Approval Date: January 12, 1998

FREEDOM OF INFORMATION SUMMARY

I. GENERAL INFORMATION

A. File Number

NADA 141-067

B. Sponsor

Biopure Corporation
11 Hurley Street
Cambridge MA 02141

C. Proprietary Name

Oxyglobin

D. Established Name

Hemoglobin glutamer-200 (bovine)

E. Dosage Form

Injectable

F. Dispensing Status

Prescription

G. Dosage Regimen

The recommended dosage of Oxyglobin® is a one-time dose of 30 mL/kg of body weight. If desired, Oxyglobin® may be warmed to 37º C prior to administration.

Remove overwrap prior to use and use within 24 hours. Oxyglobin® should be administered using aseptic technique via a standard intravenous infusion set and catheter through a central or peripheral vein at a rate of 10 mL/kg/hr. Do not administer with other fluids or drugs via the same infusion set. Do not add medications or other solutions to the bag. Do not combine the contents of more than one bag.

Use of Oxyglobin® does not require cross-matching with recipient blood. A blood transfusion is not contraindicated in dogs which receive Oxyglobin® nor is Oxyglobin® contraindicated in dogs which have previously received a blood transfusion. Oxyglobin® is intended for single dose use. Any unused Oxyglobin® should be disposed of in accordance with local requirements for handling veterinary medical waste.

NOTES

Oxyglobin® is a sterile, clear, dark purple solution containing 13 g/dL purified, polymerized hemoglobin of bovine origin in a modified Lactated Ringer's Solution.

H. Route of Administration

Intravenous at a rate of up to 10 mL/kg/hr.

I. Indication

Oxyglobin® is indicated for the treatment of anemia in dogs by increasing systemic oxygen content (plasma hemoglobin concentration) and improving the clinical signs associated with anemia for at least 24 hours, regardless of the cause of anemia (hemolysis, blood loss, or ineffective erythropoiesis).

II. EFFECTIVENESS

The effectiveness of Oxyglobin® was established by the following two studies:

A. LABORATORY DOSE RESPONSE STUDY

1) Type of Study: Dose Titration conducted in compliance with Good Laboratory Practice (GLP) regulations

   Title: A Dose Response Study with Oxyglobin® in a Model of Acute Normovolemic Hemodilution in Splenectomized Beagle Dogs

2) Principal Investigator:

   Nancy Kelly, DVM
   ITR Laboratories Canada Inc.
   19601 Clark Graham
   Baie d'Urfe, Quebec
   Canada H9X 3T1

3) Design of Study:

   a) Purpose of Study: To determine an effective dose of Oxyglobin®.

   b) Test Animals: 30 adult splenectomized Beagle dogs divided into 5 groups of 6 dogs each. Each group consisted of 3 males and 3 females.

   c) Control: Colloid control (plasma volume expander control without oxygen carrying capacity; Rheomacrodex®-saline: 10% dextran 40 and 0.9% saline)

   d) Diagnosis: Acute normovolemic hemodilution model: For each dog, blood was withdrawn and simultaneously replaced with 1.6 to 2.3 times the volume withdrawn with Lactated Ringer's Solution (LRS) while maintaining pulmonary artery wedge pressure at approximately baseline values. The procedure continued until the total hemoglobin concentration was approximately 3.0 g/dL.

   e) Dosage Form: Injectable

   f) Route of Administration: Intravenous

   g) Dosage Used: 15, 30, and 45 mL/kg Oxyglobin®, and 7 and 14 mL/kg Rheomacrodex®-saline (control) were administered once at a rate of 20

mL/kg/hr.

h) Test Duration: Dogs were splenectomized 7 days prior to treatment and hemodiluted immediately prior to treatment on Day 1. The study was terminated on Day 2.

i) Pertinent Parameters:

(1) Arterial oxygen content at 60 minutes and 24 hours post-dose

(2) Plasma hemoglobin concentration at 60 minutes and 24 hours post-dose

4) Results:

Dose	MEAN ARTERIAL OXYGEN CONTENT (mL/dL)*		
	Pre-Dose	**60 Minutes Post-Dose**	**24 Hours Post-Dose**
Control 14 mL/kg	4.6	3.6	3.8
Control 7 mL/kg	4.6	4.5	4.0
Oxyglobin® 15 mL/kg	4.9	6.6	5.0
Oxyglobin® 30 mL/kg	4.3	7.0	5.7
Oxyglobin® 45 mL/kg	4.3	7.5	6.2

* least square means

Arterial oxygen content was analyzed using analysis of covariance with pre-dose arterial oxygen content as the covariate. Pairwise comparisons showed that the arterial oxygen content at the 30 mL/kg dose and the 45 mL/kg dose was significantly increased from the control ($p<0.01$) at both 60 minutes post-dose and 24 hours post-dose. Arterial oxygen content was also analyzed using a paired t-test comparing pre-dose values with post-dose values. The 30 mL/kg and 45 mL/kg Oxyglobin® treatment groups were significantly increased ($p<0.01$) at both 60 minutes and at 24 hours post-dose. Therefore, the dose of 30 mL/kg was selected as the lowest effective dose.

Dose	MEAN PLASMA HEMOGLOBIN CONCENTRATION (g/dL)	
	60 Minutes Post-Dose	**24 Hours Post-Dose**
Control 14 mL/kg	0.0000	0.0000
Control 7 mL/kg	0.0000	0.0000
Oxyglobin® 15 mL/kg	2.5927	1.2867
Oxyglobin® 30 mL/kg	3.9452	2.4570
Oxyglobin® 45 mL/kg	4.7268	3.0753

Correlation of Arterial Oxygen Content and Plasma Hemoglobin Concentration:

Since all of the plasma hemoglobin concentration values were zero for the control dogs, only data from the Oxyglobin® treatment groups (n=18) were used to calculate the correlation between arterial oxygen content and plasma hemoglobin concentration. A positive correlation was found at both 60 minutes post-dose ($r=0.40$) and 24 hours post-dose ($r=0.59$).

5) Adverse Reactions:

Yellow, orange, or red discoloration of skin and mucous membranes, yellow or

orange discoloration of sclera, red or pink discoloration of serous nasal discharge, black or dark discoloration of feces, red mucoid-liquid material in feces, vomiting, and diarrhea were seen in Oxyglobin® treated dogs post-treatment.

6) Conclusions:

The dose of 30 mL/kg administered once was effective in increasing arterial oxygen content for 24 hours and was selected for further clinical evaluation. Additionally, a positive correlation between laboratory measured arterial oxygen content and clinically measured plasma hemoglobin concentration was established.

B. CLINICAL FIELD TRIAL

1) Type of Study: Clinical Trial

Title: A Multi-center, Randomized, Adequate and Well-Controlled Clinical Field Trial to Assess the Efficacy and Safety of Oxyglobin® in Anemic Dogs

2) Principal Investigators:

Ann Hohenhaus, DVM, The Animal Medical Center, 510 East 62nd Street, New York, NY 10021

John Jacobson, DVM, Virginia-Maryland Regional College of Veterinary Medicine, Virginia Polytechnic Institute & State University, Blacksburg, VA 24061

Urs Giger, Dr med vet and Beth Callan, VMD, University of Pennsylvania, School of Veterinary Medicine, 3850 Spruce Street, Philadelphia, PA 19104

Guillermo Couto, DVM and Mary McLoughlin, DVM, The Ohio State University, College of Veterinary Medicine, 601 Vernon L. Tharpe Street, Columbus, OH 43210

Bernard Hansen, DVM, North Carolina State University, College of Veterinary Medicine, 4700 Hillsborough Street, Raleigh, NC 27606

Robert Murtaugh, DVM and Susan Cotter, DVM, Tufts University, School of Veterinary Medicine, 200 Westboro Road, North Grafton, MA 01536

3) Design of Study:

a) *Purpose of Study*: To assess the efficacy and safety of Oxyglobin® administered once at 30 mL/kg in increasing plasma hemoglobin concentration and improving the clinical signs associated with anemia in dogs for 24 hours in a clinical setting.

b) *Test Animals*: 64 client-owned dogs of various sexes, weights, and breeds. 30 dogs were randomized to the Oxyglobin® treatment group and 34 dogs were randomized to the untreated control group (22 of these control dogs later received Oxyglobin® due to need for additional oxygen carrying support).

c) *Controls*: Untreated (negative) control

Although a negative control group was utilized in this study, two efficacy parameters, plasma hemoglobin concentration and Physical Condition Scale (PCS), did not lend themselves to analysis across test groups. Therefore, the aforementioned parameters were analyzed using each dog as its own control.

d) *Diagnosis*: Dogs were diagnosed with anemia by measuring the total hemoglobin concentration and packed cell volume (total hemoglobin concentration <7.0 g/dL, packed cell volume < 21%) and by assessing clinical signs of anemia using a defined physical condition scale (physical condition scale score < 3; see scale on next page). Dogs with disseminated intravascular coagulopathy, thrombocytopenia with active bleeding, hemoglobinemia and hemoglobinuria, autoagglutination, dehydration, oliguria or anuria, advanced cardiac disease, pregnancy, or lactation were excluded from the study.

e) *Dosage Form*: Injectable

f) *Route of Administration*: Intravenous

g) *Dosage Used*: 30 mL/kg Oxyglobin® was administered once at a rate of 15 ± 5 mL/kg/hr. (The rate was subsequently changed to 10 mL/kg/hr to improve the safe use of Oxyglobin® by preventing volume overload.)

h) *Test Duration*: Efficacy was assessed over a 24 hour period post-treatment and dogs were observed for adverse reactions over a 72 hour period post-treatment.

i) Pertinent Parameters:

(3) Plasma hemoglobin concentration, change from pretreatment

(4) Physical condition scale (PCS) score (quantitated clinical signs associated with anemia-see scale on next page), change from pretreatment

(5) Success rate (treatment success = lack of need for additional oxygen carrying support for 24 hours), treatment vs. control

(6) Time to failure (time from baseline at which the dog received additional oxygen carrying support), treatment vs. control

(7) Adverse reaction information was collected.

Physical Condition Scale (PCS)

Parameter	**1**	**2**	**3**	**4**	**5**	**Status**
Attitude	Depressed minimally responsive	Depressed but responsive	Quiet and responsive	Alert and responsive	Bright and responsive	-
Activity	Recumbent, unable to stand	Will stand, reluctant to walk	Able to walk but tires quickly	Able to walk but tires	Able to walk without tiring	-
0 Hour Resting heart rate (HR)	0 Hour Baseline HR + 30 or more bpm	0 Hour Baseline HR + (15-29 bpm)	0 Hour Baseline HR ± 14 bpm	0 Hour Baseline HR - (15-29 bpm)	0 Hour Baseline HR - 30 or more bpm	-
					Total=	-
					Number of observations graded=	-
					Total/number of observations graded= PCS=	-

4) Results:

The study was conducted as a sequential trial based on a double triangular test of the log odds-ratio (Whitehead, 1983), modified for stratification by cause of anemia with the rate of successes/failures as the criterion for stopping the trial. The trial was stopped after 64 dogs had entered the study (minimum sample size required was 60 dogs).

Of the 64 dogs entered into the study, anemia due to blood loss occurred in 25 dogs, anemia due to hemolysis in 30 dogs and anemia due to ineffective erythropoiesis in nine dogs.

The efficacy parameters were evaluated for each of two populations of dogs: the intent-to-treat population and the efficacy population. The intent-to-treat population was comprised of all dogs that were enrolled in the study (64 dogs). The efficacy population consisted of dogs which had no protocol deviations and dogs that had protocol deviations which did not affect the outcome of the study (49 dogs).

(1) Mean change in plasma hemoglobin concentration from pretreatment data:

MEAN CHANGE IN PLASMA HEMOGLOBIN CONCENTRATION FROM PRETREATMENT (g/dL)
(*EFFICACY POPULATION*)

TIME (POST-INFUSION)	**Dogs Randomized to Oxyglobin® CHANGE FROM PRETREATMENT**		
	Mean	**Standard Error**	**Number of Observations**
Immediate	4.15*	0.13	21
4 Hour	3.81*	0.14	21
12 Hour	3.45*	0.20	21
24 Hour	2.79*	0.19	21

MEAN CHANGE IN PLASMA HEMOGLOBIN CONCENTRATION FROM PRETREATMENT (g/dL)
(*INTENT-TO-TREAT POPULATION*)

TIME (POST-INFUSION)	**Dogs Randomized to Oxyglobin® CHANGE FROM PRETREATMENT**		
	Mean	**Standard Error**	**Number of Observations**
Immediate	4.16*	0.11	30
4 Hour	3.77*	0.13	29
12 Hour	3.40*	0.17	28
24 Hour	2.84*	0.18	23

* indicates statistical significance using Wilcoxon Sign Ranked Test at p<0.001
* indicates statistical significance using Wilcoxon Sign Ranked Test at p<0.001

(2) Mean change in PCS from pretreatment data:

MEAN CHANGE IN PCS FROM PRETREATMENT
(*EFFICACY POPULATION*)

TIME (POST-INFUSION)	**Dogs Randomized to Oxyglobin® CHANGE FROM PRETREATMENT**		
	Mean	**Standard Error**	**Number of Observations**
Immediate	1.18*	0.16	20
4 Hour	1.24*	0.15	20
12 Hour	1.15*	0.20	20
24 Hour	1.13*	0.20	20

* indicates statistical significance using Wilcoxon Sign Ranked Test at p<0.001

MEAN CHANGE IN PCS FROM PRETREATMENT
(*INTENT-TO-TREAT POPULATION*)

TIME (POST-INFUSION)	**Dogs Randomized to Oxyglobin® CHANGE FROM PRETREATMENT**		
	Mean	**Standard Error**	**Number of Observations**
Immediate	1.21*	0.13	28
4 Hour	1.30*	0.13	27
12 Hour	1.17*	0.16	26
24 Hour	1.11*	0.18	22

* indicates statistical significance using Wilcoxon Sign Ranked Test at p<0.001

(3) Success/Failure rate data:

SUCCESS AND FAILURE RATES OF *EFFICACY POPULATION*

(# Successes or # Failures/# Cases)

Success/Failure	**Dogs Randomized to Oxyglobin®**	**Dogs Randomized to Control**
Success	20/21 (95%)	9/28 (32%)
Failure	1/21 (5%)	19/28 (68%)

SUCCESS AND FAILURE RATES OF *INTENT-TO-TREAT POPULATION*

(# Successes or # Failures/# Cases)

Success/Failure	**Dogs Randomized to Oxyglobin®**	**Dogs Randomized to Control**
Success	22/30 (73%)	10/34 (29%)
Failure	8/30 (27%)	24/34 (71%)

The success rate of the Oxyglobinâ treatment group was statistically significantly higher than the success rate of the control group using Fisher's Exact test (p£0.001) for both the efficacy and intent-to-treat populations.

TIME TO FAILURE

(*EFFICACY POPULATION*)*

Time Interval (Hours)	**Dogs Randomized to Oxyglobin®**	**Dogs Randomized to Control**
0 - 2	0	8
>2 - 4	0	3
>4 - 12	0	5
>12 - 24	1	3

* 20 and 9 dogs randomized to the Oxyglobin® and control groups, respectively did not fail within the initial 24 hour study period, and therefore, are considered successes by definition.

TIME TO FAILURE

(*INTENT-TO-TREAT POPULATION*)**

Time Interval (Hours)	**Dogs Randomized to Oxyglobin®**	**Dogs Randomized to Control**
0 - 2	1	11
>2 - 4	2	4
>4 - 12	0	5
>12 - 24	6	4

** 22 and 10 dogs randomized to the Oxyglobin® and control groups, respectively did not fail within the initial 24 hour study period, and therefore, are considered successes by definition.

Time to failure was statistically analyzed using the Cox proportional

hazard model with test groups (Oxyglobin® or control) as an explanatory variable and with stratification for cause of anemia as a covariate. The Oxyglobin®treatment group showed a statistically significant longer time to failure compared with the control group (efficacy population: $X_1 2 = 20.43$, $p<0.001$; intent-to-treat population: $X_1 2 = 14.02$, $p<0.001$).

5) Adverse Reactions:

The table below lists the frequency of adverse reactions observed in dogs treated with Oxyglobin® (including adverse reactions in control dogs later treated with Oxyglobin® which occurred after Oxyglobin® treatment). Since 50% of the treated dogs had hemolytic anemia, the percentage of each adverse reaction in treated dogs with hemolytic anemia was also tabulated.

Frequency of Adverse Reactions in Oxyglobin® Treated Dogs

Adverse Reaction		**% of Treated Dogs with Adverse Reaction (n=52)**	**% of Adverse Reactions in Treated Dogs with Hemolytic Anemia**
Discoloration	Mucous Membranes°	69	47
	Sclera (yellow, red, brown)	56	48
	Urine (orange, red, brown)	52	41
	Skin (yellow)	12	83
Cardiovascular	Increased CVP†	33	47
	Ventricular Arrhythmia‡	15	78
	Ecchymoses/Petechiae	8	50
	Bradycardia	6	67
Gastrointestinal	Vomiting	35	72
	Diarrhea	15	50
	Anorexia	8	25
Respiratory	Tachypnea	15	50
	Dyspnea	14	71
	Pulmonary Edema	12	67
	Harsh Lung Sounds/Crackles	8	50
	Pleural Effusion	6	67
Miscellaneous	Fever	17	40
	Death/Euthanasia	15	63
	Peripheral Edema	8	25
	Hemoglobinuria*	6	67
	Dehydration	6	33

° yellow, red, purple, brown
† measured in 17 dogs only
‡ AV block, tachycardia, ventricular premature contractions
* measured in 3 dogs only

Adverse reactions occurring in 4% of the dogs treated with Oxyglobin® included: coughing, disseminated intravascular coagulopathy, melena, nasal discharge/crusts (red), peritoneal effusion, respiratory arrest, and weight loss (5-7% of body weight).

Adverse reactions occurring in less than 2% of the dogs treated with Oxyglobin® included: abdominal discomfort upon abdominal palpation, acidosis, cardiac arrest, collapse, cystitis, dark stool, discolored soft stool (red-brown), discolored tongue (purple), focal hyperemic areas on gums, forelimb cellulitis/lameness, hematemesis or hemoptysis (unable to differentiate), hypernatremia, hypotension, hypoxemia, lack of neurologic responses, left forebrain signs, nystagmus, pancreatitis, pendulous abdomen, polyuria, pulmonary thromboembolism, ptosis, reddened pinnae with papules/head shaking, reduction in heart rate, venous thrombosis, and worsening of thrombocytopenia.
Statistical analysis of the adverse reaction data (Fisher's Exact Test, a=0.1) indicated that there were no statistically significant differences in the frequency rates of an adverse reaction occurring in treated dogs receiving only Oxyglobin® and dogs receiving Oxyglobin® plus a previous and/or supplemental blood transfusion(s). Thus, in terms of adverse reactions, there was no evidence of interactions between Oxyglobin® and previous and/or post-treatment blood transfusions.

6) Conclusions:

Oxyglobin® was found to be effective and safe in increasing plasma hemoglobin concentration and improving clinical signs associated with anemia for at least 24 hours when administered once at 30 mL/kg at a rate of 10 mL/kg/hr. Relative to pretreatment, plasma hemoglobin concentration increased and clinical signs associated with anemia (lethargy/depression, exercise intolerance, and increased heart rate) improved for at least 24 hours following administration of Oxyglobin®. Treatment success, defined as the lack of need for additional oxygen carrying support for 24 hours, was 95%. Dogs treated with Oxyglobin® had a longer time to failure compared with controls (i.e., fewer dogs needed additional oxygen support within the 24 hour period following infusion with Oxyglobin®).

III. TARGET ANIMAL SAFETY

The safety of Oxyglobin® was also established in one target animal safety study. This study demonstrates that Oxyglobin®:

1) causes increases in plasma and total hemoglobin concentrations, red discoloration of plasma, increases in aspartate aminotransferase (AST) and alanine aminotransferase (ALT) with no corresponding microscopic lesions in the liver, increase in serum total protein, and hemoglobinuria,

2) causes arteriolitis, activation of tissue macrophages, renal hemosiderin deposition, renal tubular protein droplets and casts indicating saturation of tubular protein reabsorption, reversible renal tubular damage, and slight glomerulonephropathy,

3) causes production of canine immunoglobulin-G class anti-bovine hemoglobin

antibodies (anti-BvHb), and

4) common clinical signs at the recommended dose include yellow-orange discoloration of skin, ear canals, pinnae, mucous membranes (gums), and sclera, red-dark-green discoloration of feces, brown-black discoloration of urine, decreased appetite and thirst, vomiting, diarrhea, and decreased skin elasticity.

A. TARGET ANIMAL SAFETY STUDY

1) Type of Study: Laboratory Toxicity Study conducted in compliance with Good Laboratory Practice (GLP) regulations Title: A Target Animal Safety Study with Oxyglobin® Administered by Repeated Intravenous Infusions to Beagle Dogs Following Acute Hemodilution

2) Principal Investigator: E.L. Berryman
ITR Laboratories Canada Inc.
19601 Clark Graham
Baie d'Urfe, Quebec
Canada H9X 3T1

3) Design of Study:

a) *Purpose of Study*: To establish the safety of Oxyglobin® in dogs.

b) *Test Animals*: 40 healthy Beagle dogs approximately 6 months old. Eight dogs (4M, 4F) were randomly assigned to each of 5 test groups. Acute normovolemic hemodilution model: For each dog, blood was withdrawn and simultaneously replaced with 2.0 to 3.8 times the volume withdrawn with Lactated Ringer's Solution (LRS) while maintaining central venous pressure at approximately baseline values. The procedure continued until the total hemoglobin concentration was approximately 5.0 g/dL.

c) *Dosage Form*: Injectable

d) *Dosage Used*: Oxyglobin® was administered at 30, 60, and 90 mL/kg (1, 2, and 3 times the recommended dose) at a rate of 10 mL/kg/hr once a day on Days 1 and 4 13% Human Serum Albumin (HSA) Saline (protein control) was administered at 90 mL/kg at a rate of 5 mL/kg/hr once a day on Days 1 and 4. The negative control was untreated.

e) *Route of Administration*: Intravenous

f) *Test Duration*: Dogs were hemodiluted immediately prior to treatment on Day 1 and were necropsied on either Day 6 or Day 32.

g) Pertinent Parameters Measured:

Central Venous Pressure: During the hemodilution procedure and immediately prior to, every 3 hours during, and at the end of treatment infusion

Clinical Observations/Physical Examination: Twice daily from Days -14 to -1 and Days 1-11, and once daily from Days 12-32

Body Weight: Twice a week from Days -14 to -1 and once daily on Days 1,

4, 6, 7, 11, 21, and 32

Food/Water Consumption: Daily throughout the study

Electrocardiography: Days -7, 2, and 5

Hematology: CBC - Days -7, 2, 4, 5, 8, 11, 21, and 32.
Total and plasma hemoglobin concentrations Days -7, 2, 4, 5, 7, 8, 11, 18, 21, 25, and 32.
Hematocrit - Days -7, 2, 4, 5, 8, 11, 18, 21, 25, and 32.
Reticulocyte count - Days -7, 4, 8, 11, 18, 25, and 32.

Serum Chemistry: Days -7, 2, 4, 5, 6, 8, 11, 21, and 32

Coagulation: PT, APTT, FDPs, and fibrinogen - Days -7, 2, 4, 5, 8, and 32

Urinalysis: Days -7, 2, 4, 5, 6, 8, 11, 21, and 32

Creatinine Clearance: Days -7, 2, and 5

Antibody Determination: Days -7, 11, 18, 25, and 32

Bone Marrow Smears: Day 32

Gross Pathology: All organ systems - Half of the dogs in each group were necropsied on Day 6 and the remaining half on Day 32

Organ Weights: Adrenals, brain, heart, kidneys, liver, lungs & trachea, testes/ovaries, pituitary, spleen, thymus, and thyroids & parathyroids

Histopathology: Complete on all organ systems

Immunohistopathology: Both kidneys of all treated dogs in which a glomerulopathy was seen and kidneys of dogs in both control groups

4) Results:

Unless noted otherwise, the following treatment-related observations/findings were reported in dogs treated with Oxyglobin® at the recommended dosage of 30 mL/kg:

Clinical signs: yellow-orange discoloration of skin, ear canals, pinnae, mucous membranes (gums), and sclera, red-dark-green discoloration of feces, brown-black discoloration of urine, red spotting of skin and/or lips (less common finding), decreased appetite and thirst, vomiting, diarrhea, and decreased skin elasticity. All of the clinical signs were seen after one dose of Oxyglobin® and prior to the second dose. The frequency and/or intensity of the clinical signs were dose-dependent. In the 60 and 90 mL/kg groups, some feces discolored black. In addition, one of the dogs in the 90 mL/kg group had red discoloration of tears after one dose.

Clinical pathology: increases in plasma and total hemoglobin concentrations, red discoloration of plasma, 5- to 6-fold increase in aspartate aminotransferase (AST) and 2-fold increase (still within normal limits) in alanine aminotransferase (ALT) [with no corresponding microscopic lesions in the liver], increase in serum total protein, and hemoglobinuria. All of the changes were 1) seen after one dose of Oxyglobin® and prior to the second dose, 2) transient, and 3) dose-dependent. In the higher dose groups, up to 25-fold increase in AST and up to 11-fold increase in ALT were observed after one dose.

Gross pathology: dark yellow-orange-brown discoloration of the whole body (reported in all Oxyglobin® treated dogs).†* In two of the dogs each in the 2x and 3x dose groups, dark areas on the gall bladder serosa were also identified.†*

Microscopic pathology: *Lesions in Oxyglobin® treated dogs only*: eosinophilic material in arteriolar media with or without inflammation in multiple organs and tissues†g (arteriolitis), foamy histiocytes in multiple organs and tissues†** (activated, phagocytic macrophages), brown pigment (hemosiderin) in the renal cortical epithelium†***, hyaline droplets in renal cortical tubular epithelium** (indicates saturation of tubular protein reabsorption), and slight glomerulonephropathy* (limited duration and distribution). Liver sinusoidal cell distention†** (activated hepatic macrophages) and hemorrhage in the gall bladder†* were observed in the 2x and 3x dose groups only. *Lesions in both Oxyglobin® treated dogs and HSA Saline control dogs*: proteinaceous casts in renal tubules** (due to saturation of renal protein reabsorption mechanisms) and renal cortical tubular basophilia†** (slight to mild with limited distribution) (indicates regeneration subsequent to necrosis of epithelial cells).

† Dose-dependent
* 48 hours post-second dose, but not at 28 days post-second dose
** Decreased incidence at 28 days post-second dose
*** 28 days post-second dose only
g 48 hours post-second dose, but not at 28 days post-second dose in 1x and 2x dose groups, decreased incidence at 28 days post-second dose in 3x dose group

Antibody determination: Low levels of canine immunoglobulin-G class antibodies to bovine hemoglobin (anti-BvHb) were produced in 11/12 Oxyglobin® treated dogs (4 in the 1x dose, 4 in the 2x dose, 3 in the 3x dose). Due to the limited nature of the study, no relationship between anti-BvHb antibody titer and dose of Oxyglobin® administered could be demonstrated. Observed levels of IgG anti-BvHb are not expected to have any toxicological significance in dogs.

Immunohistopathology: Immunofluorescent antibody staining was performed on kidneys of Oxyglobin® treated dogs in which a glomerulopathy was identified (5/24) to detect deposition of immune complexes. Of the 5 dogs with a glomerulopathy, only one (2x dose) demonstrated focal non-specific deposits of IgG in 30% of the glomeruli (deposits in < 25% of glomeruli is considered normal in dogs).

Treatment with Oxyglobin® at all three dose levels did not interfere with the body's normal regenerative response to anemia as evidenced by reticulocytosis, hypercellularity in the bone marrow, and increased extramedullary hematopoiesis in the spleen.

5) Conclusions:

The clinical signs associated with Oxyglobin® administered at 1, 2, and 3 times the recommended dose twice 3 days apart include yellow-orange discoloration of skin, ear canals, pinnae, mucous membranes (gums), and sclera, red-dark-green-black discoloration of feces, brown-black discoloration of urine, red spotting of skin and/or lips (less common finding), decreased appetite and thirst, vomiting, diarrhea, and decreased skin elasticity. The frequency and/or intensity

of these signs increased with repeated dose and with increasing dose.

The drug-related increases in AST and ALT activities were not associated with histopathologic changes in the liver.
The other laboratory changes associated with Oxyglobin®, i.e., increases in plasma and total hemoglobin concentrations, red discoloration of plasma, increase in serum total protein, and hemoglobinuria, are physiologic effects expected with administration of a hemoglobin (protein) source. Similarly, hyaline droplets, proteinaceous casts, and hemosiderin identified microscopically in the kidney as well as active tissue macrophage phagocytosis (foamy histiocytes and sinusoidal cell distention) indicate physiologic effects of Oxyglobin®, i.e., the normal mechanisms to process pigment and foreign protein.

The arteriolitis, slight glomerulonephropathy, and reversible tubular necrosis seen microscopically are pathologic effects of Oxyglobin®. Although they were not clinically significant in the normal dogs in this study, the impact of these findings in dogs with underlying vascular or renal disease is unknown.

The antibody titers (anti-BvHb) and immunohistopathology findings suggest minimal, if any, clinical significance on the safety of single administration of Oxyglobin®. However, no data were collected to evaluate the safety or efficacy of repeat administration of Oxyglobin® in dogs.

IV. HUMAN FOOD SAFETY

Data on human safety, pertaining to consumption of drug residues in food, were not required for approval of this NADA. The drug is to be labeled for use in dogs which are non-food animals.

Human safety relative to possession, handling and administration: Labeling contains the statement, "NOT FOR HUMAN USE."

V. AGENCY CONCLUSIONS

The data in support of this NADA comply with the requirements of Section 512 of the Act and Section 514.111 of the implementing regulations, and demonstrate that Oxyglobin® (hemoglobin glutamer-200 (bovine)) when used under labeled conditions of use, is safe and effective.

The drug is restricted to use by or on the order of a licensed veterinarian because professional expertise and proper diagnosis are required to: 1) determine the need for oxygen carrying support, 2) use a drug intended for intravenous infusion which requires close monitoring and possible adjustment of infusion rate, and 3) recognize and treat, if necessary, adverse reactions to the drug.

Under section 512(c)(2)(F)(i) of the FFDCA, this approval qualifies for FIVE years of marketing exclusivity beginning on the date of approval because no active ingredient of the drug (including any ester or salt of the active ingredient) has been approved in any other application. The following patents claim Oxyglobin®: 5,084,558 (exp. 1/28/09), 5,296,465 (exp. 3/22/11) and 5,618,919 (exp. 4/8/14).

The format of this FOI Summary document has been modified from its original form to conform with Section 508 of the Rehabilitation Act (29 U.S.C. 794d). The content of this document has not changed.