed by 1 mg/kg twice daily	Slow IV injection over 5 min	Rapid improvement of cyanosis, neurological status, and hemodynamic state. 24h later, decrease of MetHb level from 50.6% to 9.8%. Patient discharged on day 3
Cause : Antibiotics					
Ferguson 1997	Dapsone (deliberate self-poisoning)	14-year-old girl	1st dose: 90 mg Total dose over 48h: 8 mg/kg	IV bolus	1h after 1st dose, MetHb level: 5.9% but rapid increase of MetHb level (exceeding 25%). MetHb level reduces over

					next 4-5 days after total dose of 8 mg/mL MB.
MacDonald 1997	Acute dapsone intoxication	3-year-old boy	4 doses of 16 mg (1 % solution, 0.1 mg/kg) with activated charcoal	IV	3 doses of MB reduced MetHb level to 6%
Prasad 2002	Dapsone	2-year-old male	1 mg/kg along with ascorbic acid (500 mg) over a period of 5 min. MB and charcoal therapy continued 8 hourly in the same dose. Day 2: 2 doses MB. MB therapy continued on days 3 and 4 (same dosage) and stopped on day 5. Cumulative MB dose: 22 mg/kg (in 4 days)	IV	After 1st dose: persistent cyanosis. MetHb level : - on day 2: 48% - on day 3: 28% - on day 4: 17% - on day 5: 8% Persistent cyanosis on day 3 and 4.
Tsai 2005	Sulfadiazine	3-year-old	1 mg/kg	IV	Decrease of MetHb to 1% within 1h
Moodambail 2005	Dapsone	19-month-old child	1 mg/kg MB 1% diluted in distilled water, 2nd dose administered 10 hrs after the 1st one Cumulative MB dose: 2 mg/kg	IV slowly over 10 min	MetHb decreased after 10 hours from 28.1 % to 21%. Cyanosis improved dramatically. After 2nd dose: MetHb level insignificant on the next 2 days and patient discharged home.
Other causes					
Merieau 2005	Metoclopramide	5-day-old newborn	1 mg/kg	IV	Cyanosis disappeared within 1 hour. MetHb level decreased from 11.2 % to 0.4%.
Attof 2006	Cerium nitrate (topical antiseptic)	16-year-old girl	1.5 mg/kg (*i.e.*100 mg) for 15 min	Not specified	MetHb down from 31.8% to 3.5% 1h after treatment
Se Eun Hyun 2009	Chinese herbal medicine	8-year-old girl	2 mg/kg MB 1% solution. Treatment repeated on 2nd day Cumulative MB dose: 4 mg/kg	IV infusion	After 1st dose: MetHb level decreased to 13.1%, cyanosis and dyspnoea improved. After 2nd dose: MetHb level decreased to 2.5% and 1.3% on the 3rd and 4th hospital days respectively. Discharged on 6th day.

Chemical products-induced methaemoglobinaemia - Case reports

In all the reported cases (2 cases in children and 10 in adults), treatment with methylthioninium chloride was effective, except in a patient with G6PD deficiency. The enzymes deficiencies of this patient have a diminished capacity to reduce methylthioninium chloride to leucomethylene blue, by performing the NADPH-dependent reduction of methaemoglobin. Thus, G6PD deficiency is a contraindication of methylthioninium chloride as indicated in the SmPC.

Table 6 and table 7 below list the chemical product induced- methaemoglobinaemia case reports found in public domain:

Table 7: Case reports of efficacy in adults with chemical products-induced methaemoglobinaemia

Reference	Cause	Patient	MB Treatment	Route	Results
Cause: Aniline products					
Demirel 1999	Aniline	Case 1: 54-year-old man Case 2: 37-year-old man	Case 1: 140 mg Case 2: 150 mg	IV	Case 1: Decrease of MetHb level to 4.9% within 1h. Decrease to 1.5% after several hours Case 2: Decrease of MetHb level to 7.5% within 2h. Complete improvement.
Ferrer-Gomez 2008	Aniline	19-year-old man	1st dose: 80 mg over 10 min 2nd dose: 1 mg/kg 3rd dose: not specified Total dose: 3 mg/kg	IV	Improvement
Kearney 1984	Aniline	32-year-old man	- 200 mg (plus 500 mg ascorbic acid) - 2nd dose: 300 mg (plus 1g ascorbic acid) - Over the next 12h: 800 mg (plus 2.5 g ascorbic acid) in 5 divided dose - 20h after poisoning occurred, 180 mg	IV	Reduction of the MetHb level from 70% to 24%. Then MetHb level declined spontaneously. Patient scheduled for quantitative G6PD studies but lost to follow-up.
Mullick 2007	Aniline-induced in patient with G6PD deficiency	23-year-old woman	50 mg (1% solution) in normal saline over 10 min	IV	No clinical improvement 1h after because of G6PD deficiency. Use of oxygen therapy.
Harvey 1983	Aniline	22-year-old man	- 1st dose: 150 mg (2 mg/kg) - 2nd dose: 150 mg	IV	Reduction of MetHb level
Liao 2002	Aniline	25-year-old man	40 mg	IV	Clinical improvement but identification of G6PD deficiency
Pizon 2009	*p*-Chloroaniline	20-year-old man	2 mg/kg	IV	Complete recovery
Cause: Other chemicals					
Anic 1999	Herbicide "Galex 500 EC" (25% of metolachlor and 25% of metobromuron dissolved in xylene)	81-year-old man	1.5 mg/kg (10 mL, 1% solution)	IV	Very effective, full recovery
Geiger 1935	Cyanide poisoning	28-year-old woman	1st dose: 50 cm^3 (1% solution) 2nd dose (50 cm^3 of 1% solution) administered 10 min after the 1st one	IV	Within 8 min after the 1st injection, respiratory function restored. Definite improvement 30 min after 2nd dose.
Maric 2008	Food additive	4 patients (age not specified)	Not specified	IV	Rapid clinical improvement

The proposal to include also chemical products-induced methaemoglobinaemia is supported by published literature. The SmPC includes a warning that in patients with aniline-induced methaemoglobinaemia, repeated doses of methylthioninium chloride Proveblue may be required. Caution should be exercised in the course of treatment with methylthioninium chloride Proveblue as this may exacerbate Heinz body formation and haemolytic anaemia. Lower doses should therefore be considered and total cumulative dose should not exceed 4 mg/kg.

• **Children**

Table 8: Case reports of efficacy in children with chemical products-induced methaemoglobinaemia

Reference	Cause	Patient	MB Treatment	Route	Results
Cause: Aniline products					
Maione 1990	Aniline shoe black	15-year-old	2 mg/kg	IV	Following administration, MetHb reduced to Hb and level of consciousness immediately improved.
Hjelt 1995	Toxic substance (probably Parachloraniline)	Premature neonates (n=13)	28 doses of MB: - 0.1-0.2 mg/kg bw (n=7) - 0.3-0.9 mg/kg bw (n=10) - 1.0-1.6 mg/kg bw (n=11)	IV	Treatment of severe methaemoglobinaemia is premature neonates with 0.3-1.0 mg/kg proved efficient. A possible side effect is an increased need for transfusion. Therefore, the smallest effective dose of MB must be used.
Golden 1998	Drinking of artificial fingernail removal fluid containing nitroethane	26-month-old boy	2 administrations of 2 mg/kg MB within 14 hrs Cumulative MB dose: 4 mg/kg	IV	MB failed to reduce MetHb due to G6PD deficiency.
Savino 2006	High concentration of courgette soup (nitrates)	2-month-infant 1-month infant	0.1 ml/kg (1% solution)	Not specified	Syndrome completely resolved after 12h
Bucaretchi 2000	See below				

In a retrospective study by Bucaretchi (2000), 17 children (1-13 years old) with methemoglobinaemia > 20% caused by dapsone exposure were described. All of the children received multiple doses of activated charcoal orally or via nasogastric tube. Twelve children were treated with methylthioninium chloride 1-2 mg/kg. Causes for refraining from methylthioninium chloride treatment not stated. There were no significant statistical differences between the results of the two treatments according to the time-course decrease in methaemoglobinaemia. However the author concludes: "Methylthioninium chloride should be the treatment of choice in urgencies of severe methaemoglobinaemia caused by acute exposure to dapsone. The therapeutic association with multiple doses of activated charcoal may reduce the chances of methaemoglobinaemia recurrence, thus reducing the need for repeated administration of methylthioninium chloride and avoiding its possible complications."

Clinical studies in special populations

Elderly

No dose adjustment is necessary in this patient population.

Renal impairment

Methylthioninium chloride Proveblue should be used with caution in patients with moderate to severe renal disease since there is limited data available and methylthioninium chloride is predominantly renally eliminated. Lower doses (<1 mg/kg) may be needed.

Hepatic impairment

There is no experience in patients with severe hepatic impairment.

Paediatric population

The posology for infants above 3 months, children and adolescents should be the same as for adults.

For infants 3 months old or younger and newborn infants, the recommended dose is 0.3-0.5 mg/kg body weight, i.e. 0.06 to 0.1 ml/kg body weight, given over a period of 5 minutes.

A repeat dose (0.3 to 0.5 mg/kg body weight, i.e. 0.06-0.1 ml/kg body weight) may be given one hour after the first dose in cases of persistent or recurrent of symptoms or if methaemoglobin levels remain significantly higher than the normal clinical range.

2.5.1. Discussion on clinical efficacy

As methylthioninium chloride is considered lifesaving for many intoxicated patients, placebo-controlled randomised studies have not been performed. The CHMP scientific advice concluded that proof of efficacy could be based on published data, mainly case reports.

No controlled clinical trials on the efficacy of Methylthioninium chloride Proveblue have been performed and current practice is only based on the cumulative experience since the first published case report with successful outcome in the 1930's.

Current medical practice in most EU Poison Centres shows that methylthioninium chloride is the first-line treatment in children and adults cases of both medicinal and chemical products induced methaemoglobinaemia. In some Member States, methylthioninium chloride is not used because it is not available.

Most cases of acquired methaemoglobinaemia reported in the adult population are related to topical anaesthetics, particularly benzocaine spray applied during surgery, the use of the antibiotic dapsone, intoxication by aniline products and nitrites or nitrates (from food, water, chemicals and medicines). In most case reports, patients with acquired methaemoglobinaemia were treated with IV infusion of methylthioninium chloride as a 10 mg/ml solution at the usual recommended dose of 1 to 2 mg/kg over 5 to 15 min.

Published literature data show that methylthioninium chloride can successfully be used in children with medicinal product induced-methaemoglobinaemia (new proposed indication) if taking into consideration the immature enzyme function in the newborn making them more disposed for developing adverse events by the substance methylthioninium chloride itself.

In all reported cases of chemical product induced- methaemoglobinaemia (new proposed indication) treatment with methylthioninium chloride was effective (2 cases in children and 10 in adults), except in one patient with G6PD deficiency.

Methylthioninium chloride Proveblue was initially proposed for use in children, but only above 3 months of age. This recommendation was based on the observation that infants below the age of approximately 3 months are more susceptible to methaemoglobinaemia produced by high doses of methylthioninium chloride and a contraindication was proposed by the Applicant to be included in the SmPC for children 3 months or younger. This contraindication was questioned by the CHMP as the methylthioninium chloride treatment option should be also available for this group, especially as infants easier can develop methaemoglobinaemia, caused e.g. by nitrate in water. The Applicant has now included dose recommendations for infants 3 months old or younger based on the very limited clinical data available for this population.

If patients fail to respond to methylthioninium chloride Proveblue, it is likely to be due to cytochrome b5 reductase deficiency, glucose-6- phosphate dehydrogenase deficiency or sulfhaemoglobinemia. In these patients, alternative treatment option should be considered.

2.5.2. Conclusions on the clinical efficacy

This medicinal product (Methylthioninium chloride Proveblue 5 mg/ml Solution for injection) has fewer impurities but the active substance is the same as for the reference product. Methylthioninium chloride is the single most used drug to treat drug- and chemical induced methaemoglobinaemia. The efficacy assessment only rests on published case reports. On this basis, the efficacy in treating methaemoglobinaemia with methylthioninium chloride must be considered to have been established.

2.6. Clinical safety

Patient exposure

The incidence of intoxications via water nitrates or via drugs is very low in developed countries and particularly in the European Community.

Table 9 below summarises the few data available addressing the incidence of acquired methaemoglobinaemia.

Table 9: Patient exposure

Reference	Cause of intoxication	Number of cases reported	Calculated incidence per year in Europe	Location
Hersh 2004	Benzocaine	60 cases since 1950	1	Worldwide
Leclerc 2008	Nitrate	3000 cases worldwide between 1945-1985	75	Europe and US (reported by WHO)
Leclerc 2008	Nitrate	1 case between 1989-1993	2.5	France (study by Zmirou on 9500 newborns)
Gruener 1970; Shuval 1972	Nitrate	2000 cases from 1941 to 1971	67	Europe and US
CSHPF 1998	Water	4 cases from 1989 to 1992	1	France

Based on these data, the maximum reported incidence of methaemoglobinaemia in Europe is 75 cases per year.

Adverse events

Intravenous administration may cause abdominal pain, headache, dizziness, tremors, anxiety, confusional state, chest pain, dyspnoea, tachycardia, hypertension, and hyperhirosis.

However, several of these are also symptoms of methaemoglobinaemia (Therapeutic Drugs 1999; Martindale 2007; Clifton 2003; Bradberry 2001).

Clifton et al 2003 mentioned dose related toxicity of methylthioninium chloride.

Table 10: Dose-related toxicity of methylene blue

Dose (mg/kg)	Toxic manifestations
2-4	Haemolytic anaemia, skin desquamation in infants
7	Nausea, vomiting, abdominal pain, chest pain, fever, haemolysis
20	Hypotension
80	Bluish discoloration of skin (similar to cyanosis)

Additionally nausea, vomiting and dysuria have been reported in patients treated with oral MB (Therapeutic Drugs 1999; Martindale 2007; Deutsch 1997).

Some cases of mild diarrhoea have occurred after oral administration of an aqueous solution of MB (Walter-Sack 2009).

Blue coloration of urine, faeces and saliva can occur.

Blue coloration of oral mucosa and teeth has also been observed after oral administration of an aqueous solution of MB (2.5%) (Walter-Sack 2009).

MB may impart a blue coloration to skin, which may hinder the diagnosis of cyanosis (Therapeutic Drugs 1999; Martindale 2007).

Photosensitisation (photo allergy or photo toxicity) may occur after administration of MB.

Patients treated with MB should be advised to take protective measures against exposure to ultraviolet or sunlight until tolerance is determined (Drug facts and comparisons 2004).

A number of adverse events associated with methylthioninium chloride treatments have been described when used in the recommended dose interval. The more severe events are linked to higher than recommended doses. Reported adverse events are covered in the SmPC.

Serious adverse event/deaths/other significant events

No case of death was reported in the literature following IV administration of MB to treat methaemoglobinaemia.

Intravenous injection of MB has occasionally caused hypotension and cardiac arrhythmias, and such disorders might prove fatal on rare occasions (Therapeutic Drugs 1999). Cardiac and blood pressure should be monitored during and after treatment with methylthioninium chloride therapy as reflected in sections 4.4 and 4.8 of the SmPC.

Necrotic ulcers have been reported at the site of injection of MB (Perry 1974). Spinal cord necrosis causing paraplegia occurred in a patient in whom MB was administered intrathecally (Sharr 1978). MB is contraindicated for intrathecal use.

As rapid injection of MB may be painful and extravasation has caused tissue necrosis, secure venous access should therefore be ensured before MB administration (Bradberry 2001).

Two case reports of anaphylactic reaction to MB infusion are described in the literature when MB was used as a marker in surgical procedures. In one case, anaphylactic reaction to MB appeared after laparoscopic chromoperturbation, the other in the framework of general anaesthesia in a surgical procedure for correction of tubal sterility (Rzymski 2003; Dewachter 2005).

The product information reflects the risk of anaphylactic reactions in section 4.8 and a contraindication for hypersensitivity in section 4.3 has been included primarily as a precautionary principle.

One case of death was reported to the Medicines and Healthcare products Regulatory Agency (MHRA) after administration of MB (MHRA 2008). Unfortunately, no information has been available concerning this case report.

Cases of deaths were reported in infants after intra-amniotic injection of MB to twin pregnant women or after IV administration of MB at large doses in two neonates (Kidd 1996; Sills 1994).

Two fatal cases were also reported death due to overdose (Sills and Zinkham report 2 cases of MB overdose in infants). One case was a neonate with trisomy 21 exposed to MB (dose undetermined) as

an intraoperative diagnostic marker and the other a neonate treated who received approx. 20 mg/kg of MB for type II glutaric acidaemia.

Laboratory tests were performed to define the occurrence of MB toxicity, phototherapy for hyperbilirubinaemia and transfusions for anaemia. Within hours after exposure to MB, the infants voided green-blue urine, followed by hyperbilirubinaemia, recurrent anaemia requiring transfusions, and red blood cell dysmorphology, including the appearance of blister cells and Heinz bodies visible in both Wright's and supravital-stained peripheral blood smears.

After the initiation of phototherapy (on day 8, for patients 1 and 2), both infants exhibited cutaneous bullae followed by desquamation. The first patient died on day 15 and the second patient on day 11. The authors attributed the death of the 2 infants to an overdose of MB (Sills 1994)

In high dose (20–30 mg/kg) methylthioninium chloride has precipitated severe intravascular haemolysis.

Lower doses may exacerbate methaemoglobinaemia and/or intravascular haemolysis, particularly following exposure to agents which themselves cause haemolysis, notably chlorates and aniline-related compounds (Bradberry 2001).

The serious adverse events noted above occurred in patients treated with higher doses of MB, unauthorised use (inrauterine injection). No fatalities occurred with IV use of MB for the treatment of methaemoglobinaemia, and two cases of anaphylaxis occurred.

Individuals without methaemoglobinaemia

The administration of large intravenous doses (≥ 7 mg/kg) of Methylthioninium chloride Proveblue to individuals without methaemoglobinaemia induces nausea and vomiting, chest tightness, chest pain, tachycardia, apprehension, severe sweating, tremor, mydriasis, blue-green staining of the urine, blue staining of the skin and mucous membranes, abdominal pain, dizziness, paraesthesia, headache, confusion, hypertension, mild methaemoglobinaemia (up to 7%) and electrocardiogram changes (T wave flattening or inversion). These features resolve generally within 2-12 hours of the injection.

Individuals with methaemoglobinaemia

Cumulative doses of Methylthioninium chloride may lead to dyspnoea and tachypnoea, presumably related to reduced oxygen availability caused by methaemoglobinaemia, chest pain, tremor, cyanosis and haemolytic anaemia.

Haemolytic anaemia has also been reported in case of severe overdose (20-30 mg/kg) in infants and adults with methaemoglobinaemia caused by aniline or chlorates. Haemodialysis may be used in patients with severe haemolysis.

Paediatric population

Hyperbilirubinaemia has been observed in infants after administration of 20 mg/kg methylthioninium chloride.

Death occurred in 2 infants after administration of 20 mg/kg methylthioninium chloride. Both infants had complex medical circumstances and methylthioninium chloride was only partially responsible.

The patient should be maintained under observation, the methaemoglobin level should be monitored and appropriate supportive measures taken as necessary.

Laboratory findings

The use of methylthioninium chloride is associated with some laboratory adverse events data:

Pulse oximetry

The presence of Methylthioninium chloride may result in an underestimation of the oxygen saturation reading (Fearnley 1995; Guay 2009). The injection of methylthioninium chloride can cause a false indication of SaO2 by interference with changes in light absorption when pulse oximetry is used as an indicator of SaO2. It is advisable to obtain an arterial blood sample to confirm PaO2 and SaO2 (Touma 2007).

A special warning has been included in Section 4.4 of SmPC: "In cases of suspected methaemoglobinemia, it is advisable to check the oxygen saturation by co-oximetry when available since pulse oximetry may provide a false estimation of oxygen saturation during administration of methylthioninium chloride."

Bispectral Index (BIS)

Methylthioninium chloride may interfere with BIS value (Matisoff 2006). Thus, anaesthesiologists should be vigilant for methaemoglobinaemia in patients receiving dapsone therapy and for BIS interference with Methylthionimium chloride Proveblue administration. This warning has been included in Section 4.4 of SmPC.

Safety in special populations

Patients with glucose-6-phosphate dehydrogenase deficiency

MB is not effective for the treatment of methaemoglobinaemia in patients with G6PD deficiency as these patients have a diminished capacity to reduce MB to LMB.

It is also potentially harmful as patients with G6PD deficiency are particularly susceptible to the haemolytic anaemia induced by MB (Liao 2002). Low grade haemolytic anaemia has also been reported in patients with G6PD deficiency after oral administration of 390 mg of MB daily (Therapeutic Drugs 1999). Therefore, MB is contra-indicated in patients with G6PD deficiency.

Patients with renal impairment

In the presence of severe renal impairment toxic blood concentrations may occur with conventional doses, since MB is eliminated predominantly by the kidney. Therefore, MB should be used with caution in patients with severe renal impairment (Therapeutic Drugs 1999; Martindale 2007; Bradberry 2001)

Methylthioninium chloride Proveblue should be used with caution in patients with moderate to severe renal disease since there is limited data available and methylthioninium chloride is predominantly renally eliminated. Lower doses (<1 mg/kg) may be needed.

Use in children

Extreme caution should be exercised when administering to newborns and infants below the age of 3 months due to lower concentrations of NADPH-methaemoglobin reductase necessary for reducing methaemoglobin to haemoglobin, making these infants more susceptible to methaemoglobinaemia produced by high doses of methylthioninium chloride.

Safety information for children aged 3 to 18 years in published literature does not indicate that MB in doses below 7 mg/ml seem to cause any serious side effects except in infants and in patients with G6PD deficiency.

High doses (50 mg iv every 4 hours until the symptomatology receded) of MB have been administered to several children 2-17 years in treatment of ifosfamide-induced encephalopathy. No death or neurological sequelae were noted.

Use in the elderly

No specific toxic effects have been reported on the elderly without renal function deficiency (Therapeutic Drugs 1999).

Extrinsic factors

In the literature, no potential interactions with extrinsic factors such as use of tobacco, use of alcohol or food habits associated with the use of MB have been reported.

Use in Pregnancy and Lactation

There is no published data on IV injection of MB during pregnancy. However, several cases of intra-amniotic administration of MB to pregnant women have been reported demonstrating the teratogenicity of MB following this route of administration in pregnant women.

The use of MB in pregnant patients is therefore restricted to life threatening methaemoglobinaemia as it has been shown that MB is teratogenic.

There is no published data on whether or not the drug crosses into breast milk. Breast feeding should therefore be interrupted if acute treatment is necessary.

MB is not indicated for intrauterine injection for diagnosis of premature rupture of membranes or for amniocentesis.

Patients with hyperglycaemia or diabetes mellitus

If diluted in glucose 50 mg/ml (5%) solution for injection, methylthioninium chloride must be used with caution in patients with hyperglycaemia or diabetes mellitus, as these conditions may be exacerbated by the glucose solution.

Safety related to drug-drug interactions and other interactions

Methylthioninium chloride should be used with caution in the treatment of aniline-induced methaemoglobinaemia since it may precipitate Heinz body formation and haemolytic anaemia. Methylthioninium chloride may reduce methaemoglobin concentrations, but repeated doses could aggravate haemolysis without further reducing methaemoglobinaemia.

Methylthioninium chloride can also exacerbate dapsone-induced haemolytic anaemia because of the formation of the dapsone reactive metabolite hydroxylamine, which oxidises haemoglobin.

Methylthioninium chloride should not be used to treat methaemoglobinaemia induced by sodium nitrite during the treatment of cyanide poisoning, since cyanide binding will be reduced with resultant increased toxicity. Thus, sodium nitrite-induced methaemoglobinaemia is a contraindication

Methylthioninium chloride is also contra-indicated in methaemoglobinaemia due to chlorate poisoning as the more toxic hypochlorite may be formed.

Methylthioninium chloride should preferably be avoided in patients receiving drugs that enhance serotonergic transmission including SSRIs, bupropion, buspirone, clomipramine, mirtazapine, and venlafaxine. Drug interactions between methylthioninium chloride and SSRIs can cause potentially fatal serotonin toxicity.

An *in vitro* study showed that methylthioninium chloride is a potent inhibitor of CYP450 1A2, 2B6, 2C9 and 2C19. The clinical relevance of this finding is unknown but it cannot be excluded that the systemic exposure of medicinal products being substrates for these isoenzymes may be increased on concomitant administration with methylthioninium chloride.

2.6.1. Discussion on clinical safety

Based on available data from EU, the maximum reported incidence of methaemoglobinaemia in Europe is estimated to be 75 cases per year.

Intravenous administration has been reported to cause abdominal pain, headache, dizziness, tremors, anxiety, confusional state, chest pain, dyspnoea, tachycardia, hypertension, and hyperhirosis. However, several of these are also symptoms of methaemoglobinaemia. Intravenous injection has occasionally caused hypotension and cardiac arrhythmias, and such disorders have been fatal on rare occasions. Blue coloration of urine, faeces and saliva can occur. Methylthioninium chloride may impart a blue coloration to skin, which may hinder the diagnosis of cyanosis. Photosensitisation (photoallergy or phototoxicity) may occur after administration of methylthioninium chloride. A Drug Analysis Print covering adverse reaction reports (all routes of administration) to the UK Medicines Agency, during the period July 1963 to July 2010, related some additional adverse events such as aphasia, agitation, hypoxia and urticaria to Methylthioninium chloride.

If overdosed, methylthioninium chloride can instead oxidize ferrous irons to ferric ions, thus converting haemoglobin to methaemoglobin and cause haemolysis. In severe over dosage, 20-30 mg/kg, methylthioninium chloride can cause severe intravascular haemolysis and haemolytic anaemia may occur.

Methylthioninium chloride is not effective for the treatment of methaemoglobinaemia in patients with G6PD deficiency as these patients have a diminished capacity to reduce methylthioninium chloride to leucomethylene blue.

The epidemiologic evidence for teratogenicity of methylthioninium chloride is strong. Studies in animals have shown reproductive toxicity.

Drug-drug interactions between methylthioninium chloride and drugs that enhance serotonergic transmission e.g. SSRIs have been described.

2.6.2. Conclusions on the clinical safety

A number of adverse events associated with methylthioninium chloride treatments have been described when used in the recommended dose interval. The more severe events are linked to higher than recommended doses. Reported adverse events are covered in the proposed SmPC and the applicant has now listed additional reported events to section 4.8 (aphasia, agitation, hypoxia and urticaria) and also included a wording concerning monitoring of cardiac and blood pressure during and after treatment with methylthioninium chloride therapy in section 4.4.

2.7. 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 11: Summary of the risk management plan

Safety issue	Proposed pharmacovigilance activities	Proposed risk minimisation activities
Important identified safety issues		
Rapid injection of Methylthioninium chloride Proveblue	Routine pharmacovigilance activities.	Routine risk minimizations activities Section 4.2 and 4.4 of the SmPC state that Methylthioninium chloride Proveblue must be injected very slowly over a period of 5 minutes. The Patient Information Leaflet (PIL) and preparation guide for healthcare professionals also includes a warning to this effect. It is recommended to include this warning on the outer carton to ensure that in an emergency situation, the information is not missed by the healthcare professional administering the injection.
Dilution with sodium chloride (NaCl)	Routine pharmacovigilance activities.	Routine risk minimizations activities Section 6.6 of the SmPC states Do not dilute with sodium chloride (NaCl) solution for injection because it has been demonstrated that chloride reduces the solubility of methylthioninium chloride (common ion effect). The warning is repeated in the preparation guide section of the PIL for healthcare professionals.
Neonates and infants below 3 months of age	Routine pharmacovigilance activities.	Routine risk minimizations activities Section 4.4 of the SmPC states that extreme caution should be exercised when administering Methylthioninium chloride Proveblue to newborns and infants below 3 months of age. The same warning is also included in the PIL.
Hypersensitivity to methylthioninium chloride, or any other thiazine dyes	Routine pharmacovigilance activities.	Routine risk minimizations activities Section 4.3 of the SmPC provides for a strict contraindication for patients with known hypersensititivity to the active substance or any other thiazine dyes. Section 4.8 of the SmPC also includes 'anaphylactic reactions' as an adverse event. The same warning is

		included in the PIL.
Sodium nitrite-induced methaemoglobinaemia during the treatment of cyanide poisoning	Routine pharmacovigilance activities.	Routine risk minimizations activities Section 4.3 of the SmPC states that Methylthioninium chloride Proveblue is contraindicated for use in sodium nitrite-induced methaemoglobinaemia. The same warning is included in the PIL.
Methaemoglobinaemia resulting from chlorate poisoning	Routine pharmacovigilance activities.	Routine risk minimizations activities Section 4.3 of the SmPC states that Methylthioninium chloride Proveblue is contraindicated for methaemoglobinaemia due to chlorate poisoning. The same warning is included in the PIL.
Patients with Glucose-6-phosphate dehydrogenase deficiency (G6PD)	Routine pharmacovigilance activities.	Routine risk minimizations activities Section 4.3 of the SmPC states that Methylthioninium chloride Proveblue is contraindicated for the treatment of methaemoglobinaemia in patients with glucose-6-phosphate dehydrogenase deficiency (G6PD) due to the risk of haemolytic anaemia. A similar warning is included in the PIL.
Deficiency in NADPH reductase	Routine pharmacovigilance activities.	Routine risk minimizations activities Section 4.3 of the SmPC states that Methylthioninium chloride Proveblue is contraindicated for deficiency in NADPH reductase. A similar warning is included in the PIL.
Subcutaneous and intra-thecal injection	Routine pharmacovigilance activities.	Routine risk minimizations activities Section 4.2 of the SmPC states that Methylthioninium chloride Proveblue must not be administered by subcutaneous and intra-thecal injection. The same warning is included in the PIL.
Patients with aniline-induced methaemoglobinaemia	Routine pharmacovigilance activities.	Routine risk minimizations activities Section 4.4 of the SmPC states In patients with aniline-induced methaemoglobinaemia, repeated doses of methylthioninium chloride may be required. Caution should be exercised in the course of treatment with methylthioninium chloride as this may exacerbate Heinz body formation and haemolytic anaemia. Lower doses should

		therefore be considered and total cumulative dose should not exceed 4 mg/kg. An appropriate warning is included in the PIL.
Patients with dapsone-induced haemolytic anaemia (and/or patients with severe anaemia)	Routine pharmacovigilance activities.	Routine risk minimizations activities Section 4.4 of the SmPC states Methylthioninium chloride Proveblue can exacerbate dapsone-induced haemolytic anemia because of the formation of the dapsone reactive metabolite hydroxylamine which oxidises haemoglobin. It is recommended not to exceed a cumulative dose of 4 mg/kg in these patients. An appropriate warning is also included in the PIL.
Patients with moderate or severe renal impairment (and/or kidney disease)	Routine pharmacovigilance activities.	Routine risk minimizations activities Section 4.2 of the SmPC states that in patients with moderate to severe renal impairment, doses of Methylthioninium chloride Proveblue may need to be reduced as it is predominantly renally eliminated. An appropriate warning is also included in the PIL.
Diagnosis of cyanosis	Routine pharmacovigilance activities.	Routine risk minimizations activities Section 4.4 of the SmPC advises clearly that Methylthioninium chloride Proveblue imparts a blue colour to skin which may hinder a diagnosis of cyanosis.
Interaction with serotonergic drugs resulting in CNS toxicity	Routine pharmacovigilance activities.	Routine risk minimizations activities Section 4.5 of the SmPC advises clearly that Methylthioninium chloride administration should be avoided in patients receiving serotonergic drugs due to the risk of CNS toxicity. An appropriate warning is also included in the PIL.
Pregnancy, breast-feeding and fertility	Routine pharmacovigilance activities.	Routine risk minimizations activities Section 4.6 of the SmPC advises clearly that Methylthioninium chloride Proveblue administered intravenously has not been investigated in pregnancy and should not be used unless clearly necessary. Section 4.6 and 5.3 of the SmPC provides appropriate guidance for consideration in relation to reduced sperm motility.

		Section 4.6 also advises breast-feeding to be discontinued for up to 6 days after treatment with Methylthioninium chloride Proveblue. Corresponding advice as appropriate is also clearly communicated within the PIL.
Haemolytic anaemia	Routine pharmacovigilance activities.	Routine risk minimizations activities Section 4.3 of the SmPC states a contraindication for Patients with Glucose-6-phosphate dehydrogenase deficiency (G6DP) due to the risk of haemolytic anaemia. Additional warning statements can be found for haemolytic anaemia within section 4.4 for patients with chemically induced methaemoglobinaemia.
Overdose	Routine pharmacovigilance activities	Routine risk minimizations activities Several precautions and warnings have been included in the SmPC: • In section 4.2, it specifies that 'Methylthioninium chloride Proveblue is for administration by a healthcare professional'. Healthcare professionals are trained how to administer injections correctly and have many years of experience, thus reducing the likelihood of incorrect administration. • Section 4.2 specifies the maximum recommended cumulative dose for adults (7 mg/kg) and stresses that the recommended dose should not be exceeded. In patients with renal impairment/disease, smaller doses of Methylthioninium chloride may be required. A warning to this effect is included in section 4.2 to reduce the chance of overdose occurring in these patients. Sections 4.2 and 4.4 also state that in the case of aniline- or dapsone-induced methaemaglobinaemia, the maximum recommended cumulative dose for the course of treatment is 4 mg/kg. The PIL includes an appropriate statement in consideration that the product is a hospital administered product. The packaging is distinct and clearly states

		the product name to ensure healthcare professionals will not accidentally administer the product, resulting in overdose.
Important potential safety issues		
Photosensitivity	Routine pharmacovigilance activities.	Routine risk minimizations activities Provepharm to monitor the literature and post-marketing data to ascertain the incidence of photosensitivity and if necessary update section 4.8 of the SmPC in line with the PSUR cycle.
For all AEs with an unknown frequency	Routine pharmacovigilance activities.	Routine risk minimizations activities On the basis of post-marketing data, section 4.8 of the SmPC can be updated with AE frequencies in line with the PSUR cycle.
Long term administration	Routine pharmacovigilance activities.	Routine risk minimizations activities The product remains an antidote for use in emergency situations. Nevertheless, Section 4.2 of the SmPC has been updated to state that "Treatment should not usually exceed one day."
Misdiagnosis as a result of underlying cardiac, pulmonary, or haematologic diseases	Routine pharmacovigilance activities.	Routine risk minimizations activities Within a critical care setting, clinicians will be aware of standard protocols which require them to ascertain the patients medical history and differential diagnosis prior to treatment with Methylthioninium chloride.
Patients with diabetes mellitus or hyperglycaemia	Routine pharmacovigilance activities.	Routine risk minimizations activities Section 4.4 of the SmPC contains precautionary wording regarding the administration of glucose solution to diabetic and hyperglycaemic patient groups. It is noted that this advice would be concurrent with the precautions ordinarily taken by clinicians when treating such patients.
Patients with hereditary (congenital) methaemoglobinaemia	Routine pharmacovigilance activities.	Routine risk minimizations activities Within a critical care setting, clinicians will be aware of standard protocols which require them to ascertain the patients medical history and make a clinical diagnosis prior to treatment with Methylthioninium chloride.
Methaemoglobinaemia rebound (resulting from	Routine pharmacovigilance	Routine risk minimizations activities

intermittent infusion methylthioninium blue or in patients with dapsone-induced methaemoglobinaemia)	activities.	In a clinical care setting, clinicians will be aware of standard protocols which require them to monitor patients before and after treatment.
Missing information		
Ethnicity	Routine pharmacovigilance activities.	Routine risk minimizations activities
Incidence and prevalence of medicinal and chemical product-induced methaemoglobinaemia	Routine pharmacovigilance activities.	Routine risk minimizations activities Methaemoglobinaemia remains a rare condition and based on the literature data currently available.

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.

User consultation

The results of the user consultation with target patient groups on the package leaflet submitted by the applicant show that the package leaflet meets the criteria for readability as set out in the *Guideline on the readability of the label and package leaflet of medicinal products for human use.*

2.8. Benefit-Risk Balance

Benefits

- Beneficial effects

Methylthioninium chloride is the single most documented and clinically used substance to treat drug- and chemical induced methaemoglobinaemia. The clinical experience of methylthioninium chloride over the last 70 years has demonstrated its efficacy to reverse most cases of induced methaemoglobinaemia.

The applicant of Methylthioninium chloride Proveblue has aimed at producing a solution for injection packaged in a suitable form for IV injection to be in conformity with the general monographs of the Ph.Eur. Methylthioninium chloride Proveblue is claimed to be essentially similar to the reference product Methylthioninium Chloride Injection USP 1% w/v by Martindale Pharmaceuticals Limited. The methylthioninium chloride of the Proveblue product is very pure and complies with the purity criteria of the Ph. Eur. monograph for methylthioninium chloride.

This antidote could be life saving.

The applied new indications

- chemical induced methaemoglobinaemia in adults and
- drug- and chemical induced methaemoglobinaemia in newborn infants, infants, children and adolescents

are supported by the submitted literature references.

- Uncertainty in the knowledge about the beneficial effects.

Methylthioninium chloride Proveblue is provided in a different strength compared with the reference product due to a difference in the solubility. However, the difference in solubility is small, and the applicant has convincingly demonstrated that the potential difference in solubility between Methylthioninium chloride Proveblue and the reference product will not impact on the pharmacokinetic features. A bioequivalence study is not required and it can be concluded that treatment with Methylthioninium chloride Proveblue will result in similar benefits as that of the reference product

Methylthioninium chloride is the single most used drug to treat drug- and chemical induced methaemoglobinaemia. All poison centres in the EU have since long adopted methylthioninium chloride as the drug of choice for treating this condition. No controlled clinical trials according to modern standards have been performed with methylthioninium chloride. This also applies to the product assessed here, Methylthioninium chloride Proveblue. The efficacy assessment rests only on published case reports. Based on these, the efficacy in treating methaemoglobinaemia with methylthioninium chloride must be considered to have been established. There is limited experience for Methylthioninium chloride treatment in infants, but sufficient to establish the efficacy of the product in this patient population. In addition, available data for other alternative treatment for methaemoglobinaemia in this population are almost non-existing.

Risks

- Unfavourable effects

A number of adverse events associated with methylthioninium chloride treatments have been described when used in the recommended dose interval. The more severe events (e.g. cardiovascular events) are linked to higher than recommended doses. When used as recommended in the SmPC text the risk level is estimated to be low, especially as the patients usually are treated in high care facilities. Renal excretion is the major elimination pathway and impaired renal function may lead to increased systemic exposure.

- Uncertainty in the knowledge about the unfavourable effects

As mentioned above, the applicant has convincingly demonstrated that the reduced solubility does not lead to pharmacokinetic differences compared with the reference product and safety data can be extrapolated from the reference product.

As for efficacy, safety data are based on a limited number of case reports. However, the long clinical experience has created a safety data base that must be considered to be reliable. Although clinical data in infants is limited, the recommended reduced dose should mitigate the risks in this patient population.

Benefit-risk balance

- Importance of favourable and unfavourable effects

Long clinical experience has demonstrated that the anti-dote methylthioninium chloride is effective in treating externally induced methaemoglobinaemia. The reference product Methylthioninium Chloride Injection USP 1% w/v by Martindale Pharmaceuticals Limited has successfully been used for many years, and the treatment is considered as life saving. Alternative treatments exist, but have been found less effective. Severe adverse events linked to methylthioninium chloride treatments are few. The methylthioninium chloride of the Proveblue product is very pure, which appears to be a potential

advantage with this product. The Applicant has demonstrated that potential differences in quality between Methylthioninium chloride Proveblue and the reference product is of no relevance for the pharmacokinetic features (indirectly efficacy and safety) and provided sufficient information regarding the synthesis of the starting material. Thus, the favourable effects of the treatment are considered as undisputable and clearly outweigh the risks.

- Benefit-risk balance

Based on previous experience with the reference product, the benefit-risk balance for methylthioninium chloride in the treatment of methaemoglobinaemia is positive. The applicant has demonstrated that potential differences in quality between Methylthioninium chloride Proveblue and the reference product is of no relevance for the pharmacokinetic features (indirectly efficacy and safety) of this product and provided sufficient information regarding the synthesis of the starting material. Thus, the benefit-risk balance can be considered as positive.

2.8.1. Discussion on the benefit-risk balance

The long positive clinical experience is presently sufficient for establishing benefits and risks related to methylthioninium chloride and since quality and quality related pharmacokinetics issues are solved this should also be applicable to Methylthioninium chloride Proveblue.

2.8.2. Risk management plan

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
- no additional risk minimisation activities were required beyond those included in the product information

2.9. Recommendation

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considered by consensus that the risk-benefit balance of Methylthioninium chloride Proveblue in the acute symptomatic treatment of medicinal and chemical products- induced methaemoglobinaemia was favourable and therefore recommended the granting of the marketing authorisation.