PRODUCT PROFILE

Product name:	Oxyglobin
Procedure No.:	EMEA/V/C/045
Applicant company :	Biopure Netherlands B.V. Prinses Irenestraat 59 N1-1077 WV Amsterdam The Netherlands
Active substances	Bovine haemoglobin glutamer 200
Proposed International Non-proprietary Name:	-
Pharmaceutical form:	Solution for infusion
Strength	16.2 g/125 ml
Presentation:	125 ml polyolefin infusion bags
Package size:	A box with two (2) polyolefin infusion bags (125 ml), each within an overwrap.
Target species:	Dogs
Withdrawal period:	N/a
Route of administration:	Intravenous use
Product type:	Pharmaceutical
Therapeutic indication:	Oxyglobin provides oxygen carrying support to dogs improving the clinical signs of anaemia for at least 24 hours, independent of the underlying condition.

SCIENTIFIC DISCUSSION

1. INTRODUCTION

Oxyglobin is a haemoglobin-based oxygen carrying solution, which increases plasma and total haemoglobin concentrations, and consequently arterial oxygen content. The active substance is haemoglobin glutamer-200 (bovine), a glutaraldehyde polymerised haemoblobin. Prior to polymerisation, the haemoglobin is isolated from bovine red blood cells and highly purified by diafiltration, centrifugation and chromatographic techniques. The product contains haemoglobin glutamer-200 (bovine) 16.2g in 125 ml modified Lactated Ringer's Solution containing sodium lactate. A small amount of acetylcysteine is present to prevent the oxidation of the haemoglobin to physiologically inactive methaemoglobin. The solution is isotonic and isosmotic. The product is stable between 2-30°C with a shelf life of 2 years. It should be used within 24 hours when the package is opened for infusion.

Oxyglobin's plasma half-life is 30-40 hours and elimination from the plasma is complete in 5-7 days. A small amount of unstabilised tetrameric haemoglobin (<5%) may be excreted in the kidneys resulting in transient haemoglobinuria for <4 hours.

The target species is dogs. Oxyglobin provides oxygen carrying support and expands plasma volume to improve clinical signs of anaemia for at least 24 hours regardless of the underlying condition. The product does not need cross matching with blood and fulfils a need in veterinary practice.

The product is contraindicated in dogs likely to have circulatory overload with conditions such as advanced cardiac disease, or otherwise severely impaired cardiac function, or renal impairment with oliguria or anuria. This should be particularly considered when administering adjunctive intravenous fluids. Signs of circulatory overload should be monitored or central venous pressure measured. In cases of concern the infusion of Oxyglobin should be discontinued temporarily and reinstated at a slower rate when signs abate and/or Central Venous Pressure (CVP) decreases.

Treatment results in a mild decrease in PCV (packed cell volume) immediately after infusion due to haemodilution.

The safety in pregnant or lactating bitches has not been determined and hence the use in such dogs is not recommended.

2. OVERVIEW OF PART II OF THE DOSSIER: ANALYTICAL ASPECTS

2.1 QUALITATIVE AND QUANTITATIVE PARTICULARS OF THE CONSTITUENTS

Oxyglobin contains Haemoglobin glutamer 200 (bovine) as active substance at a concentration of 16.2 g/125 ml. The function is oxygen delivery and an in house specification is followed.

The solution is supplied in pre-sterilised Stericon infusion bags, which are composed of a laminated bilayer film, with polyolefin polymer on the product contact/sealing surface and nylon on the outside surface. The bags are of 125mL capacity and are protected by sealed outer polyethylene/aluminium foil/polyester film overwrap pouches.

2.1.1 Development Pharmaceutics

The native bovine haemoglobin molecule (tetramer) has been treated with glutararaldehyde, a cross-linking agent stabilise the tetramer form and then to produce a polymer. This reaction is controlled to ensure a reproducible molecular weight. The product is formulated in a Ringer Lactate Solution to provide an isotonic vehicle for the parenteral administration of the product, and a shelf life of 2 years

at 2-30°C is claimed. It is intended for single dose administration. The applicant has addressed the choice of the formulation and in particular the role of N-acetylcysteine in maintenance of the potency of the product limiting the conversion of haemoglobin to methhaemoglobin inactive.

2.2 METHOD OF PREPARATION

The batch size is 250L with a yield of 2,000 bags. The manufacturing process is fully descibed and a flow chart was provided. The active ingredient is not isolated and so further details are included in section 2.3 below.

The outline of the filling process is described and the flow charts are included in the dossier, together with the relevant in-process controls.

Microbiological validation of the filling process was conducted using media fills on 4 occasions using the bag as intended for marketing and otherwise mimicking the actual filling process. Incubation took place at 20-25°C, which is justified since this temperature range can support the growth of all mesophilic micro-organisms. The validation studies will be repeated at a justified frequency.

Details of the validation of the sterilising filters were provided in the dossier.

A bioburden sample is routinely taken at the end of the first day of filling from final bulk (designated T-990) and then taken again half way through the second day of filling. Each day of filling is considered a separate lot of product. This sampling schedule represents the highest bioburden load that the sterilizing filter is exposed to during a fill. The specification for these samples is not more than 2 CFU/mL.

2.3 CONTROL OF STARTING MATERIALS

2.3.1 Active Ingredient

The active substance is haemoglobin glutamer 200 (bovine) and a specification for the bulk oxyglobin solution is provided in the dossier.

The Applicant has provided specifications of all materials used. The specifications in general appear to be satisfactory. The majority of analytical methods are based on pharmacopoeial methods. Of the in-house methods used the details appear to be satisfactory. Adequate details of sanitisation of tanks have been provided. The process uses clean steam to steam sanitise/sterilise various process vessels, lines, filters and equipment items that have been previously cleaned.

Starting material (bovine blood) is obtained from carefully controlled herds. The donor cattle are not segregated from other cattle. However, controls are in place to assure compliance with the requirements of EMEA/CVMP/145/97 Final, 'Note for Guidance for minimising the Risk of Transmitting Animal Spongiform Encephalopathy Agents via Veterinary Medicinal Products'. Animals are identified as potential donors at least 90 days prior to slaughter by an identification system that includes the individual animal number and the lot number. Typically, the identification is in the form of an ear tag, although the system may include electronic implants or tattoos. Animals that have been medicated within 90 days prior to donation are not utilised.

Biopure currently uses USP quality sodium citrate and this has been acceptably justified. Biopure has performed a storage study on the citrate anticoagulant solution and found that it may be stored for up to 14 days before exceeding specifications.

The company has not performed any screening or clearance studies for mycoplasma since they consider that no cellular fraction is used in the production process; any mycoplasma present are unlikely to survive, neither are they likely to cross the species barrier. However the company proposed testing 5 lots of intermediate, compound 500, as a validation exercise to demonstrate freedom from mycoplasma in representative production batches. This approach has been approved and

the company may apply, post authorisation, to delete this testing requirements by way of a variation, with a report on the outcome of the validation study.

The company does not perform viral screening on incoming material, but has performed viral clearance studies and has agreed to perform viral screening on BVD virus and Blue Tongue on the final product of every batch that is marketed in the first year post-authorisation. The situation will then be further reviewed by CVMP. The assay techniques will be based on cell culture.

The manufacture of the active substance takes place in several stages. The first product is a cell free haemoglobin intermediate. It is stated that this solution may be stored for up to 4 weeks at 2-10° C before further processing and data have been supplied to support this claim. The next stage is purified haemoglobin and data have been supplied to support the claimed storage for up to 90 days at 17-22°C after sterile filtration. Treatment of this final intermediate with glutaraldehyde polymerises the haemoglobin, and after stabilisation with sodium borohydride and diafiltration, the final bulk oxyglobin is sterile filtered through double 0.22µ filters and stored in a 275 L portable batch holding tank. Certain reprocessing conditions are described. The Applicant has confirmed that no other reprocessing criteria will be considered without prior approval.

Purity of the active substance was assessed in three separate lots by sodium dodecyl sulfate – polyacrylamide gel electrophoresis SDS-PAGE which showed absence of major blood proteins, IgG, albumin and carbonic anhydrase, confirmed by immunoblot. Background information on the procedures for SDS-PAGE and Western blotting techniques are provided together with relevant validation by way of response. The Applicant does not test for IgG in each batch.

Structure elucidation and degree of polymerisation was conducted by HPSEC, LDMS, IEF, RPHPLC, aminoacid analysis, and UV-visible absorbance spectroscopy.

Other test procedures are detailed in the dossier in regard to the finished product.

3 batches of active substance demonstrated compliance with specifications.

Stability of intermediates during the processing place is addressed.

TSE prion clearance studies have addressed the risk of transmission of BSE taking into consideration the source herds, the risk category for blood and the clearance studies. Clearance studies are summarised in the dossier and show some significant levels of removal in the diafiltration and anion exchange stages but no clear TSE - prion inactivation - this is as expected since very drastic conditions are needed to inactivate prion protein. Virus validation studies - see below.

Batch analytical data will cover 5 consecutive production scale batches and address all parameters in the specification.

2.3.2 Other Ingredients

Excipients comply with requirements of the Ph. Eur where applicable.

2.4 CONTROL AT INTERMEDIATE STAGES OF THE MANUFACTURING PROCESS

Not applicable.

2.5 CONTROL OF THE FINISHED PRODUCT

Specifications of the finished product

A full specification for the time of release has been provided. Many of the tests necessary to control the finished product are in fact conducted on the bulk Oxyglobin solution or as in-process basis. This approach is accepted in principle, however the specification, with which each batch must comply if tested, should be clearly stated even if on a batch by batch basis, the tests are not carried out on the product in the final container. Testing is included on process impurities, glutaraldehyde and N-acetylcysteine, ion analysis and HP-SEC tests being conducted at the bulk stage.

The applicant has confirmed that the key parameters affecting oxygen carrying capacity are Total Haemoglobin with a valid upper limit on the content of the inactive species methaemoglobin.

The release and shelf life limit has been tightened to <5% of met-haemoglobin needs.

The following specifications for N-acetylcysteine have been chosen and are considered acceptable:

Release	0.22 - 0.13%
Shelf life	0.22 - 0.02%

Analytical Validation

Potency determination is conducted by co-oximetry/spectrophotometry to provide simultaneous determination of haemoglobin, oxy-haemoglobin and met-haemoglobin content. A full explanation of the scientific principles and operation of the technique has been supplied. Satisfactory validation in terms of limit of quantification, range, sensitivity and robustness has also been provided.

Cross validation of the accuracy of determination of met-haemoglobin and total haemoglobin has been described in full.

Determination of osmolality is carried out by freezing point depression, and validated for precision, linearity and accuracy. The endotoxin determination has been validated for precision, accuracy and linearity. This test and the validation studies conform with the requirements of the Ph. Eur. monograph. It is noted that the sterility test methodology is in accordance with the requirements of the Ph. Eur. Test for Sterility. However the reference organisms used in the validation studies deviate from those specified in the Ph. Eur. test, in particular in relation to *Staph. Aureus* and *Pseudomonas aeruginosa* where environmental isolate organisms were used. The test will be re-validated in accordance with Ph. Eur. Methodology before being used to release batches onto the market within Europe.

VALIDATION OF VIRAL CLEARANCE

Choice of viruses

The Applicant has chosen four model viruses, namely Bovine Parvovirus (BPV), Ecotropic Murine Leukemia Virus (MuLV), Infectious Bovine Rhinotracheitis Virus (IBR) and Bovine Viral Diarrhoea Virus (BVDV), for use in experimental studies intended to validate the capacity of the production process to eliminate viral contamination.

The range of viruses studied encompasses a reasonable range of physico-chemical types (RNA/DNA, enveloped/non-enveloped), though only one non-enveloped virus is represented (BPV), and these are frequently the most difficult class of virus to remove or inactivate. The physico-chemical properties of each virus are summarised in the table below.

VIRUS	GENOME	ENVELOPE	SIZE (nm)	FAMILY	SUSCEPTIBILITY TO PHYSICAL & CHEMICAL INACTIVATION
Ecotropic Murine Leukemia Virus (MuLV)	RNA	Yes	80-100	Retroviridae	High
Bovine Parvovirus (BPV)	DNA	No	20-25	parvoviridae	Low
Bovine Viral Diarrhea (BVD)	RNA	Yes	40-70	Togaviridae	High
Infectious Bovine Rhinotracheitis (IBR)	DNA	Yes	150-250	Herpesviridae	Medium

Detailed descriptions of the strains etc. of the viruses have been provided on the model viruses used in the clearance experiments, including strain designations, taxonomy, literature references, characteristics, tissue sources, host ranges, and propagation substrates. All the virus preparations were obtained from ATCC. Bearing in mind the possible virus contaminants of bovine starting materials, the viruses also appear to be reasonable choices as relevant and model viruses.

Virus validation studies

The Applicant has addressed the question of whether the presence of antibodies to the model viruses could be contributing to the virus load reductions observed in the validation studies by citing immunoglobulin clearance rates which are reported in detail elsewhere in the dossier. Four process steps likely to contribute to virus elimination were studied.

The virus validation experiments were conducted by Microbiological Associates in the USA in accordance with the requirements of Good Laboratory Practice.

The overall reductions demonstrated for each virus are fairly substantial (> 8 to > 14). However, the following reservations apply to the data:

- few of the process steps have been shown to reduce infectivity by a factor of more than 4 logs,
- the effectiveness of the process steps against the one non-enveloped virus studied was > 8 logs,
- two steps involve virus removal/partitioning which is generally less appealing than virus inactivation, however, the two steps do represent chemical inactivation.

As a result an undertaking from the Applicant for completing the following specific obligations was received:

1. Sampling for BVD virus and Blue Tongue virus will be performed on the final product of every batch that is marketed in the first year post-authorisation.

2. The assay techniques for BVD virus and Blue Tongue virus will be based on cell culture.

3. An experiment [validated scaled-down manufacturing process] will be performed by Biopure to assess the integrity of the viral inactivation techniques employed in the manufacturing process by 14 July 2000. This will involve processing five separate batches of raw material, containing blood derived from at least one animal that has been shown to be viraemic with BVD virus. Testing of the final product from each of these five separate batches by the cell culture technique outlined previously will subsequently be performed, to assess whether or not the virus survived the inactivation steps.

4. By 14 July 2000, the results of the virus validation study outlined in Point 3 will be submitted to the CVMP. Should the data provide sufficient guarantees of BVD virus inactivation, consideration will then be given by the CVMP to reducing the frequency of product testing to one batch of final product per year.

- Biopure submitted the results of a study addressing the specific obligations raised in Points 3 and 4 on 11 July 2000.
- The Rapporteur submitted his report on the study on 9 October 2000.
- Preliminary discussions took place during the CVMP meeting on 10 October 2000.
- The company was asked to explain the departure from the originally agreed protocol in their study on 10 October 2000.
- Biopure provided a number of justifications on 10 October 2000.
- Comments from CVMP members on the annual reassessment were received by 20 October 2000.
- CVMP gave its opinion on the annual reassessment on 8 November 2000.

The dossier consisted of a validation report accompanied by several appendixes and an expert opinion from the company's Product Development Director indicating successful conclusion to this study.

However, the CVMP had required the company to conduct a study by processing five separate batches of raw material (bovine blood) containing blood derived from at least one animal shown to be viraemic with BVD virus. Testing of product derived from each of these five separate batches by the agreed cell culture technique was to be performed to assess whether the virus survived the manufacturing process. This approach was not followed in the present study since a standard positive control culture of BVD virus was utilised instead of a wild type virus from a viraemic animal.

BVDV Clearance Study

BVD virus is an enveloped RNA virus belonging to the Flaviviridae family and is 40 to 70 nm in size with medium resistance to physical/chemical reagents. The present study was undertaken to address the ability of the normal Oxyglobin production process to effectively remove/inactivate this model virus. All studies were conducted in accordance with international viral inactivation regulatory guidelines (for example CPMP/BWP/278/95) by the contract laboratory Bio Reliance, Rockville, Maryland.

Study Methods

BVD virus from two separate lots ATCCVR534 lots BD060497P and BD010997P were used in the study. The average virus titre of 2.3×10^7 PFU per ml was utilised. The various starting materials and intermediates in the purification process were examined from the perspective of potential interference and cytotoxity during the study. The effect of freezing was also examined in regard to potential antiviral effects of the process. A scaled down version of the process was used for the validation study and data validating the size of the scaling down were also provided.

The process was challenged by spiking the test virus (2.3 x 107 pfu/ml) in to the lysed bovine red blood cells. The principle processes being challenged in the process (Figure 2) were - 100 kd ultrafiltration step

- Anion exchange chromatograph
- 30 KD Diafiltration
- Glutaraldehyde polymerisation of the Hb
- Sodium borohydride reduction

Four sampling points were identified for vital culture assay. However, a single initial spike of virus was conducted to assess the overall capacity of the process for virus removal/inactivation.

Process Detail and Controls

A synopsis of the process is provided in the attached summary. For each run a control hold (medium control) was prepared by spiking stock BVD virus into EMEM, and held under the same conditions as the process step being challenged and used to subtract artefacts of the medium.

Results

Good consistency between the runs was noted and an average virus clearance of 7 logs was recorded.

The study demonstrated a consistent and reproducible viral reduction of 6-7 logs for the total manufacturing process. This is a lower cumulative log reduction than that obtained in the original studies which challenged the 4 key processing steps separately and summed the resulting clearance values as is the usual procedure in accordance with EU guidance. Since no virus was detected in Output 3 or Final Output samples in any of the 6 runs then the results presented herein probably represent an underestimate of the actual virus inactivation capacity of the process. From this perspective the results gave adequate reassurance about the effectiveness and consistency of virus elimination during the process.

The approach adopted by the company should not present any adverse implications with respect to the data outlined in the study. Although the company has provided sound reasons, it is also agreed that the study plan should not have been changed (using ATCC strains in stead of wild type virus) without agreement with the CVMP. However, the study was accepted as fulfilling the specific obligations raised in Points 3 and 4 of Annex IIC.

2.6 STABILITY

2.6.1 Active Ingredient

The Applicant has presented data available from the literature to support the studies conducted on the finished product. The Oxyglobin stability studies followed the key structural characteristics, methaemoglobin, molecular weight distribution and total concentration of the active substance (haemoglobin) because it is known that denaturation of the product can not occur without these parameters changing.

2.6.2 Formulated Product

Stability studies were performed with three (3) batches of the finished product in the market packaging, at three different storage temperatures (2-8°, RT, and 37 or 40° C) for up to 24 months. Data from additional batches will be provided, on an ongoing basis, to support the stability claim of 24 months when stored equal to or below 30° C.

Details of the general safety test have been provided. RT (room temperature) was confirmed by the company to have been conducted at a constant 25°C in accordance with ICH principles.

Analysis of retained/production batches H4C108 and H4C109, stored for 45 months at ambient temperature are provided as supportive data.

After the granting of a Marketing Authorisation the Applicant submitted a Type I No 20 variation to extend the shelf life to 3 years for the product when stored at temperatures not exceeding 30°C. This Type I variation was approved by the EMEA on 1 September 2000.

3. OVERVIEW OF PART III OF THE DOSSIER: TOXICOLOGICAL AND PHARMACOLOGICAL ASPECTS

3.1 Introduction

Oxyglobin is a haemoglobin (Hb) glutamer-200 (bovine) with oxygen carrying ability. Oxyglobin is formulated as a sterile solution for infusion (125 ml/bag containing bovine haemoglobin 13 g/dl in modified Lactate Ringer's solution).

Oxyglobin is a haemoglobin based oxygen carrier manufactured by stabilising purified bovine Hb. The purification process involves several diafiltration, centrifugation and chromatography steps. Native Hb is a 65 kD tetramer, which dissociates rapidly into dimers in plasma. To prevent rapid excretion by the kidneys and prolong half-life, the Hb is chemically cross-linked to form stable polymers, up to 500 kD,

with <5% as unstable tetramer. The formulation contains Hb at physiologic concentration, 13 g/dl, and has acceptable oncotic pressure 20-25 mmHg. In its long development history, various formulations of bovine Hb have been tested in several species. Abstracts and summaries of these studies have been included in the dossier and form a background for the main studies. In this assessment report only those studies using the formulation that is the subject of this application have been examined in detail. Pivotal studies for Part III of the dossier were carried out in dogs, the target species. Whilst few comply with GLP guidance, the studies conducted were sufficient in number and type, and contain adequate group sizes to assess the effects of Oxyglobin in dogs and permitted determination of the drug's safety.

3.2 Pharmacology

3.2.1 Pharmacodynamics

A series of studies were carried out to show that Oxyglobin can provide oxygen carrying support in a model of acute anaemia in dogs. Normovolaemic haemodilution was established by withdrawal of blood and simultaneous replacement with Lactate Ringer's Solution (LRS) until total haemoglobin (Hb) was 3 g/dl, cardiovascular, blood gas and blood pressure parameters were monitored.

A series of studies compared Oxyglobin to Rheomacrodex (Dextran 40), LRS and homologous packed red blood cells (PRBC) dogs were treated with: (1) Oxyglobin or Rheomacrodex at 60 ml/kg/hr; (2) Oxyglobin 35 ml/kg at 60 ml/kg/hr, 35 ml/kg at 20 ml/kg/hr, 41 ml/kg at 20 ml/kg/hr or Rheomacrodex 15 ml/kg at 10 ml/kg/hr; (3) Oxyglobin 45 ml/kg at 20 ml/kg/hr, 41.5 ml/kg at 20 ml/kg/hr, 20 ml/kg at 20 ml/kg/hr, 15 ml/kg at 20 ml/kg/hr, Rheomacrodex 16ml/kg at 10 ml/kg/hr, 35 ml/kg at 20 ml/kg/hr or PRBC (13 g/dl) 45 ml/kg at 20 ml/kg/hr; (4) PRBC (24 g/dl) 18.8 ml/kg at 120 ml/kg/hr; (5) Oxyglobin 13-27-52-106 ml/kg or LRS 53 ml/kg at 120 ml/kg/hr. In the third study dogs were splenectomised at least 3 days prior to treatment. In Oxyglobin treated dogs serous nasal discharge, red/yellow discoloration of the skin and mucous membranes, soft faeces and dehydration were seen. Other observations were seen in all animals and are thought to result from the haemodilution procedure, e.g. a fall in RBC and WBC count, a general dilution of serum chemistry values, dose related increase in cardiac output and blood pressure. Haematological and blood gas parameters were monitored for 24 hours after treatment. Oxyglobin treated dogs showed good recovery from induced anaemia and in general this was moderately better than Rheomacrodex or LRS treated dogs but not as good as for PRBC treated dogs.

A GLP dose response study was carried out using the same model, i.e. splenectomised, normovolaemic, haemodiluted dogs (3/sex/group). The animals received 7-14 ml/kg Rheomacrodex or 15-30-45 ml/kg Oxyglobin iv at 20 ml/kg/hr. Pale skin and serous nasal discharge were observed during the bleed. On treatment with Oxyglobin, there was a dose related red discoloration of skin, mucous membranes and nasal discharge. Two of the high volume control dogs died, probably due to severe haemodilution. Blood chemistry, coagulation factors and haematocrit showed evidence of haemodilution. Blood pressure and cardiac output increases were also thought to be volume effects. These effects lasted up to 24 hours in higher volume treatments. There was an increase in oxygen delivery, but not consumption, at 1 but not 24 hours in Oxyglobin treated vs. Rheomacrodex treated animals.

A brief report is included of a multi-centre, randomised field trial in dogs with anaemia and the full report is presented in Part IV. In the dogs admitted to the trial the anaemia resulted from blood loss (25), haemolysis (30), or ineffective erythropoeisis (9). Fifty-two of the 64 dogs entered into the trial were treated with Oxyglobin at 30 ml/kg iv at 15 ml/kg/hr. Thirty dogs were randomised to receive Oxyglobin. Twenty-two of 34 control dogs (no treatment/LRS) subsequently received Oxyglobin. Successful treatment was recorded if no other oxygen support was required in the 24 hours post-treatment. In this study the success rate was 95% for treated vs. 32% for untreated (blood loss, 90 vs. 45%; haemolysis 100 vs. 9%; ineffective erythropoiesis 100 vs. 50%). In addition, there was a longer time to failure in treated dogs and improved physical condition scores over 24 hours compared to controls. Adverse events were recorded in control and treated dogs. These included: death 23%, gastrointestinal effects 52%, cardiovascular a44%, respiratory 36%, central venous pressure 100% (of the 18 animals measured), clinical pathology 75% and miscellaneous 38%. Most adverse events were related to the underlying disease and were transient or mild/moderate rather than severe.

3.2.2 Pharmacokinetics

One pilot pharmacokinetic study was carried out in four Beagle dogs. Two animals each received a single i.v. dose of 8.5 or 42.5 ml/kg at 7 ml/kg/hr. Plasma levels of haemoglobin and methaemoglobin were measured over 7 days. Total plasma Hb peaked at 1.67-5.25 g/dl in 1-12 hours and returned to baseline in 72-120 hours with a half-life of 32-59 hours, respectively. At the lower dose the kinetics appear to be first order, while at higher doses evidence suggests zero order kinetics. Methaemoglobin levels reached 10% at 7.5-10 hours respectively. The ratio of oxidised to reduced Hb at 1 hour was 2-1.4 and at 48 hours was 2.9-2.5 respectively. In a second pilot study one female dog was splenectomised 15 days prior to administration of a top loading dose of Oxyglobin, 30 ml/kg iv at 20 ml/kg/hr (ITR 298-92[3:446]). The Hct, total Hb and plasma Hb were measured over 14 days. The haematocrit decreased from 31% to 25% after treatment and normalised at 8 hours implying this was a dilution effect. The plasma Hb increased from 0.0047 to 3.108 g/dl post infusion, T½ = 42-48 hr, and returned to baseline at 288 hours. Total Hb remained slightly elevated throughout.

Some sampling for kinetic purposes was carried out as part of one study (full report in Part IV). Beagles (6M/group) received 42.5 ml/kg human serum albumin (HSA) (13% w/v) or 8.5-21-42.5 ml/kg Oxyglobin iv at 7 ml/kg/hr, 3 were sacrificed at 48hrs and 3 at 8 days. Serial blood samples were taken in all groups. Analysis of plasma was by SEC in high salt dissociating conditions, thus only the stabilised Hb of Oxyglobin was eluted intact, and spectrophotometry. The levels of Hb tetramer, Hb octamer, Hb >octamer and methaemoglobin were measured. Urinary excretion was complete 4 hours post-dose (0-0.14-0.45%) respectively.

The tissue distribution of Oxyglobin is assumed to be almost total, as evidenced by the widespread tissue discoloration seen at post mortem. No attempt has been made to determine the fate of the administered Hb in terms of metabolism or excretion, apart from the observation that < 1% is excreted in the urine in the 4 hours post-dose.

3.3 Toxicology

3.3.1 Single dose toxicity

Three similar, non-GLP, acute toxicity studies were carried out in dogs, with similar results. The normovolaemic model of acute haemodilution was used. In the first study animals received Oxyglobin solution 30-42.5 ml/kg or Rheomacrodex 19 ml/kg at 7 ml/kg/hr as a single i.v. dose followed by 7 days observation. In the second study (ITR 301-92[6:1302]) animals received no treatment or Oxyglobin solution 15-30-45 ml/kg at 7 ml/kg/hr as a single iv dose followed by 7 days observation. In the third study (ITR 316-92[6:1410]) animals received Oxyglobin solution 8.5-21 ml/kg at 7 ml/kg/hr as a single iv dose followed by 7 days observation. All animals showed some effects of haemodilution, i.e. reduced activity, and appetite, mild oedema, low haematocrit, RBC and WBC counts, extra-medullary haematopoeisis, circulating nucleated RBC, increased heart rate and cardiac output. In addition, Oxyglobin treated animals had some vomiting and loose faeces, a dose related discoloration of the skin and mucous membranes, dose related increases in aminoaspartate transferase (AST) and creatine kinase (CK) activity and discoloured urine. All these effects appeared to have reversed in 2-3 days. At post mortem, dose related observations included: discoloration of tissues; arteriolar inflammation in many tissues (e.g. gastrointestinal tract, bladder, kidneys, ovaries) characterised by endothelial swelling, hyaline and macrophage infiltration; gall bladder haemorrhage (possibly secondary to arteriolar inflammation); randomly distributed hyaline degeneration of hepatocytes, some associated bile stasis; mild bilateral renal tubular basophilia. It is hypothesised that the arteriolar inflammation, and other associated changes, result from the large volumes of protein rich solution administered, e.g. hyaline may contain the test article and/or metabolites. There is no evidence to support or refute this hypothesis.

These reports are all listed as pilot studies, the impression given is that they are dose range finding. The main concerns arising from the studies would be of low animal numbers and failure to establish the maximum tolerated dose (MTD), in fact the therapeutic dose was exceeded by only 50%. However, the adverse effects seen are consistent in all studies, occur within the therapeutic dose range, are largely predictable and/or transient and do not appear to have major short term clinical significance.

3.3.2 Repeated dose toxicity

Four similar, non-GLP, repeated dose toxicity studies were carried out in dogs, with similar results. The normovolaemic model of acute haemodilution was used, blood was withdrawn and replaced with LRS until a Hb = 5 g/dl was reached, animals were then treated with test or control article, subsequent top loading doses were administered on days 3 and 5. In the first study animals received Oxyglobin solution 25-35-50 ml/kg, Rheomacrodex 25 ml/kg or 50 ml/kg PRBC (13g/dl) in three iv doses as described, followed by 7-14 days observation. In the second study animals received no treatment, 45 ml/kg HSA (13% w/v) or Oxyglobin solution 30-45 ml/kg at 15 ml/kg/hr in three iv doses as described, followed by 7-19 days observation. In the third study animals received 90 ml/kg HSA (13% w/v) or Oxyglobin solution 60-90 ml/kg at 15 ml/kg/hr in three iv doses as described, followed by 7-19 days observation. In the fourth study animals received 90 ml/kg HSA (13% w/v) (reduced to 60 ml/kg for top loading doses) or 60-90 ml/kg Oxyglobin solution at 15 ml/kg/hr in three iv doses as described, followed by 7-28 days observation. All animals showed some effects of haemodilution, i.e. reduced activity, and appetite, mild oedema, low haematocrit, RBC and WBC counts, hyper-cellular bone marrow extra-medullary haematopoeisis, circulating nucleated RBC, increased heart rate and cardiac output, increased urine volume and reduced specific gravity. In addition, Oxyglobin treated animals had some vomiting and loose faeces, a dose related discoloration of the skin and mucous membranes, dose related increases in AST activity and discoloured urine. All these effects appear to have reversed by day 7-14.

Treatment related findings observed post mortem included: (1) foamy histiocytes with fine pink/brown granules in most tissues, possibly resulting from phagocytosis and incomplete degradation of the test substance; (2) hepatic sinusoidal cell distension with foamy cytoplasm, single cell necrosis at higher doses, hepatic intra-canalicular cholestasis, brown/pink amorphous material adhering to gall bladder mucosa; (3) arteriolar inflammation and/or eosinophilic material in arteriolar/arterial media with widespread tissue distribution, not evident by day 33; (4) on day 7 hyaline droplets and/or vacuolation in renal cortical tubular epithelium, bilateral tubular basophilia with increased mitotic activity dilatation and casts; (5) myocardial degeneration (papillary muscle and septum) and micro-thrombosis formation in several tissues associated with large volume infusions and premature death in one HSA and one Oxyglobin treated dog. The margin of safety (i.e. MTD) was not established in any of these studies; it is thought that this may ultimately depend on the clinical picture, i.e. the intravascular volume changes, rather than on any specific toxic event.

One pivotal toxicity study was carried out to GLP standards. In this study the model of acute anaemia (using normovolaemic haemodilution to Hb=5g/dl) was used. Beagle dogs (4/sex/group) were dosed on day 1 and day 4 with no treatment, 90 ml/kg HSA (13% w/v) at 5ml/kg/hr, or 30-60-90 ml/kg Oxyglobin at 10ml/kg/hr. Half the animals were sacrificed on day 7, the rest on day 33. In life observations included: pallor of the skin and mucous membranes for 2-3 weeks in negative control groups, dose related yellow/orange discoloration of the skin and mucous membranes of treated animals, resolving at day 11, 19 or 33 respectively; loss of skin elasticity in HSA and Oxyglobin treated groups, this resolved by day 12 in all but two top dose animals; decreased activity and swelling of the nictitating membrane and pinnae in control and top dose groups more than at lower doses; dark/discoloured faeces, occasionally loose, and sporadic vomiting in all treated groups, this had completely resolved by day 8; there was no significant differences between the groups with respect to body weight gain, food/water consumption, central venous pressure or ECG. Many of these observations are compatible with haemodilution effects, as are the measured haematological parameters, i.e. the fall in RBC count, Hb and Hct, recovered by day 21 in treated and untreated groups but not by day 33 in HSA treated controls. Serum chemistry analyses revealed increased ALT and AST in treated dogs compared to controls, levels returned to baseline by day 8-10 except in top dose animals. Urine showed a slight dose related brown discoloration, this was normal by day 8, some Hb was detected in the 24 hours post-dose. Creatinine clearance was slightly decreased in mid- and top dose animals in the 24 hours post-dose, this was not considered significant.

At post mortem a slight increase in the relative weight of the kidneys was seen in treated animals compared to the no treatment control, this was within the normal range but was probably a treatment related effect. There was wide spread non-pathologic tissue discoloration in treated animals. Darkened areas in the gall bladder serosa and bladder mucosa, seen in mid- and top dose animals at day 7 but not day 33, were

thought to be due to perivascular leakage. Histological examination showed: foamy histiocytes in many tissues of treated animals (these were mainly in the arteriolar media and often associated with inflammation); sinusoidal cell distension in liver at mid-and top doses; hepatic intracanalicular cholestasis and red/brown amorphous material in the gall bladder lumen (top dose only); hyaline droplets in the renal tubules and a slight, non-dose related proliferative glomerulonephrophathy; some pigment in the renal tubules. The incidence of these observations was reduced by day 33 (except hepatic effects). Administration of high volumes (i.e. HSA, mid- and top dose animals) was associated with myocardial degeneration, renal casts and renal cortical tubular basophilia, again this was seen predominantly at day 7 not day 33.

It is thought that histiocyte formation and sinusoidal distension may be part of normal degradation of Oxyglobin, by phagocytosis. Hyaline droplets in the kidney may be due to glomerular filtration and re-uptake of Hb or its metabolites. Other events of note are:

- slight recoverable proliferative glomerulonephropathy.
- myocardial necrosis and/or degeneration, apparently recoverable, associated with large volumes of proteinaceous material, HSA or bovine Hb.
- cholestasis etc., seen in one top dose animal at day 7, this is somewhat unusual and possibly treatment related.
- increase in vomiting etc.(possibly due to general poor health in anaemic dogs).

There is no evidence to support or refute these hypotheses. However, the more serious events occur at 2-3-times the therapeutic dose and most animals had recovered within a month. This would indicate that the risks associated with use of Oxyglobin in the manner described in this application should be minimal.

3.3.3 Tolerance in the target species

These studies have been assessed and are reported in 4.1.2 of this report.

3.3.4 Reproductive toxicity (including teratogenicity)

No specific reproductive toxicity studies were carried out. An abstract of a preliminary study in rats is included in the dossier. This would suggest that continuous i.v. infusion of bovine Hb solution (6 g/45 ml/kg) on days 6-17 of gestation is associated with severe maternal toxicity and significant increases in external malformations of the foetus. The protocol and species used do not compare well to the proposed clinical usage and, in the absence of other information, Oxyglobin is not recommended in breeding dogs and pregnant or lactating bitches. The SPC mentions "The safety of Oxyglobin for use in pregnant or lactating bitches has not been determined. The use in such animals is not recommended". This advice is considered acceptable but given the conditions of use of the product (emergency), a risk benefit assessment by the veterinarian is always possible.

3.3.5 Mutagenicity

The bacterial reverse mutation assay (Ames test) was carried out in *S. typhimurium* strains TA98, TA100, TA1535 and TA1537 and in *E. coli* WP2 *uvr*A, in the presence and absence of Aroclor-induced rat liver microsomal enzymes (S9 mix), using test article (bovine Hb, Oxyglobin) in the concentration range 0.1-5.0 mg/plate and suitable positive and negative control substances (G97BE22.502[11:2846]). All criteria for a valid study were met and there was no increase in mutation rate with the test article compared to negative controls. Bovine Hb (Oxyglobin) is non-mutagenic under the conditions of this assay. The mammalian cell forward mutation assay was carried out in the mouse lymphoma cell line L5178Y/TK$^{+/-}$, in the presence and absence of Aroclor-induced rat liver microsomal enzymes (S9 mix), using test article (bovine Hb, Oxyglobin) in the concentration range 1.0-5.0 mg/ml and suitable positive and negative control substances. All criteria for a valid study were met, there was no increase in mutation rate with the test article compared to negative controls. Bovine Hb (Oxyglobin) is non-mutagenic under the conditions of this assay. An *in vivo* assay was carried out to evaluate the potential induction of micronucleated polychromatic erythrocytes in the bone marrow of mice, following iv injection of the test article (oxyglobin) at doses of 1-1950 mg/kg. No mortality or clinical signs were observed. Slight reductions in the ratio of polychromatic to total erythrocytes were seen in treated groups compared to negative control

animals, but there was no significant increase in micronucleated polychromatic erythrocytes. All criteria for a valid study were met, bovine Hb (Oxyglobin) was shown to be non-clastogenic under the conditions of this assay.

3.3.6 Carcinogenicity

The lack of mutagenic potential and the fact that this substance is endogenous (in cattle) and intended for a single treatment is sufficient to consider carcinogenicity studies irrelevant.

3.4 Studies of other effects

3.4.1 Immunotoxicity/Antigenicity

As part of one study, immunofluorescence staining for immune complexes of IgG, IgM, IgA and complement was carried out on kidneys taken from controls and treated animals where glomerulonephropathy was seen. Untreated controls were normal, i.e. none had focal deposits in >25% of the glomeruli in the outer cortex. Treated controls had focal to diffuse deposits of IgG, IgM and complement in 25-50% of glomeruli in all areas of the cortex, mainly in the media of small arteries. One Oxyglobin treated animal had focal deposits in >30% of glomeruli in the outer cortex. Incidences of immune complex formation in the kidneys were not drug or glomerulonephropathy (graded slight in treated dogs) linked.

As part of one study, antibody determination was carried out on the sera of all animals. There were no detectable anti-Oxyglobin antibodies in either control group. There was a dose related (with respect to time of onset and magnitude immune response) increase on day 11-18 after dosing with Oxyglobin. No antibody complexes were seen in the kidneys, indicating probable clearance by the reticuloendothelial system. The clinical significance of this finding is uncertain.

3.5 Ecotoxicity

An environmental risk assessment was provided. Based on the nature of the chemical structure (protein), the proposed dose (single i.v. administration of 30 ml/kg), the proposed indication (oxygen carrying support in intensive care implying low number of cases) and the target species (dogs being companion animals), Oxyglobin is exempt from extensive testing and would not be expected to cause any environmental risk.

3.6 Operator Safety

The Applicant has indicated that no safety issues exist for the user regarding accidental self-injection. No dermal studies have been conducted using Oxyglobin solution; however, haemoglobin is an inert biological molecule with no expected inherent toxicities. A haemoglobin formulation intended for human use (Hemopure) was repeatedly injected intradermally into humans without any harmful effects or signs of toxicity. This assessment was part of a larger study to evaluate potential hypersensitivity reactions toHemopure. The data from this study are relevant since Hemopure and Oxyglobin are both polymerised bovine haemoglobin solutions meeting the same level of purity, and the only difference between the two solutions relates to the molecular weight distributions of the polymerised haemoglobin.

A second study used *in vitro* models of non-infectious inflammation to predict hypersensitivity reactions in man. This would be the major concern following accidental self-injection by the operator. There was no evidence of induction of histamine release from basophils and the models suggest no direct effect and no indirect, complement or IgE mediated effect. No *in vivo* data was included. Nonetheless, the models used are highly predictive of the *in vivo* response and no hypersensitivity reactions are anticipated in man. Once normal needle precautions are used, no extra warnings should be necessary.

3.2 Residues:

The application is for a non-food producing species, and therefore residues documentation is not applicable.

4. OVERVIEW OF PART IV OF THE DOSSIER: CLINICAL ASPECTS

4.1 Pre-clinical studies:

4.1.1 Pharmacology

4.1.1.1 Pharmacodynamics:

See part 3.

4.1.1.2 Pharmacokinetics:

See part 3.

4.1.2 Tolerance in the target species:

Target Animal Study with Oxyglobin administered by Repeat Intravenous Infusion to Beagle Dogs following Acute Haemodilution

The objective of this study was to assess the margin of safety of Oxyglobin under conditions of repeated use in the target species and to assess the safety of Oxyglobin under conditions of repeated use. Five groups of four male and four female Beagle dogs were allocated to this study. On Day 1, each animal was subjected to a haemodilution procedure. Following haemodilution, dogs of Group 2 received 90 ml/kg 13% HSA saline, Group 3 received 30 ml/kg Oxyglobin, Group 4 received 60 ml/kg Oxyglobin and Group 5 received 90 ml/kg Oxyglobin. (Control dogs of Group 1 received no treatment following haemodilution). On Day 4, the treatment regimen was repeated (without the haemodilution procedure). Forty eight hours following completion of infusion on Day 4, two males and two females per group were sacrificed. Remaining animals were sacrificed 28 days following the completion of the infusion on Day 4.

Investigations included clinical examination, body weight, food consumption, water consumption, measurement of central venous pressure, electrocardiography, haematological parameters, indices of coagulation, blood chemistry, urinalysis, kidney terminal investigations (including function, creatinine clearance and macroscopic examination), organ weights and microscopic examination of specified tissues. In addition, bone marrow cellularity was examined and serum samples were sent to the sponsor for antibody analysis.

Due to the procedures to which animals have been subjected, observations have been classified as either i) related to treatment,. ii) arising as a result of the experimental procedure, or iii) incidental.

This study concentrated on the safety aspects of this application and the increase in plasma haemoglobin levels in treated animals was the only real indication that the test compound may contribute to an increase in oxygen carrying capacity of the animal. However, there were no measurements of arterial oxygen content to support this assumption. Some treated and control animals had a transient prolongation in activated partial thromboplastin time (APTT) coagulation tests, presumably due to a dilution phenomenon. This effect was most marked at the highest Oxyglobin treatment dose level.

The Applicant was asked to discuss in greater detail the normovolaemic haemodilution model used in the tolerance studies on the target species specifically with regard to anaemia's of different aetiology. The model chosen was adequate to establish the potential efficacy of the product and its safety in the target species.

The formulations used in the tolerance studies were identical, meeting the same specifications as detailed in the specifications of batches of Oxyglobin solution used in studies provided in the Marketing Application.

The Applicant was asked to comment on how the occasional lesions of kidneys, liver, heart, arterioles and gall bladder shown in the toxicology studies might affect an Oxyglobin treated dog, which has decreased capacity. Adverse events associated with Oxyglobin appear to relate to the administration of large volumes of protein solution. These excessive volumes are unlikely to arise in clinical practice, therefore the observed effects are likely to be very rare and of little, long term, clinical significance. There are warnings in the SPC relating to circulatory overload and, as stated by the Applicant, the individual risk benefit determination will always have to be made by the veterinarian.

As the formulation contains reasonably high levels of N-acetylcysteine (NAC), which is associated with severe adverse reactions in humans, the Applicant was asked to comment on the possible adverse effects in the target species. A number of publications were submitted concerning the effects of NAC in various species. Considering the severity of the proposed indication and the limited adverse effects in the dog, the safety margin is considered adequate.

4.1.3 Resistance

Not applicable.

4.2 Clinical studies:

Review Articles from the Veterinary Literature

Nine articles were presented to outline the practical difficulties in blood transfusion practices in veterinary medicine and to assess the likely causes of anaemia in the canine. Whilst there was much information of background interest in this area of medicine, there was no specific data relating to the use of Oxyglobin in any of the test articles, and so no meaningful conclusions on test compound efficacy could be drawn from such information.

Bovine haemoglobin is more potent than autologous red blood cells in restoring muscular tissue oxygenation after profound isovolaemic haemodilution in dogs

This study compared the effects of stored red cells, freshly donated blood and ultrapurified bovine haemoglobin (HBOC) on haemodynamic variables, oxygen transport capacity and muscular tissue oxygenation after acute and almost complete isovolaemic haemodilution in a canine model. Twenty-four Foxhounds (15 male and 9 female, mean age 2 +/- 0.5 yr, mean weight 30 +/- 14 kg) were included in the study.

Anaesthesia was induced with 5 $mg\text{-}kg^{-1}$ ketamine hydrochloride and 2 $mg\text{-}kg^{-1}$ i.m. xylazine , and maintained by continuous infusion of 0.025 $mg\text{-}kg^{-1}\text{-}hr^{-1}$ fentanyl, 0.4 $mg\text{-}kg^{-1}\text{-}hr^{-1}$ midazolam and 0.2 $mg\text{-}kg^{-1}\text{-}hr^{-1}$ vecuronium. Mechanical ventilation was performed with 30% oxygen in air after endotracheal intubation. Temperature, ECG and heart rate (HR) were monitored continuously. Catheters were inserted into both right femoral arteries and veins for measurement of mean arterial pressure (MAP), mean pulmonary artery pressure, (PAP), central venous pressure (CVP) and pulmonary capillary wedge pressure (PCWP), as well as for arterial and mixed-venous blood gas sampling. Cardiac output (CO) was determined by the thermodilution method (average of four measures). Skeletal muscle oxygen tension (tPO_2) was measured in the left sartorius muscle by a micro-processor controlled fast responding (T_{90} < 500 msec) polarographic needle probe of 12.5µ diameter. At every respective measure point, 200 single tPO_2 values were determined over a period of five minutes in a conical tissue area of 2-3 cm^3. After insertion in the skeletal muscle in a depth of 20mm, the probe was driven forward through the tissue in different directions in steps of 0.7 mm each followed by a reverse step of 0.3 mm under control of the microprocessor. This procedure was

repeated every 30 sec following measurements of 20 single tPO_2 values and guaranteed relief of tissue pressure at the tip of the probe and avoided compression of capillaries.

Haematocrit values (Hct) were determined five minutes after centrifugation of arterial blood. Free haemoglobin in plasma (Hbf) was measured using ethylene diamine tetra-acetate (EDTA) stabilised arterial blood after five minutes centrifugation. Total haemoglobin (Hbtot) in heparinized arterial blood and Hbf were measured using a six wave length oximeter. An oxygen-specific fuel cell was used for measurement of arterial ($Ca\text{-}O_2$) and mixed-venous oxygen content ($Cv\text{-}O_2$). Arterial and mixed-venous lactate were measured photometrically after dilution using a specific test kit. Following variables were calculated: cardiac index (CI), systemic vascular resistance (SVR). pulmonary vascular resistance (PVR). oxygen delivery (DO_2), oxygen consumption (VO_2), oxygen extraction ratio ($ER\text{-}O_2$) = VO_2/DO_2.

Eight dogs which were randomly allocated to Group 1 underwent phlebotomy under sedation for autologous blood donation three weeks before the experiment. Fifteen $ml\text{-}kg^{-1}$ blood were withdrawn using 63 ml of CPD solution as anticoagulant and substituted by an equal volume of Ringer's solution. The blood was immediately separated into plasma and red cell concentrate (RBC) by centrifugation (4000g, 15 min). The RBCs were stored at +4°C. On the day of the experiment all animals received a PCWP controlled isovolaemic haemodilution to haematocrit values of 2O%, 15% and 10%. Blood exchange was performed using 6% hetastarch. This entailed progressive removal of blood with infusion of hetastarch in volumes sufficient to monitor PCWP in a range of 8 +/- 2 mm Hg. In eight dogs of Group 2 the blood volume removed by isovolaemic haemodilution was stored in CPD bags at room temperature. When a haematocrit of 10% was reached, stepwise transfusion was started in all groups to achieve haemoglobin target values of +1 $g\text{-}dl^{-1}$, +2 $g\text{-}dl^{-1}$ and +3 $g\text{-}dl^{-1}$ compared with the respective haemoglobin value at haematocrit 10%. Animals of Group 1 received their own stored red cells. In Group 2, the dogs received the freshly donated blood. In Group 3, animals received ultrapurified, polymerized bovine haemoglobin (HBOC, Biopure) with a haemoglobin concentration of 13 +/- 1 $g\text{-}dl^{-1}$ and an oncotic pressure of 17 mmHg. The HBOC was prepared from bovine red cells by lysis, filtration, chromatography and polymerisation with glutaraldehyde.

Data were reported as mean values +/- SD. Skeletal muscle tPO_2 values are plotted as 10th-, 50th- and 90th-percentiles. The tPO_2-values were tested using the Mann Whitney U-test. Differences within groups of other variables were tested by one-way ANOVA and post-hoc comparison by Student's t-test. Differences between groups were rested by two-way ANOVA and post-hoc comparison with Bonferoni's correction for alpha. All differences were considered significant at $P < 0.05$.

Blood gases
In all groups, temperature and arterial blood gases did not change over time. During haemodilution pH, standard bicarbonate (SB) and base excess (BE) decreased when compared with baseline ($P < 0.05$). The SB and BE continued to remain at lower values than baseline during transfusion in all groups ($P < 0.05$). However, this effect was more pronounced in Groups 1 (SB: 15.1 +/- 2.3; BE: -11.5 +/- 3.2) and 2 (SB: 16.1 +/- 1.5; BE –10.3 +/- 2.0) than in Group 3 (SB: 17.2 +/- 0.8; BE: -8.7 +/- 0.9, $P < 0.05$)

Haemodynamics
Haemodynamic variables were presented. The HR and CO content increased during haemodilution and remained at a higher level than baseline during transfusion in all groups ($P < 0.05$). The PCWP as a parameter for isovolaemic conditions did not change over time in all groups. The SVR was continuously decreased during haemodilution ($P < 0.05$) and increased during transfusion but remained below baseline ($P < 0.05$).

Oxygen transport
The mean haemoglobin concentration of banked packed RBCs was 29.1+/-1.2 g-dl^{-1}, of freshly donated blood 9.0+/-1.8 g-dl^{-1} and of HBOC 13.2+/- 0.5 g-dl^{-1} ($P < 0.01$ Group 1 vs. 2, 3; $P < 0.01$ group 2 vs 3). The HBOC solution had a higher pH (7.5 +/- 0, $P < 0.05$) than RBC units (pH = 6.3+/-0.1) and fresh blood (pH = 6.8+/-0.1).

The Hct and Hbtot values decreased and increased, respectively, in parallel to haemodilution and transfusion in Groups 1 and 2 (Table II). In contrast, Hct remained unchanged at 10% during increasing Hbf values of 0.6 +/- 0.5 g-dl^{-1}, 1.5 +/- 0.5 g-dl^{-1} and 2.7 +/- 1.2 g-dl^{-1} ($P<0.01$ vs baseline). The HBOC showed a contribution to arterial oxygen content of 17% after the first augmentation of Hbtot, 23% after the second and 42% after the final augmentation. The calculated oxygen transport capacity (Huefner index) was 1.37 g-dl^{-1} for canine erythrocytes and 1.16 g-dl^{-1} for HBOC. Arterial and mixed venous oxygen content as well as DO_2 decreased during haemodilution ($P < 0.05$) and slightly increased during transfusion in all groups, while VO_2 only increased in Group 3 after final HBOC transfusion ($P < 0.05$). The ERO_2 increased during haemodilution in all groups ($P <0.05$). In contrast to Groups 1 and 2, ERO_2 remained elevated during transfusion of HBOC in Group 3 ($P < 0.05$) with lower arterial and venous oxygen contents than in Groups 1 and 2 ($P < 0.05$). Arterial and mixed-venous lactate concentrations did not change in groups.

Muscular tissue oxygenation
All pooled tPO_2 histograms, consisting of 1,600 single measurements, showed a shift to the left because of continuously decreasing tPO_2 values during severe acute isovolaemic anaemia. In all groups, the mean tPO_2 decreased at Hct 10% when compared with baseline ($P < 0.01$). Transfusion provided a shift of the pooled histograms to the right in all groups, but mean tPO_2 values were higher in Group 3 than in Groups 1 and 2 ($P < 0.01$). There was a more pronounced shift of the histogram to the right in Group 3 than in other groups. In contrast to Groups 1 and 2, no tPO_2 value <7.5 mmHg was seen in Group 3 during transfusion of HBOC. The % increases of tPO_2 during transfusion were higher in Group 3 than in Groups 1 and 2 when compared with baseline ($P <0.0I$). In Group 3, the baseline tPO_2 was restored by a haemoglobin elevation of 0.7 g-dl^{-1} while in Groups 1 and 2 a haemoglobin rise of 2.7 g-dl^{-1} and 2.1 g-dl^{-1} was required ($P < 0.01$).

This was a published article and hence, all raw data were not available for assessment, Nevertheless, the numbers of animals used allowed for meaningful comparison of the different groups. Arterial and venous oxygen content were changed in parallel to changes of haematocrit and haemoglobin concentrations but were lower in Group 3 than in Groups 1 and 2 ($P < 0.05$) during transfusion. In contrast, the oxygen extraction ratio was higher in Group 3 (59 +/- 8%, $P < 0.01$) at the end of transfusion than in Group 1 (37 +/- 13%) and 2 (32 +/- 5%). In Group 3, mean tissue oxygen tension increased from 16 +/- 5 mmHg after haemodilution to 56 +/- 11 mmHg after transfusion ($P < 0. 01$) and was higher than in Group 1 (41 +/- 9, $P < 0.01$) and Group 2 (29 +/- 11, $P < 0.01$). While in Group 3 an augmentation of 0.7 g dl^{-1} haemoglobin resulted in restoring baseline tissue oxygenation, higher doses of 2.7 g dl^{-1} and 2.1 g - dl^{-1} were needed in Groups 1 and 2 to reach this level ($P < 0.01$).

The data showed that smaller doses of HBOC resulted in higher tissue oxygen tensions after severe isovolaemic haemodilution than those observed with transfusing autologous stored red cells. The lower arterial oxygen content and oxygen transport capacity of HBOC compared with groups receiving RBC transfusion seemed to be due to a different oxygen saturation curve of HBOC which showed only 85% saturation at a PO2 of 100 mmHg. Despite this lower oxygen transport capacity, the tPO2 was higher in HBOC treated animals. In addition, the oxygen extraction ratio and the final oxygen consumption were higher in animals with HBOC transfusion. This data suggested a lower oxygen affinity of bovine haemoglobin than that of autologous RBCs. HBOC had a higher P_{50} of 34 mmHg when compared with the physiological P_{50} of canine haemoglobin of 30 mmHg. The more complete off-loading of oxygen to the tissues may overcompensate for the lower oxygen transport capacity of HBOC in comparison to circulating viable canine red cells, and provide faster restoration of baseline tPO_2. Increases in tPO_2 remained at a higher level in HBOC treated animals compared to other groups with a maximal rise after the first 1g dl^{-1} augmentation of haemoglobin. The Applicant has subsequently clarified that the dosage level of bovine haemoglobin used in this study was approximately 7 ml/kg delivering 0.7g/dl in the plasma. This compares to the recommended therapeutic dosage (RTD) of 30 ml/kg of Oxyglobin Solution, which delivers approximately 4g/dl plasma haemoglobin. The results of this study support the fact that bovine haemoglobin has a pharmacological effect at doses of less than 1/4 the recommended dose.

Dose Response Study With Oxyglobin in a Model of Acute Normovolaemic Haemodilution in Splenectomized Beagle Dogs

The purpose of this study was to determine the drug effect and dose response of Oxyglobin in splenectomized beagle dogs 60 minutes and 24 hours following acute normovolemic hemodilution as compared to a synthetic colloid solution with respect to arterial oxygen content per gram of red blood cell haemoglobin and oxygen delivery.

Dogs were splenectomized a minimum of 7 days prior to commencement of treatment. The experimental design consisted of simultaneous blood withdrawal and volume replacement with warmed Lactate Ringer's Solution (LRS) under general anaesthesia while maintaining pulmonary arterial wedge pressure. The blood withdrawal/volume replacement was performed until the haemoglobin concentration approximated 30 g/L. Following this haemodilution, three dogs/sex in each of the three treatment groups were administered Oxyglobin at a dose volume of 15, 30 and 45 ml/kg for the low (Group 3), mid (Group 4) and high (Group 5) dose groups, respectively. Three dogs/sex in each of the two control groups were administered Rheomacrodex 10%-Saline at a dose volume of 14 and 7 ml/kg for the colloid mid dose group (Group 1) and the colloid control low dose group (Group 2), respectively.

Frequently observed clinical signs recorded during the experimental procedures consisted of nasal discharge (clear, pink or red) and swelling of mucous membranes. Animals were also pale in colour as a result of the blood withdrawal. On study day 2, extensive subcutaneous swellings were noted in all groups in the caudal (lower) abdominal and/or inguinal areas. The nasal discharge and swellings were probably the result of the rapid administration and extravascular redistribution of Lactated Ringer's Solution (LRS). The skin, gums and/or sclera of Oxyglobin-treated groups were discoloured red, yellow or orange. The discoloration of the nasal discharge and of the skin, gums and/or sclera was encountered in Oxyglobin-treated groups, particularly in the mid and high dose groups and was thought to be related to the nature of the test article (haemoglobin-based solution). The faeces were black or dark and were sometimes soft, liquid, or loose in most animals of Groups 4 and 5 (Oxyglobin mid and high dose) on study day 2. These changes in the colour and consistency of the faeces appeared product-related.

During the blood withdrawal the pulmonary arterial wedge pressure was maintained but the systemic arterial pressures (systolic, diastolic and mean) significantly decreased in all groups. The systolic blood pressure was maintained greater than or equal to 50 mmHg throughout the withdrawal phase with the exception of two animals in Group 1 (colloid control mid dose) and one animal in Group 5 (OS high dose). Both control animals were unresponsive to treatment with Rheomacrodex 10%-Saline and died during or shortly following treatment from severe haemodilution while the high dose female

was successfully resuscitated with Oxyglobin. Macroscopic and histopathological examinations were performed on the two animals of Group 1 (1002B and 1502B). The cause of death in control dogs could not be ascertained by morphologic assessment alone. However, cardiac changes noted in animal 1002B supports an acute cardiovascular failure, possibly of ischemic origin.

The blood withdrawal/volume replacement procedure resulted in the decrease of measured blood components (platelets, total haemoglobin, red blood cell haemoglobin, hematocrit, albumin and globulin) and the increase in activated partial thromboplastin time (APTT) and prothrombin time (PT). The acute reduction in total haemoglobin (HGB) resulted in decreases in venous Po_2, in oxygen content (arterial and venous) and in the oxygen delivery to the tissues, resulting in increased oxygen extraction ratio when compared to prewithdrawal values. Despite the acute reduction in haemoglobin, the oxygen consumption was maintained throughout the blood withdrawal period.

Following dosing with Oxyglobin, increases in total haemoglobin and plasma haemoglobin concentrations were observed at 60 minutes and 24 hours post treatment when compared to colloid control groups with a linear dose-response in Oxyglobin treated groups. These increases were attributed to the haemoglobin contribution of the Oxyglobin. At 60 minutes post dosing, this contribution of haemoglobin improved oxygenation in Oxyglobin treated groups, as indicated by the statistically significant drug effect and increased linear dose-response in arterial O_2 content/gram of red blood cell (a02ct/RBC), oxygen delivery and oxygen content (arterial and venous) compared to colloid control values. In addition, the O_2 extraction ratio showed a statistically significant drug effect and a decreasing linear dose-response compared to colloid controls. The oxygen delivery and extraction ratio at 24 hours post dose did not differ significantly between Oxyglobin treated and colloid control groups. However, a significant drug effect was seen for arterial and venous oxygen content in all Oxyglobin treated groups when compared to colloid control groups. An increasing linear dose-response was observed for a02ct/RBC but values were not quite statistically significant ($P=0.057$). Additionally, arterial oxygen content and a02ct/RBC values for Oxyglobin mid and high dose groups were significantly increased compared to predose values.

Significant increases in the systemic blood pressure (systolic, diastolic and mean) were observed immediately post dose in all groups, including both control groups, compared to predose values. At 60 minutes post dose, significant increases were maintained in OS-treated groups and values were increased compared to colloid control groups. The pulmonary artery pressure (PAP) (systolic, diastolic and mean) were also significantly increased in the OS mid and high dose groups compared to predose values, and the diastolic and mean PAP values for all OS-treated groups were significantly increased compared to colloid control groups. The variations among OS-treated and control groups at 60 minutes post dose were considered to be a direct drug effect.

At 24 hours post dose, the colloid control low dose (Group 2) showed statistically significant increases in the systemic blood pressure and significant increases in the mean pulmonary pressure compared to pre-dose values. Although statistical significance was seen in the colloid control low dose, no statistically significant differences were seen among groups at 24 hours post dose: therefore no clinical significance was assigned to these changes. Although no dose or drug effect was observed for the systemic and pulmonary arterial pressures in Oxyglobin treated groups, significant increases were maintained for systemic blood pressure in all Oxyglobin treated groups compared to pre-dose and a significant increase in the diastolic pulmonary pressure was observed in the Oxyglobin mid dose compared to pre-dose values.

At 60 minutes post dose, statistically significant differences in PAWP and cardiac output were observed among groups. Differences seen in the colloid control and Oxyglobin treated groups were attributed to a volume effect due to the absence of a significant drug effect.

Results from this study demonstrated that the administration of Oxyglobin maintained an adequate haemoglobin concentration essential to improve oxygenation as demonstrated by a significant drug effect and increasing linear dose-response in oxygen delivery to tissues and in arterial oxygen content per gram of red blood cell when compared to colloid control groups at 60 minutes post dose. Therefore, of the time points measured, the maximum improvement in oxygenation occurred at 45

ml/kg at 60 minutes post dose. At 24 hours post dose, the a02ct/RBC was not quite significantly different in Oxyglobin treated groups when compared to colloid control groups, although a significant drug effect and increasing linear dose response was found for a02ct.

This was a GLP compliant study. The number of animals used did allow meaningful interpretation of data. The fact that only 2 time points were assessed is a limitation to this study, as certain beneficial effects of the test article may have been lost well before the 24 hour time-point. Several side effects of the test compound were recorded, including discoloration of skin and mucous membranes as well as the presence of dark faeces. The increase in total and plasma haemoglobin concentrations when compared to colloid control animals is understandable and expected. Whilst certain parameters (arterial oxygen and venous oxygen content) were statistically significantly improved at the 24 hour time-point when compared to controls, other important parameters were not (oxygen delivery to tissues and oxygen extraction ratio). The latter 2 parameters were significantly improved at the 60 minute time-point, but this effect was lost at some time point between 1 hour and 24 hours. A significant dose response effect was not always seen in this study, although mid- and high-dose oxyglobin animals had values significantly improved for many parameters when compared to pre-dose values. It should be noted that the 45ml/kg dose level performed better than the RTD of 30ml/kg and a question on selection of the RTD was posed to the applicant. The applicant's response concentrated on a dose selection that was a balance between efficacy on the one hand, and safety (i.e. circulatory overload) on the other. Although an adequate screen of haematological and coagulation tests were performed, it would have been highly desirable to have carried out more blood gas measurements to assess consistency in the response to medication over the course of the day. The report states that APTT coagulation tests were not possible post treatment as blood samples would not coagulate. On further questioning in relation to this point, it was accepted that such an effect was likely to be the result of a haemodilution factor. Whilst it is accepted that this was a pre-clinical study and that certain parameters were improved by the test compound, it is difficult to know if a significant improvement in tissue oxygenation for any meaningful period of time was obtained in this study.

Multicentre Randomised Clinical Field Trial to Assess the Efficacy and Safety of Oxyglobin in Anaemic Dogs

The objective of this study was to assess the efficacy and safety of Oxyglobin in increasing plasma haemoglobin concentration and improving the clinical signs associated with anaemia in dogs due to conditions such as blood loss, haemolysis or ineffective erythropoiesis.

The trial was a randomised, adequate and well-controlled multicentre study conducted in 64 dogs diagnosed with acute or chronic anaemia prior to entry into the study. It included six sites at four distinct geographic locations. Fifty-two dogs received Oxyglobin (30 ml/kg body weight) followed by a 24 hour efficacy observation period and a 72 hour safety monitoring period. Two methods of control were used: an untreated (negative) control and the dog as its own control.

Prior to treatment, haematology, coagulation, serum chemistry and urinalysis samples were taken and a physical examination including assessment of the baseline physical condition scale (PCS) was performed. The PCS was designed to quantitate parameters which change as a result of improvement or deterioration of clinical signs associated with anaemia. Upon entrance into the treatment phase of the study, dogs were stratified by cause of anaemia and randomised to the treatment or control group. Dogs randomised to the treatment group received 30 ml/kg of Oxyglobin at 15 $\pm$ 5 ml/kg body weight/hr and dogs randomised to the control group were monitored for up to 24 hours or until a change in their condition occurred. Dogs in both groups were monitored for a decrease in total haemoglobin concentration or PCS at which time they received additional oxygen carrying support. If additional oxygen carrying support was needed, treated dogs received packed red blood cells and control dogs received Oxyglobin. The measures of efficacy were comparisons between treated and control dogs for failure rates and time to failure (time at which additional oxygen carrying support was needed) and changes in plasma haemoglobin concentrations and the PCS post- infusion in Oxyglobin treated dogs relative to pre-treatment. Treatment success was defined as the lack of a need for additional oxygen carrying support for 24 hours. The incidence and severity of adverse events over 72

hours were recorded as a measure of safety. At study termination, a complete physical examination and haematology, coagulation, serum chemistry, and urinalysis were performed.

Sixty-four dogs were entered into the study: 30 dogs with anaemia due to haemolysis, 25 dogs with blood loss, and nine dogs with ineffective erythropoiesis. The percentage of successes in the Oxyglobin treated group was 95% compared with 32% in the control group. Time to failure controlling for cause of anaemia was statistically significantly longer in the Oxyglobin treated group than in the control group ($p \leq 0.001$). A consistent, post-infusion efficacious effect was also seen in the plasma haemoglobin and PCS parameters. Plasma haemoglobin concentration and PCS were statistically significantly increased ($p \leq 0.001$) at every time point post-infusion compared with baseline values. Variation in baseline and post-treatment haematology, coagulation, and urinalysis parameters was relatively equal between dogs receiving Oxyglobin and control dogs. Although dogs with various diseases with expected increases in serum enzyme activity were represented in the study population, an increase in aspartate transaminase (AST) activity appeared to be treatment related. An increase in total protein concentration in 25% of the treated dogs occurred. It was likely to be due to the presence of Oxyglobin haemoglobin protein in the serum.

Adverse events (n = 277) were reported in 60 (control and treated groups); 175 events were associated or had unknown association (n = 95 and n = 80, respectively) with Oxyglobin. The number of adverse events was not unexpected considering the severity of disease of dogs in the study. The majority of adverse events observed were of a mild and transient nature. Of the reported adverse events < 1% of the associated adverse events and < 4% of the unknown adverse events were described as severe. Of the ninety-five associated adverse events, 85 were mild in severity including 63 transient, occurrences of discoloured mucous membranes and urine. Fifteen of the 85 mild events were transient, increased central venous pressure (CVP) due to the plasma expanding properties of Oxyglobin. There were also eight moderate and two severe, associated adverse events. Both severe associated adverse events were pulmonary edema resolving by 9.5 and 56 hours post infusion. Of the 80 adverse events having unknown association to Oxyglobin, 70 were mild or moderate in severity and 10 were severe. The dogs in this study were critically ill by virtue of the fact that they met the entry criteria of a need for a blood transfusion and had a hematocrit of $\leq$ 21%. Fifteen dogs died or were euthanised during the study and four additional dogs which were prematurely discontinued from the study were euthanised after discontinuation. Sixteen of the 19 dogs, which died or were euthanased, received Oxyglobin, three were untreated, control dogs. The deaths of fifteen of the Oxyglobin treated dogs were considered to be related to their underlying disease and not the administration of Oxyglobin. The death of one dog with chronic anaemia due to ineffective erythropoiesis may have been complicated by circulatory overload related to the administration of Oxyglobin.

The study provided significant clinical and statistical support for the conclusion that Oxyglobin had an efficacious effect on the study parameters when assessed in anaemic dogs and it had a safe treatment profile. Transient treatment related effects included mild, transient discoloration of mucous membranes and urine, moderate expansion of intravascular volume (expressed as an increase in CVP) and mild increases in AST activity.

Treatment with Oxyglobin resulted in a statistically significantly higher success rate (95%) compared with the control success rate (32%) ($p = 0.05$); a statistically significantly longer time to failure compared with the control group ($p \leq 0.001$); a statistically significant increase in plasma haemoglobin concentration at every time point post-infusion compared with baseline values ($p \leq 0.001$) and a statistically significant increase in physical condition score at every time point post-infusion compared with baseline values ($p \leq 0.001$).

The statistically significantly different success rates between Oxyglobin treated and control groups demonstrated a strong decrease in the requirement for additional oxygen carrying support in the 24 hour period following Oxyglobin infusion. Results of the time to failure analysis further support the conclusion that Oxyglobin treated dogs were less likely to require additional oxygen carrying support at all time points in the 24 hour period following infusion.

It was initially considered that the overall number of animals included was not extensive particularly when one takes into account the different aetiologies for the anaemia. In the initial protocol, there were 30 animals receiving treatment and 34 negative controls. 22 of the negative controls required treatment and were subsequently administered oxyglobin. The dose rate used was the RTD. The parameters measured in this study were limited i.e. Physical Condition Score (PCS) and total haemoglobin concentration. The former parameter was subjective and could be highly influenced by the cause and severity of the anaemia e.g. the PCS could be markedly different for an animal with anaemia due to chronic renal failure as against anaemia due to acute major haemmorhage from a road traffic accident. The definition of treatment success was considered favourable to the test compound as many anaemic dogs simply rested in a kennel may not require extra oxygen carrying support. In fact, the success rate of over 30% for the negative control group demonstrates this. Whilst the improvement in PCS scores for the oxyglobin treated animals is noted, additional parameters to demonstrate efficacy would have been desirable. The inherent intravenous administration of fluids involved in giving oxyglobin to the dogs i.e. 30ml/kg b.w., could have also significantly improved the PCS by helping to hydrate the debilitated patients as compared to the negative controls who received nothing. Many adverse events were recorded, the most serious being pulmonary oedema, although the frequency of such an effect was low. A significant increase in AST values was seen in treated animals. A total of 15 dogs died or were euthanised during the study, further reducing the number of animals that could be analysed at study termination. Out of the 15 animals who died, 6 were oxyglobin treated cases and 7 out of the 9 nine control animals who died also received oxyglobin prior to death / euthanasia. Consideration of these case numbers takes away from the claimed success rate of 95% for the oxyglobin treated animals.

An initial assessment of the submitted efficacy data was inconclusive. The applicant was requested to submit any further supportive data and to re-analyse the results of the multi-centred clinical field trial. A large number of supportive studies and a revised version of the field trial were subsequently presented. Clarification was also received on the extrapolation of dose rates used in the study by Standl et al (1996) to the RTD proposed by the applicant. Further data collated from clinical cases occurring post-licencing in the USA was also submitted.

Of all the data subsequently submitted, the studies performed by Standl et al (1996) and Page et al (1998) provide the most useful information. The former study clearly showed an improvement in tissue oxygenation in dogs following the use of HBOC in an isovolaemic haemodilution model. This test compared the amount of haemoglobin required to restore the baseline tissue oxygen concentration by using bovine haemoglobin, stored RBCs or freshly donated blood. The results obtained for bovine haemoglobin were significantly better than for the other two groups. This study is particularly important because it directly measured the oxygen levels in muscle with an invasive probe. As it is now evident that the dose rate used was approximately ¼ the RTD for this compound in the dog, this study attains more significance. The second study provides evidence in an *in-vitro* model of oxygen transport, that HBOC is capable of both taking up and releasing oxygen in the test microcapillary model, and that the results obtained were better than for RBC suspensions. These studies establish evidence for a pathway as follows; treatment with HBOC leads to an increase in the plasma haemoglobin concentration (see previous data), which in turn leads to an increase in arterial oxygen concentration as this haemoglobin has been shown to be functional (both *in vivo* and *in vitro*), which in turn leads to increased oxygenation of the tissues. If this pathway can be accepted by combining various parts of the data, then it follows that demonstration of an increase in the plasma haemoglobin concentration in later data can be accepted as likely proof of efficacy. The other studies provided merely complement the initial work by providing evidence of oxygen support in severely compromised animals, and also by demonstrating an increase in arterial oxygen concentration in a target species study. It is accepted that measurement of blood gas concentrations on their own would be influenced by other factors such as pulmonary function. Furthermore, at a clinical field level, the use of invasive probes to measure tissue oxygen concentration in sick dogs would pose ethical problems.

Conclusions

It is accepted that the multi-centre clinical trial was technically difficult to perform. The target population contained many critically ill patients, so ethical constraints were a major limiting factor. A

high mortality rate was a likely outcome irrespective of the treatment protocol adopted. The use of invasive probes to measure tissue oxygen concentrations was not deemed appropriate to a trial involving privately owned pet animals, and this is a reasonable approach for the Applicant to adopt. The short period of observation (i.e. 24 hours) made it difficult initially to assess whether or not treatment had been of significant benefit, but this situation has been somewhat rectified by the fact that in many cases, further data was available up to the 72 hour time-point. Case numbers were low but the Applicant argued that once statistical analysis had demonstrated a positive benefit of treatment, it was not necessary to include any additional dogs simply to inflate the numbers of animals included. Despite the Applicant's arguments, the CVMP would still have preferred a positive control group (most likely consisting of animals treated with packed red cells or blood where appropriate) in order to compare the efficacy with the standard treatment currently employed. Notwithstanding the points raised above, the CVMP can accept the Applicant's argument that any future trial would also suffer from most of the limitations previously encountered. The design of such a study involving critically ill patients, with different underlying causes would always pose logistical and ethical problems. What can be stated with confidence is that this trial did demonstrate a statistically significant increase in the plasma haemoglobin concentration in treated animals over negative controls. Combining this result with the conclusions of the pre-clinical studies allows us to extrapolate that the increase in plasma haemoglobin concentrations would have led to an increase in the arterial oxygen content, and that this oxygen would have been available to the peripheral tissues. Such a connection can be supported when all the data is considered together.

The data derived from the post-licensing use of this product in 71 dogs in the U.S.A. show that some of the cases had meaningful diagnostic work-ups, with repeat clinical examinations and blood results. Each case can only be considered on its individual merits, as there were no controls or consistent approaches adopted between cases. A major limitation in analysing such data was the use of combination therapy in most cases, but this was deemed unavoidable, as this practice represents the clinical reality for diseases such as immune-mediated haemolytic anaemia. In such cases, the use of Oxyglobin is designed to support oxygen transport whilst the underlying disease process is being addressed. Despite the obvious limitations, some useful data is available from these case reports, and an overall assessment of the 71 dogs does show a beneficial effect of Oxyglobin treatment.

Postauthorisation Developments resulting from Pharmacovigilance

Information became available to demonstrate that the contra-indication for the use of Oxyglobin in dogs with renal impairment (Section 5.3 of the SPC), had been misinterpreted as meaning the product is unsuitable for use in dogs with renal failure. The concern at the time of initial licensing was that animals with oliguria or anuria should not be treated with Oxyglobin, as this would predispose these dogs to circulatory overload. Consequently, the CVMP supported the Applicant's argument that the term "renal impairment" should be replaced by the terms "oliguria or anuria". This initial aspect of the variation was therefore recommended.

Nystagmus had been reported infrequently in the initial clinical trials that accompanied this application, and in the first PSUR for this product. Consequently, the CVMP recommended that a warning in relation to nystagmus should be added under Section 5.4 of the SPC (undesirable effects). Consequently, the CVMP recommended acceptance of this aspect of the variation, in order to comply with the previous decision of the CVMP.

The request to add warnings on a possible loss of appetite and fever following the use of Oxyglobin is based on information relating to adverse effects observed in the US. The CVMP recommended that such warnings should be included under Section 5.4 of the SPC. A re-wording of the warning in relation to circulatory overload, with the inclusion of tachypnoea, dyspnoea, harsh lung sounds and pulmonary oedema has been supported by clinical data, and was recommended by the CVMP on safety grounds.

The addition of new information under Section 5.5 of the SPC to alert the user to possible interference that may occur in the results of various clinicopathological tests following the use of Oxyglobin was

supported by the submission of corroborative data. The CVMP recommended acceptance of this proposal.

The final part of the application sought to allow for a more flexible dosage regime for Oxyglobin (i.e. a dose rate of 15-30 ml/kg bodyweight, instead of the current SPC recommendation of 30 ml/kg bodyweight). This application was supported by data that includes a new pharmacokinetic study in the dog, the results of field data collation and literature references. Various other papers were additionally submitted in support of this application, many of which were previously assessed by the committee. A pivotal study at the time of initial assessment of Oxyglobin was the work performed by Standl *et al* (1996). This study demonstrated that dose levels of bovine haemoglobin equivalent to 7 ml Oxyglobin/kg bodyweight were effective in restoring baseline muscle oxygenation concentrations in dogs that had suffered severe isovolaemic haemodilution. The author was also able to calculate the plasma haemoglobin concentration necessary to obtain such an effect (0.7 g/dl), a point that will be referred to below in relation to the new kinetic data.

Following the assessment of the original clinical trial data, the Applicant subsequently submitted the results of clinical data derived from 70 dogs treated with Oxyglobin following licensing of the product in the US. Oxyglobin was being primarily used to provide additional oxygen carrying support to dogs with anaemia, and also served to expand the circulatory volume on account of its route of administration. This data revealed that veterinarians in the field were employing a wide dosage rate band when utilising the product. The rationale for this approach was that the requirement for additional oxygen-carrying support often varied significantly between animals on the basis of their haematocrit, available circulatory volume, speed of onset of disease/ clinical signs etc. The dose rate was also being adjusted to the animal's individual needs in order to avoid inducing circulatory overload, thereby attempting to counteract an important safety issue. Analysis of the data appeared to support the Applicant's argument that dose levels within the new suggested band were clinically effective at field level. This adjusting of the dose according to individual animal requirements was also performed routinely at field level for similar agents administered intravenously, such as blood and plasma volume expanders.

A new pharmacokinetic study investigated the kinetics of Oxyglobin at dose levels of 7-15 ml/kg bodyweight in the target species. Splenectomized beagle dogs were haemodiluted to a haematocrit of 15% by withdrawing blood while simultaneously replacing it with approximately 2.5 times the volume withdrawn with Lactated Ringers solution. Following haemodilution, dogs were treated with Oxyglobin solution at dosage levels of 7, 10 or 15 ml/kg (6-7 dogs/dose group), transfused with packed RBCs at a dosage level of 10 ml/kg (4 dogs), or left untreated (4 control dogs).

This study demonstrated that Oxyglobin dose rates within the range of 10-30 ml/kg bodyweight were effective at maintaining plasma haemoglobin concentrations at levels above those shown to be effective in restoring tissue oxygenation status in the study be Standl *et al* (1996) i.e. a plasma haemoglobin concentration ≥ 0.7-1g/dl. However, the duration of the desired effect did vary according to dose as detailed in the tables below (tables A and B). The minimum duration of effect based on a plasma concentration of $\geq$ 1 g/dl extends from 12 to 78 hours at a dosage rate of 10 to 30 ml/kg (Table A).

Table A

Dose (ml/kg)	Peak Plasma Concentration (g/dl)	Duration (hours) Oxyglobin levels over 1 g/dl	Half-Life (hours)	Cleared from Plasma (Days)
15	2.0 – 2.5	23 – 39	19 – 30	4 – 6
21	3.0 – 3.3	66 – 70	25 – 34	5 – 7
30	4.0	74 - 82	22 – 43	5 – 9

Table B

Time at Which Plasma Haemoglobin = 1 g/dl

Dosage (ml/kg)	Plasma Haemoglobin Concentration (g/dl)	Time (Hours post end of infusion)
7	1.09 ± 0.08	3
10	1.14 ± 0.18	12
15	1.18 ± 0.12	24

On the basis of the data submitted by the Applicant, the request to vary the recommended therapeutic dose rate within a band (15-30 ml/kg bodyweight) that can be adjusted according to the animal's individual requirements appeared scientifically valid.

This variation was accompanied by sufficient data to support all the proposed changes. Consequently, the conditions laid down in Annex II to Commission Regulation (EC) No 542/95 for the requested variation have been met.

The CVMP, therefore, recommended that this variation, accompanied by the submitted documentation in accordance with Commission Regulation (EC) No 542/95, be granted.

5. RISK-BENEFIT ASSESSMENT AND CONCLUSION

The main concerns of the CVMP relating to Oxyglobin concerned the adequacy of the normovolaemic haemodilution model in predicting efficacy and/or adverse effects in anaemia's of different aetiology, e.g. hypovolaemic, haemolytic, and the tolerability of Oxyglobin and it's metabolites on re-administration to a dog or on accidental administration to the operator. The company has shown that the product can provide additional oxygen carrying support to dogs with normovolaemic haemodilution induced anaemia, at doses which do not directly cause serious adverse effects. This is sufficient to illustrate the potential for efficacy and the probable safety of Oxyglobin in the treatment of anaemia in general. The extent to which anaemia of alternative aetiology differs from this model may influence the outcome of treatment with Oxyglobin. However, at this point in the development of the product it would be impractical and unethical to attempt to develop an experimental model of every form of anaemia, thus the evaluation of efficacy and safety in anaemia of alternative aetiology can only be done with well conducted clinical trials and/or thorough pharmacovigilance.

The metabolite profile of Oxyglobin has not been well characterised, but it is difficult to conceive of any metabolites other than the those arising from normal haemoglobin degradation. This being the case no tolerance problems would be expected, except in the case of overload, e.g. hyperbilirubinaemia. This issue was partially addressed by the use of some hyperbilirubinaemic dogs in the clinical trials. Although the

number of dogs treated is low, results suggest a good benefit risk analysis. Some repeated dose studies were carried out which suggest that multiple dosing will be tolerated, and, since Oxyglobin is intended for a one off administration, further consideration of the problems associated with re-administration will not be necessary. Accidental self-administration by the operator is considered unlikely. Some information has been submitted which suggests that allergic/local tolerance problems are unlikely.

Several pivotal questions were posed to the Applicant in the area of clinical efficacy. The Company has succeeded in clarifying many aspects of the study that were previously unclear. Furthermore, the pre-clinical data package has been expanded to include studies that were not previously presented, or on which interpretation was difficult. This pre-clinical data is of paramount importance as it provides scientific evidence that HBOC is capable of taking up, transporting and releasing oxygen in species other than cattle. The data further show that this oxygen can be off-loaded to peripheral tissues such as muscle, as evidenced by the work of Standl et al (1996). Further evidence was provided by the study that demonstrated restoration of normal tissue oxygenation by the use of bovine haemoglobin in dogs with arterial stenosis. Other studies were presented in which animals of various species (principally the sheep and the dog) were shown to survive with Oxyglobin infusions despite having PCV values below 3%, a figure which would not be considered compatible with life. These latter studies are supportive, but are not definitive.

The overall conclusion is that sufficient data is now available to support a beneficial role for Oxyglobin as a supportive treatment in dogs with anaemia. This conclusion can only be supported by combining the results of both the pre-clinical and clinical studies. This is a niche product for a relatively small and highly select target population. The current treatment options available for oxygen support in anaemic patients have their own difficulties (poor availability of blood, cross-matching etc.), and similar limitations to their success rates. Further clinical trials with Oxyglobin would be likely to encounter the same ethical and logistical problems that the original one did. Interpretation of data where combination therapies are employed would continue to pose a significant problem.

Pivotal questions were also posed to the applicant on quality issues, particularly in relation to the viral safety of the product. Having considered the results of the virus validation studies, the CVMP was of the opinion that screening of the product for certain specified viruses would be required.

Based on the original and complementary data presented, the Committee for Veterinary Medicinal Products concluded that the quality, safety and efficacy of the product were considered to be in accordance with the requirements of Council Directive 81/852/EEC and supported the claims proposed by the applicant.