NON-GLP FINAL REPORT

Testing Facility Study No. 20086532

A Rising-dose and Multiple-dose Tolerance Study of OX-66 by Oral Gavage in Rats

SPONSOR:
Baylor University
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United States

TESTING FACILITY:
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640 N. Elizabeth Street
Spencerville, OH 45887
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17 February 2016

TABLE OF CONTENTS

LIST OF APPENDICES

1. RESPONSIBLE PERSONNEL

1.1. Testing Facility

Study Director	Alene T. McCoy, PhD
Site Director	Rusty E. Rush, MS, DABT
Director of Research/Scientific Report Review	Mark A. Morse, PhD, DABT
Supervisor, Study Coordination/ Study Coordinator	Jody R. Hohenbrink, MS
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Lead Archivist	Rebecca R. English, BS

2. SUMMARY

The objective of this study was to determine the potential toxicity of OX-66, an oxygenating therapeutic, when given orally as a single dose to rats followed by a minimum 24-hour observation period repeated for up to 4 cycles. In addition, the potential toxicity of OX-66 was determined when given orally for 10 days to rats.

The study design was as follows:

Text Table 1
Experimental Design for the Rising-dose Study

Group No.	Test Material	Dose Level (mg/kg/day)	Dose Volume (mL/kg)	Dose Concentration (mg/mL)	Number of Animals Rising-dose Study	
					Males	Females
1	OX-66	250	5	50	3	3
2	OX-66	500	5	100	3	3
3	OX-66	750	5	150	3	3
4	OX-66	1000	5	200	3	3

The following parameters and end points were evaluated in the rising-dose phase of this study: clinical signs, body weights, and body weight changes.

Text Table 2
Experimental Design for the Multiple-dose Study

Group No.	Test Material	Dose Level (mg/kg/day)	Dose Volume (mL/kg)	Dose Concentration (mg/mL)	No. of Animals Multiple-dose Study	
					Males	Females
1	OX-66	1000	5	200	5	5

The following parameters and end points were evaluated in the multiple-dose phase of this study: clinical signs, body weights, body weight changes, clinical pathology parameters (hematology, coagulation, and clinical chemistry), and gross necropsy findings.

Administration of OX-66 by once daily oral gavage was well tolerated in rats at a single dose of up to 1000 mg/kg, or for 10 days at a level of 1000 mg/kg/day. There were no abnormal clinical observations or changes in body weight or body weight gains during either phase of the study. There were also no changes in hematology, coagulation, or clinical chemistry parameters after 10 days of daily administration of 1000 mg/kg/day, compared to historical controls. Based on these results, the no-observed-effect level (NOEL) was considered to be 1000 mg/kg/day.

3. INTRODUCTION

The objective of this study was to determine the potential toxicity of OX-66, an oxygenating therapeutic, when given orally as a single dose to rats followed by a minimum 24-hour observation period repeated for up to 4 cycles. In addition, the potential toxicity of OX-66 was determined when given orally for 10 days to rats.

The design of this study is based on the study objective(s), the overall product development strategy for the test article, and the following study design guidelines: Committee for Human Medicinal Products (CHMP) and ICH Harmonised Tripartite Guideline M3 (R2).

The initial study protocol, the last protocol amendment, and deviations are presented in Appendix 1.

Study Initiation Date:	15 Oct 2015
Initiation of Dosing (Rising-dose):	26 Oct 2015
Completion of In-life (Rising-dose):	16 Nov 2015
Initiation of Dosing (Multiple-dose):	10 Nov 2015
Completion of In-life (Multiple-dose):	20 Nov 2015

4. MATERIALS AND METHODS

4.1. Test and Control Articles

4.1.1. Test Article

Identification:	OX-66
Batch (Lot) No.:	OX66-082015
Receipt Date:	22 Oct 2015
Expiration Date:	Concomitant assessment, ongoing
Physical Description:	White powder
Purity:	N/A
Storage Conditions:	Kept in a controlled room temperature area
Supplier:	Baylor University

4.1.2. Control Article

Identification:	0.9% Sodium Chloride, Injection
Batch (Lot) No.:	C949883
Expiration Date:	29 Feb 2016
Physical Description:	Clear colorless liquid
Storage Conditions:	Kept in a room temperature area
Supplier:	Baxter Healthcare

4.2. Test Article Characterization

The Sponsor provided to the Testing Facility documentation of the identity, strength, purity, and composition for the test article. An MSDS was provided to the Testing Facility and is presented in Appendix 2.

4.3. Analysis of Test Article

The stability of the bulk test article was not determined during the course of this study.

4.4. Test Article Inventory and Disposition

Records of the receipt, distribution, storage, and disposition of test article (including empty containers) were maintained. All unused Sponsor-supplied bulk test article will be returned to the Sponsor (after issuance of the final reports of all studies using these materials). All empty containers were maintained for the duration of the study.

4.5. Dose Formulation and Analysis

4.5.1. Preparation of Test Article

Test article dosing formulations were prepared at appropriate concentrations to meet dose level requirements. For both phases of the study, the dosing formulations were prepared on each day of dosing and stirred continuously during dosing.

Details of the preparation and dispensing of the test article have been retained in the Study Records.

4.5.2. Sample Collection and Analysis

Samples for dose formulation analysis were not collected by the Testing Facility.

4.6. Test System

4.6.1. Receipt

On 20 Oct 2015 (rising-dose study animals) and 03 Nov 2015 (multiple-dose study animals), a total of 22 male and 22 female Sprague Dawley Crl:CD(SD) rats (16 animals/sex for rising-dose study and 6 animals/sex for multiple-dose study) were received from Charles River Laboratories, Raleigh, NC. The animals were examined and weighed on the day following receipt. The animals were 8 to 9 weeks old and males weighed between 224 g and 317 g and females weighed between 180 g and 211 g at the initiation of dosing for each study phase.

4.6.2. Justification for Test System and Number of Animals

The Sprague Dawley rat was chosen as the animal model for this study because it is an accepted rodent species for preclinical toxicity testing by regulatory agencies.

The total number of animals used in this study was considered to be the minimum required to properly characterize the effects of the test article. This study was designed such that it did not require an unnecessary number of animals to accomplish its objectives.

At this time, studies in laboratory animals provide the best available basis for extrapolation to humans and are required to support regulatory submissions. Acceptable alternative models, which do not use live animals, currently do not exist.

4.6.3. Animal Identification

Each animal was identified by a cage card and metal ear tag after randomization.

4.6.4. Environmental Acclimation

The animals were acclimated to their designated housing for at least 6 days before the first day of dosing.

4.6.5. Selection, Assignment, and Disposition of Animals

Animals assigned to study were assigned to groups by a stratified randomization scheme designed to achieve similar group mean body weights. Males and females were randomized separately. Animals in poor health or at extremes of body weight range were not assigned to groups.

The disposition of all animals was documented in the Study Records.

4.6.6. Husbandry

4.6.6.1. Housing

On arrival, animals were individually housed until randomization. Following randomization, animals were group housed (up to 3 animals of the same sex and same dosing group together) in polycarbonate cages containing appropriate bedding equipped with an automatic watering valve. Housing and care were as specified in the USDA Animal Welfare Act (9 CFR, Parts 1, 2, and 3) and as described in the *Guide for the Care and Use of Laboratory Animals* from the National Research Council.[1]

4.6.6.2. Environmental Conditions

Temperatures of 69°F to 72°F (21°C to 22°C) with a relative humidity of 46% to 57% were maintained. A 12-hour light/12-hour dark cycle was maintained, except when interrupted for designated procedures. Ten or greater air changes per hour with 100% fresh air (no air recirculation) were maintained in the animal rooms.

4.6.6.3. Food

PMI Nutrition International Certified Rodent Chow No. 5CR4 (14% protein) was provided ad libitum throughout the study, except during designated procedures. The feed was analyzed by the supplier for nutritional components and environmental contaminants. Results of the dietary analyses were provided by the manufacturer for each lot of diet and are on file at the Testing Facility. It is considered that there are no known contaminants in the feed that would interfere with the objectives of the study.

4.6.6.4. Water

Municipal tap water, after treatment by reverse osmosis and ultraviolet irradiation, was freely available to each animal via an automatic watering system (except during designated procedures). Periodic analysis of the water is performed, and results of these analyses are on file at the Testing Facility. It is considered that there are no known contaminants in the water that could interfere with the outcome of the study.

4.6.6.5. Animal Enrichment

For psychological/environmental enrichment, animals were provided with items such as a chewing object, except when interrupted by study procedures/activities.

4.6.6.6. Veterinary Care

Veterinary care was available throughout the course of the study; however, no examinations or treatments were required.

4.7. Experimental Design

Text Table 3
Experimental Design for the Rising-dose Study

Group No.	Test Material	Dose Level (mg/kg/day)	Dose Volume (mL/kg)	Dose Concentration (mg/mL)	Number of Animals Rising-dose Study	
					Males	Females
1	OX-66	250	5	50	3	3
2	OX-66	500	5	100	3	3
3	OX-66	750	5	150	3	3
4	OX-66	1000	5	200	3	3

Text Table 4
Experimental Design for the Multiple-dose Study

Group No.	Test Material	Dose Level (mg/kg/day)	Dose Volume (mL/kg)	Dose Concentration (mg/mL)	No. of Animals Multiple-dose Study	
					Males	Females
1	OX-66	1000	5	200	5	5

4.7.1. Administration of Test Materials

For the rising-dose study, the test article was administered to the appropriate animals by oral gavage. A minimum 24-hour observation period was maintained before proceeding to the next dose level. The dose volume for each animal was based on the most recent body weight measurement. The doses were given using a syringe with attached gavage cannula. The first day of dosing for each group was designated as Study Day 1.

For the multiple-dose study, the test article was administered to the appropriate animals by once daily oral gavage from Days 1 to 10. The dose volume for each animal was based on the most recent body weight measurement. The doses were given using a syringe with attached gavage cannula. The first day of dosing was designated as Study Day 1.

The dosing formulations were stirred continuously during dose administration.

4.7.2. Justification of Route and Dose Levels

The oral route of exposure was selected because this is the intended route of human exposure.

For the rising-dose study, the dose levels were selected to match those used in a PK study that was performed by the Sponsor. In another previous rat study performed by the Sponsor, there were no adverse effects noted in animals dosed orally for up to 7 days at 100 mg/kg/day.

For the multiple-dose study, the dose level was selected based on the results of the rising-dose study.

4.8. In-life Procedures, Observations, and Measurements – Rising-dose Study

4.8.1. Mortality/Moribundity Checks

The animals were observed for general health/mortality and moribundity twice daily, once in the morning and afternoon, throughout the study.

4.8.2. Clinical Observations

4.8.2.1. Cage Side Observations

Cage side observations were performed once daily, beginning during Week -1 and continuing throughout the dosing period, 1 to 3 hours postdose on the days of dosing. Cage side observations were not required on the days of detailed clinical observations during the pretest (prior to Day 1) period.

4.8.2.2. Detailed Clinical Observations

The animals were removed from the cage and a detailed clinical observation was performed on the day of randomization and at least once weekly, beginning on Day 1.

4.8.3. Body Weights

Each animal was weighed on the day of randomization, at least once weekly beginning on Day 1, and on Day 15.

4.9. Terminal Procedures – Rising-dose Study

4.9.1. Unscheduled Deaths

No rising-dose study animals died during the course of the study.

4.9.2. Scheduled Euthanasia

Rising-dose study animals surviving until scheduled euthanasia were euthanized by carbon dioxide inhalation on Day 15 and discarded.

4.10. In-life Procedures, Observations, and Measurements – Multiple-dose Study

4.10.1. Mortality/Moribundity Checks

The animals were observed for general health/mortality and moribundity twice daily, once in the morning and afternoon, throughout the study.

4.10.2. Clinical Observations

4.10.2.1. Cage Side Observations

Cage side observations were performed once daily, beginning during Week -1 and continuing throughout the dosing period, 1 to 3 hours postdose during the dosing period. Cage side observations were not required on the days of detailed clinical observations during the pretest (prior to Day 1) period.

4.10.2.2. Detailed Clinical Observations

The animals were removed from the cage and a detailed clinical observation was performed on the day of randomization and on Days 2, 7, and 10.

4.10.3. Body Weights

Each animal was weighed on the day of randomization and on Days 1, 4, 7, and 10. A fasted weight was recorded on the day of necropsy.

4.11. Laboratory Evaluations – Multiple-dose Study

4.11.1. Clinical Pathology

4.11.1.1. Sample Collection

Blood was collected from the vena cava (under isoflurane anesthesia at gross necropsy). After collection, samples were transferred to the clinical pathology laboratory for processing.

Multiple-dose study animals were fasted overnight before scheduled clinical pathology sample collections, but had access to water ad libitum. Samples were collected according to Text Table 5.

Text Table 5
Samples for Clinical Pathology Evaluation

Group Nos.	Time Point	Hematology	Coagulation	Clinical Chemistry
1	Day 11	X	X	X

X = sample collected.

4.11.1.2. Hematology

Blood samples were analyzed for the parameters specified in Text Table 6.

Text Table 6
Hematology Parameters

Red blood cell count	White blood cell count
Hemoglobin concentration	Neutrophil count (absolute)
Hematocrit	Lymphocyte count (absolute)
Mean corpuscular volume	Monocyte count (absolute)
Red blood cell distribution width	Eosinophil count (absolute)
Mean corpuscular hemoglobin concentration	Basophil count (absolute)
Mean corpuscular hemoglobin	Large unstained cells
Reticulocyte count (absolute)	Other cells (as appropriate)
Platelet count	

Blood smear slides were prepared for all animals for possible RBC morphology evaluation. One slide per animal was prepared, stained, and archived; however, no evaluations were performed.

4.11.1.3. Coagulation

Blood samples were processed for plasma, and plasma was analyzed for the parameters listed in Text Table 7.

Text Table 7
Coagulation Parameters

Activated partial thromboplastin time Fibrinogen	Prothrombin time

4.11.1.4. Clinical Chemistry

Blood samples were processed for serum, and the serum was analyzed for the parameters specified in Text Table 8.

Text Table 8
Clinical Chemistry Parameters

Alanine aminotransferase	Total protein
Aspartate aminotransferase	Albumin
Alkaline phosphatase	Globulin (calculated)
Gamma-glutamyltransferase	Albumin/globulin ratio
Creatine kinase	Glucose
Total bilirubin	Cholesterol
Urea nitrogen	Triglycerides
Creatinine	Sodium
Calcium	Potassium
Phosphorus	Chloride

4.12. Terminal Procedures – Multiple Dose Study

Terminal procedures are summarized in Text Table 9.

Text Table 9
Terminal Procedures

Group No.	No. of Animals		Scheduled Euthanasia Day	Necropsy Procedures	
	Male	Female		Necropsy	Tissue Collection
1	5	5	11	X	X

X = procedure conducted

4.12.1. Unscheduled Deaths

No multiple-dose study animals died during the course of the study.

4.12.2. Scheduled Euthanasia

Multiple-dose study animals surviving until scheduled euthanasia were weighed, samples for evaluation of clinical pathology parameters were collected as specified in Section 4.11, and the animals were euthanized by isoflurane inhalation, followed by exsanguination. Animals were fasted (overnight) before their scheduled necropsy.

4.12.3. Necropsy

Multiple-dose study animals were subjected to a complete necropsy examination, which included evaluation of the carcass and musculoskeletal system; all external surfaces and orifices; cranial cavity and external surfaces of the brain; and thoracic, abdominal, and pelvic cavities with their associated organs and tissues.

4.12.4. Tissue Collection and Preservation

Representative samples of the tissues identified in Text Table 10 were collected from all animals and preserved in 10% neutral buffered formalin, unless otherwise indicated.

Text Table 10
Tissue Collection and Preservation

Animal identification	Large intestine, colon
Artery, aorta	Large intestine, rectum
Body cavity, nasal	Larynx
Bone marrow smear	Liver
Bone marrow	Lung
Bone, femur	Lymph node, mandibular
Bone, sternum	Lymph node, mesenteric
Brain	Muscle, skeletal
Cervix	Nerve, optic[a]
Epididymis	Nerve, sciatic
Esophagus	Ovary
Eye[a]	Pancreas
Gland, adrenal	Skin
Gland, harderian	Small intestine, duodenum
Gland, mammary	Small intestine, ileum
Gland, parathyroid	Small intestine, jejunum
Gland, pituitary	Spinal cord
Gland, prostate	Spleen
Gland, salivary	Stomach
Gland, seminal vesicle	Testis[b]
Gland, thyroid	Thymus
Gross lesions/masses	Tongue
Gut-associated lymphoid tissue	Trachea
Heart	Urinary bladder
Kidney	Uterus
Large intestine, cecum	Vagina

[a] Preserved in Davidson's fixative.
[b] Preserved in Modified Davidson's fixative.

5. COMPUTERIZED SYSTEMS

Critical computerized systems used in the study are listed below or presented in the appropriate Phase Report. All computerized systems used in the conduct of this study have been validated; when a particular system has not satisfied all requirements, appropriate administrative and procedural controls were implemented to assure the quality and integrity of data.

Text Table 11
Critical Computerized Systems

System Name	Version No.	Description of Data Collected and/or Analyzed
Provantis	8	Applicable in-life, clinical pathology, and necropsy data
Systems 600 Apogee Insight System	3.11	Temperature and/or humidity (animal rooms, refrigerators, freezers, and compound storage, as applicable)
Instem Life Science Systems, DISPENSE	8	Test material receipt, accountability and/or formulation activities
Bayer Advia 120® Automated Hematology Analyzer	3.1.8.0	Hematology data
Olympus AU640e	8.1	Clinical chemistry data
Stago STA Compact Analyzer	107.03	Coagulation data

The following computer study numbers were used to collect data for the various study phases: 20086532, rising-dose and multiple-dose study data; and 20086532 PRE, acclimation data. The tables and appendices within this report display the applicable computer study number.

6. STATISTICAL ANALYSIS

Data are presented as individual values by animal. The individual data tables also include the calculated means and standard deviations for each group.

7. RETENTION OF RECORDS, SAMPLES, AND SPECIMENS

All study-specific raw data, electronic data, documentation, protocol, retained samples and specimens, and final reports from this study are the property of the Sponsor. These materials were available at the Testing Facility during the progress of the study. When the Final Report is issued, all study-specific raw data, documentation, protocol, samples, specimens, and final reports will be archived at the Testing Facility and then transferred to the archive at Charles River Laboratories, Inc., Horsham, PA. One year after issue of the Draft Report, the Sponsor will be contacted to determine the disposition of these materials.

Electronic data generated by the Testing Facility were archived as noted above, except that the data collected using Provantis 8 and Dispense 8 and reporting files stored on SDMS were archived at the Charles River Laboratories facility location in Wilmington, MA.

8. RESULTS

8.1. Rising-dose Study

8.1.1. Mortality

(Appendix 3)

No mortality occurred during this study. All of the animals survived until the scheduled euthanasia.

8.1.2. Clinical Observations

(Appendix 4)

No abnormal clinical observations were made during this study. All animals appeared healthy throughout the course of the study.

8.1.3. Body Weights and Body Weight Gains

(Appendix 5 and Appendix 6)

Individual body weight gains during the rising-dose study ranged from 71 to 116 g in males, and 4 to 36 g in females. There were no significant differences in body weight between dose groups.

8.2. Multiple-dose Study

8.2.1. Mortality

(Appendix 7)

No mortality occurred during this study. All of the animals survived until the scheduled euthanasia.

8.2.2. Clinical Observations

(Appendix 8)

No abnormal clinical observations were made during this study. All animals appeared healthy throughout the course of the study.

8.2.3. Body Weights and Body Weight Gains

(Appendix 9 and Appendix 10)

Body weight gains during the multiple-dose study ranged from 44 to 82 g in males, and 29 to 45 g in females.

8.2.4. Hematology and Coagulation

(Appendix 11)

Historical control data are presented in Appendix 13.

There were no apparent test article-related changes in any hematology or coagulation end points. All values were within the ranges of historical controls.

8.2.5. Clinical Chemistry

(Appendix 12)

Historical control data are presented in Appendix 13.

There were no apparent test article-related changes in any clinical chemistry end points. All values were within the ranges of historical controls.

8.2.6. Gross Pathology

(Appendix 14)

There were no gross pathology observations in the multiple-dose study animals, except in 1 male (Animal No. 9600). Gross findings in this animal included multiple dark foci in the left lobe of the lung.

9. CONCLUSION

In conclusion, administration of OX-66 by once daily oral gavage was well tolerated in rats at a single dose of up to 1000 mg/kg, or for 10 days at a level of 1000 mg/kg/day. There were no abnormal clinical observations or changes in body weight or body weight gains during either phase of the study. There were also no changes in hematology, coagulation, or clinical chemistry parameters after 10 days of daily administration of 1000 mg/kg/day, compared to historical controls. Based on these results, the no-observed-effect level (NOEL) was considered to be 1000 mg/kg/day.

10. REFERENCE

1. Guide for the care and use of laboratory animals. Washington, D.C.: National Academy Press. NRC (National Research Council); 2011.

11. REPORT APPROVAL

Date: 17FEB2016

Alene T. McCoy, PhD
Study Director

Appendix 1

FINAL PROTOCOL

Testing Facility Study No. 20086532

A Rising-dose and Multiple-dose Tolerance Study of OX-66 by Oral Gavage in Rats

SPONSOR:
Baylor University
One Bear Place
Waco, TX 76798
United States

TESTING FACILITY:
Charles River Laboratories, Inc.
640 N. Elizabeth Street
Spencerville, OH 45887
United States

15 October 2015

Appendix 1

TABLE OF CONTENTS

Appendix 1

Appendix 1

1. OBJECTIVE(S)

The objective of this study is to determine the potential toxicity of OX-66, an oxygenating therapeutic, when given orally as a single dose to rats followed by a minimum 24-hour observation period repeated for up to 4 cycles. In addition, the potential toxicity of OX-66 will be determined when given orally for 10 days to rats.

1.1. Study Classification

Study Category:	Toxicology
Study Type:	Repeat Dose Toxicity
Study Design:	Parallel
Primary Treatment CAS Registry Number:	Not Available
Primary Treatment Unique Ingredient ID:	Aluminum hydroxyl
Class of Compound:	Not Available

2. PROPOSED STUDY SCHEDULE

Proposed study dates are listed below. Actual applicable dates will be included in the Final Report.

Animal Arrival/Transfer:	20 Oct 2015 (Rising Dose Phase) 03 Nov 2015 (Multiple Dose Phase)
Initiation of Dosing (Rising-dose Study):	26 Oct 2015 (Group 1) 28 Oct 2015 (Group 2) 30 Oct 2015 (Group 3) 02 Nov 2015 (Group 4)
Completion of In-life (Rising-dose Study):	09 Nov 2015 (Group 1) 11 Nov 2015 (Group 2) 13 Nov 2015 (Group 3) 16 Nov 2015 (Group 4) (Last date of scheduled euthanasia)
Initiation of Dosing (Multiple-dose Study):	10 Nov 2015
Completion of In-life (Multiple-dose Study):	20 Nov 2015 (Last date of necropsy)
Draft Report:	22 Jan 2016

3. GUIDELINES FOR STUDY DESIGN

The design of this study was based on the study objective(s), the overall product development strategy for the test article, and the following study design guidelines:

Appendix 1

- Committee for Human Medicinal Products (CHMP). *Guideline on Repeated Dose Toxicity.* CPMP/SWP/1042/99 Rev 1 Corr.
- ICH Harmonised Tripartite Guideline M3 (R2). *Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals.*

4. REGULATORY COMPLIANCE

This study is not within the scope of regulations governing the conduct of nonclinical laboratory studies and is not intended to comply with such regulations.

5. SPONSOR

Sponsor Representative

Erica Bruce, PhD
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BSB A456R-Bruce Lab
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6. RESPONSIBLE PERSONNEL

Study Director

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Management Contact

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Fax: 419.647.6560
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Appendix 1

7. TEST AND CONTROL ARTICLES

7.1. Test Article(s)

Identification: OX-66

Batch (Lot) Number: OX66-082015

Expiration Date: Concomitant assessment, ongoing

Physical Description: White powder

Correction Factors:

Name	Base/Salt Conversion	Purity	Hygroscopic Water	Total Correction (base/salt×purity×hygroscopic water)
OX-66	N/A	N/A[a]	N/A	100% (assumed for calculation purposes)

[a] Dose calculations will not be corrected for purity.

Storage Conditions: Kept in a controlled room temperature area

7.2. Control Article(s)

Identification: 0.9% Sodium Chloride, Injection

Supplier: To be included in the Final Report

Batch (Lot) Number: To be included in the Final Report

Expiration Date: To be included in the Final Report

Physical Description: Liquid

Storage Conditions: Kept in a room temperature area

7.3. Test Article Characterization

The Sponsor will provide to the Testing Facility documentation of the identity, strength, purity, composition, and stability for the test article, if available. A Certificate of Analysis or equivalent documentation will be provided for inclusion in the Final Report, if available.

The Sponsor has appropriate documentation on file concerning the method of synthesis, fabrication or derivation of the test article, and this information is available should it be requested.

7.4. Analysis of Test Article

The stability of the bulk test article will not be determined during the course of this study. Information to support the stability of each lot of the bulk test article will be provided by the Sponsor, if available.

Appendix 1

7.5. Test Article Inventory and Disposition

Records of the receipt, distribution, storage, and disposition of test article (including empty containers) will be maintained. All unused Sponsor-supplied bulk test article will be returned to the Sponsor (after issue of the Final Reports of all studies using these materials, unless otherwise instructed by the Sponsor). All empty containers will be maintained for the duration of the study.

Shipping Contact
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8. SAFETY

The following safety instructions apply to this study:

Standard laboratory safety procedures will be employed for handling the test and control article(s). Specifically, laboratory gloves, laboratory coat, and eye protection will be worn. Safety information on the test article will be provided by the Sponsor in the form of a Material Safety Data Sheet or equivalent, if available.

9. DOSE FORMULATION AND ANALYSIS

9.1. Preparation of Test Article

Test article dosing formulations will be prepared at appropriate concentrations to meet dose level requirements. For the rising dose phase, the dosing formulations will be prepared on the day of dosing. For the multiple dose phase, the dosing formulations will be prepared daily. The dosing formulation will also be stirred continuously during dosing.

Any residual volumes will be discarded unless otherwise requested by the Study Director.

9.2. Sample Collection and Analysis

Samples for dose formulation analysis will not be collected by the Testing Facility.

10. TEST SYSTEM

Species:	Rat
Strain:	Crl:CD(SD) Sprague-Dawley rat

Appendix 1

Source:	Charles River Laboratories
Number of Males Ordered:	16 (rising dose phase) 6 (multiple dose phase)
Number of Females Ordered:	16 (rising dose phase) 6 (multiple dose phase)
Target Age at the Initiation of Dosing:	At least 8 weeks
Target Weight at the Initiation of Dosing:	200 to 300 g (males)/150 to 250 g (females)

The actual age, weight, and number of animals received will be listed in the Final Report.

10.1. Justification of Test System and Number of Animals

The Sprague Dawley rat was chosen as the animal model for this study as it is an accepted rodent species for preclinical toxicity testing by regulatory agencies.

The total number of animals to be used in this study is considered to be the minimum required to properly characterize the effects of the test article. This study has been designed such that it does not require an unnecessary number of animals to accomplish its objectives.

At this time, studies in laboratory animals provide the best available basis for extrapolation to humans and are required to support regulatory submissions. Acceptable models which do not use live animals currently do not exist.

10.2. Animal Identification

At study assignment, each animal will be identified using a metal ear tag. If required, animals may be temporarily identified using an approved identification method such as indelible ink.

Each animal will be identified by a cage card and metal ear tag after randomization.

10.3. Environmental Acclimation

The animals will be acclimated to their designated housing for at least 6 days before the first day of dosing.

10.4. Selection, Assignment, Replacement, and Disposition of Animals

Animals will be assigned to groups by a stratified randomization scheme designed to achieve similar group mean body weights. Males and females will be randomized separately. Animals in poor health or at extremes of body weight range will not be assigned to groups.

Before the initiation of dosing, any assigned animals considered unsuitable for use in the study will be replaced by alternate animals obtained from the same shipment and maintained under the same environmental conditions.

Appendix 1

After initiation of dosing, study animals may be replaced during the replacement period with alternate animals in the event of accidental injury, non-test article-related health issues, or similar circumstances.

The alternate animals may be used as replacements on the study within 3 days.

The disposition of all animals will be documented in the study records.

11. HUSBANDRY

11.1. Housing

On arrival, animals will be individually housed until randomization. Following randomization, animals will be group housed (up to 3 animals of the same sex and same dosing group together) in polycarbonate cages containing appropriate bedding equipped with an automatic watering valve. These housing conditions will be maintained unless deemed inappropriate by the Study Director and/or Clinical Veterinarian. The room(s) in which the animals will be kept will be documented in the study records.

Animals will be separated during designated procedures/activities. Each cage will be clearly labeled with a color-coded cage card indicating study, group, animal number(s), and sex. Cages will be arranged on the racks in group order. Where possible, control group animals will be housed on a separate rack from the test article treated animals.

11.2. Environmental Conditions

The targeted conditions for animal room environment will be as follows:

Temperature:	68°F to 79°F (20°C to 26°C)
Humidity:	30% to 70%
Light Cycle:	12 hours light and 12 hours dark (except during designated procedures)
Ventilation:	10 or more air changes per hour

11.3. Food

PMI Nutrition International Certified Rodent Chow No. 5CR4 (14% protein) will be provided ad libitum throughout the study, except during designated procedures. The same diet in meal form may be provided to individual animals as warranted by clinical signs (e.g., broken/damaged incisors or other health changes).

The feed is analyzed by the supplier for nutritional components and environmental contaminants. Results of the analysis are provided by the supplier and are on file at the Testing Facility.

It is considered that there are no known contaminants in the feed that would interfere with the objectives of the study.

Appendix 1

11.4. Water

Municipal tap water after treatment by reverse osmosis and ultraviolet irradiation will be freely available to each animal via an automatic watering system (except during designated procedures). Water bottles and/or supplemental water gel can be provided, if required.

Periodic analysis of the water is performed, and results of these analyses are on file at the Testing Facility.

It is considered that there are no known contaminants in the water that could interfere with the outcome of the study.

11.5. Animal Enrichment

Animals will be socially housed for psychological/environmental enrichment and will be provided with items such as a hiding device and/or a chewing object, except when interrupted by study procedures/activities.

11.6. Veterinary Care

Veterinary care will be available throughout the course of the study and animals will be examined by the veterinary staff as warranted by clinical signs or other changes. All veterinary examinations and recommended therapeutic treatments, if any, will be documented in the study records.

In the event that animals show signs of illness or distress, the responsible veterinarian may make initial recommendations about treatment of the animal(s) and/or alteration of study procedures, which must be approved by the Study Director. All such actions will be properly documented in the study records and, when appropriate, by protocol amendment. Treatment of the animal(s) for minor injuries or ailments may be approved without prior consultation with the Sponsor representative when such treatment does not impact fulfillment of the study objectives. If the condition of the animal(s) warrants significant therapeutic intervention or alterations in study procedures, the Sponsor representative will be contacted, when possible, to discuss appropriate action. If the condition of the animal(s) is such that emergency measures must be taken, the Study Director and/or attending veterinarian will attempt to consult with the Sponsor representative prior to responding to the medical crisis, but the Study Director and/or veterinarian has authority to act immediately at his/her discretion to alleviate suffering. The Sponsor representative will be fully informed of any such events.

Appendix 1

12. EXPERIMENTAL DESIGN

Experimental Design for the Rising-dose Study

Group No.	Test Material	Dose Level (mg/kg/day)	Dose Volume (mL/kg)	Dose Concentration (mg/mL)	Number of Animals Rising-dose Study	
					Males	Females
1	OX-66	250	5	50	3	3
2	OX-66	500	5	100	3	3
3	OX-66	750	5	150	3	3
4	OX-66	1000	5	200	3	3

Experimental Design for the Multiple-dose Study

Group No.	Test Material	Dose Level (mg/kg/day)	Dose Volume (mL/kg)	Dose Concentration (mg/mL)	Number of Animals Multiple-dose Study	
					Males	Females
1	OX-66	TBD	TBD	TBD	5	5

TBD = To be determined based on the results of the Rising-dose Study.

12.1. Administration of Test Article

For the rising-dose study, the test article will be administered to the appropriate animals by oral gavage. A minimum 24-hour observation period will be maintained before proceeding to the next dose level. The dose volume for each animal will be based on the most recent body weight measurement. The doses will be given using a syringe with attached gavage cannula. The first day of dosing for each group will be designated as Study Day 1.

For the multiple-dose study, the test article will be administered to the appropriate animals by once daily oral gavage from Days 1 to 10. The dose volume for each animal will be based on the most recent body weight measurement. The doses will be given using a syringe with attached gavage cannula. The first day of dosing will be designated as Study Day 1 (exception: alternate animals used for replacement after Day 1 will assume the day of the animal being replaced).

The dosing formulations will be stirred continuously during dose administration.

12.2. Justification of Route and Dose Levels

The oral route of exposure was selected because this is the intended route of human exposure.

For the rising-dose study, the dose levels were selected based on information provided by the Sponsor. The dose levels will match a PK study that will be performed by the Sponsor. In a previous rat study, there were no adverse effects noted in animals dosed orally for up to 7 days at 100 mg/kg/day.

For the multiple-dose study, the dose levels will be selected based on the results of the rising-dose study.

Appendix 1

13. IN-LIFE PROCEDURES, OBSERVATIONS, AND MEASUREMENTS– RISING-DOSE STUDY

13.1. Mortality/Moribundity Checks

Frequency:	Twice daily, once in the morning and once in the afternoon, throughout the study.
Procedure:	Animals will be observed for general health/mortality and moribundity. Animals will not be removed from cage during observation, unless necessary for identification or confirmation of possible findings.

13.2. Clinical Observations

13.2.1. Cage Side Observations

Frequency:	Once daily, beginning Week -1 and throughout the dosing period; 1 to 3 hours postdose on the days of dosing. Cage side observations are not required on the days of detailed clinical observations during the pretest (prior to Day 1) period.
Procedure:	Animals will not be removed from cage during observation, unless necessary for identification or confirmation of possible findings.

13.2.2. Detailed Clinical Observations

Frequency:	Day of randomization and at least once weekly beginning on Day 1
Procedure:	Animals removed from the cage for examination.

13.3. Body Weights

Frequency:	Day of randomization, at least once weekly beginning on Day 1, Day 15
Procedure:	Animals will be individually weighed. Terminal body weights will not be collected from animals found dead or euthanized moribund.

14. TERMINAL PROCEDURES-RISING-DOSE STUDY

14.1. Unscheduled Deaths

If a rising-dose study animal dies on study, a necropsy will be conducted. If necessary, the animal will be refrigerated to minimize autolysis.

Rising-dose study animals may be euthanized for humane reasons as per Testing Facility SOPs. These animals will undergo necropsy. If necessary, the animal will be refrigerated to minimize autolysis.

Appendix 1

14.2. Scheduled Euthanasia

Rising-dose study animals surviving until scheduled euthanasia will be euthanized by carbon dioxide inhalation on Day 15 and discarded. Animals may be anesthetized with isoflurane prior to euthanasia by carbon dioxide inhalation.

14.3. Necropsy

Rising-dose study animals that are found dead or euthanized moribund will be subjected to a complete necropsy examination, which will include evaluation of the carcass and musculoskeletal system; all external surfaces and orifices; cranial cavity and external surfaces of the brain; and thoracic, abdominal, and pelvic cavities with their associated organs and tissues. No tissues will be retained.

Necropsy procedures will be performed by qualified personnel with appropriate training and experience in animal anatomy and gross pathology.

Images may be generated for illustration of or consultation on gross observations. Generation of such images will be documented. Images and associated documentation will be retained and archived.

15. IN-LIFE PROCEDURES, OBSERVATIONS, AND MEASUREMENTS-MULTIPLE-DOSE STUDY

15.1. Mortality/Moribundity Checks

Frequency:	Twice daily, once in the morning and once in the afternoon, throughout the study.
Procedure:	Animals will be observed for general health/mortality and moribundity. Animals will not be removed from cage during observation, unless necessary for identification or confirmation of possible findings.

15.2. Clinical Observations

15.2.1. Cage Side Observations

Frequency:	Once daily, beginning Week -1 and throughout the dosing period; 1 to 3 hours postdose during the dosing period. Cage side observations are not required on the days of detailed clinical observations during the pretest (prior to Day 1) period.
Procedure:	Animals will not be removed from cage during observation, unless necessary for identification or confirmation of possible findings.

Appendix 1

15.2.2. Detailed Clinical Observations

Frequency: Day of randomization and Days 2, 7, and 10

Procedure: Animals removed from the cage for examination.

15.3. Body Weights

Frequency: Day of randomization and Days 1, 4, 7, and 10

Procedure: Animals will be individually weighed. A fasted weight will be recorded on the day of necropsy. Terminal body weights will not be collected from animals found dead or euthanized moribund.

16. LABORATORY EVALUATIONS-MULTIPLE-DOSE STUDY

16.1. Clinical Pathology

16.1.1. Sample Collection

Blood will be collected from the vena cava (under isoflurane anesthesia at gross necropsy). Blood for unscheduled euthanasia animals may be collected under isoflurane anesthesia from the jugular vein or orbital plexus. Additional blood samples may be obtained (e.g., due to clotting of non-serum samples) if permissible sampling frequency and blood volume are not exceeded. After collection, samples will be transferred to the appropriate laboratory for processing.

Multiple-dose study animals will be fasted overnight before scheduled clinical pathology sample collections (fasting of the animals is not required for hematology determinations), but will have access to water ad libitum. Samples will be collected according to the following table:

Samples for Clinical Pathology Evaluation

Group Nos.	Time Point	Hematology	Coagulation	Clinical Chemistry
1	Day 11	X	X	X
Unscheduled euthanasia (when possible)	Before euthanasia	X	X	X

X = sample to be collected; - = not applicable.

Any residual/retained clinical pathology samples will be discarded before issue of the Final Report.

16.1.2. Hematology

Target Volume: 0.5 mL

Anticoagulant: K_2EDTA

Appendix 1

Hematology Parameters

Red blood cell count	White blood cell count
Hemoglobin concentration	Neutrophil count (absolute)
Hematocrit	Lymphocyte count (absolute)
Mean corpuscular volume	Monocyte count (absolute)
Red Blood Cell Distribution Width	Eosinophil count (absolute)
Mean corpuscular hemoglobin concentration	Basophil count (absolute)
Mean corpuscular hemoglobin	Large unstained cells
Reticulocyte count (absolute)	Other cells (as appropriate)
Platelet count	

One blood smear will be prepared from each hematology sample. The slide will be labeled, stained, and archived. Slide review will only be performed on samples that meet flagging criteria in order to confirm accurate hematology analyzer results. If additional examination of blood smears is deemed necessary, the smears may be subsequently evaluated at additional cost by protocol amendment.

16.1.3. Coagulation

Target Volume: 1.8 mL

Anticoagulant: Sodium citrate

Processing: To plasma

Coagulation Parameters

Activated partial thromboplastin time	Prothrombin time
Fibrinogen	

16.1.4. Clinical Chemistry

Target Volume: 2 mL

Anticoagulant: None

Processing: To serum

Clinical Chemistry Parameters

Alanine aminotransferase	Total protein
Aspartate aminotransferase	Albumin
Alkaline phosphatase	Globulin
Gamma-glutamyltransferase	Albumin/globulin ratio
Creatine Kinase	Glucose
Total bilirubin[a]	Cholesterol
Urea nitrogen	Triglycerides
Creatinine	Sodium
Calcium	Potassium
Phosphorus	Chloride

[a] When total bilirubin is > 0.5 mg/dL, direct bilirubin will be measured and indirect bilirubin will be calculated.

Appendix 1

17. TERMINAL PROCEDURES-MULTIPLE-DOSE STUDY

Terminal procedures are summarized in the following table:

Terminal Procedures for Multiple-dose Study Animals

Group No.	Number of Animals		Scheduled Euthanasia Day	Necropsy Procedures			Histology	Histopathology
	M	F		Necropsy	Tissue Collection	Organ Weights		
1	5	5	11	X	X	-	-	-
Unscheduled Deaths				X	X	-	-	-
Replaced animals (prestudy)				X	-	-	-	-
Replaced animals (after dosing start)				X	X	-	-	-

X = procedure to be conducted; - = not applicable.
[a] See Tissue Collection and Preservation table for listing of tissues.

17.1. Unscheduled Deaths

If a multiple-dose study animal dies on study, a necropsy will be conducted and specified tissues will be saved. If necessary, the animal will be refrigerated to minimize autolysis.

Multiple-dose study animals may be euthanized for humane reasons as per Testing Facility SOPs. Samples for evaluation of clinical pathology parameters will be obtained if possible as specified in Section 16 (priority is hematology > clinical chemistry > coagulation). These animals will undergo necropsy, and specified tissues will be retained. If necessary, the animal will be refrigerated to minimize autolysis.

17.2. Scheduled Euthanasia

Multiple-dose study animals surviving until scheduled euthanasia will have a terminal body weight recorded; samples collected for evaluation of clinical pathology parameters as specified in Section 16; and the animals will be euthanized by isoflurane inhalation, followed by exsanguination. Animals will be fasted (overnight) before their scheduled necropsy.

17.3. Necropsy

Multiple-dose study animals will be subjected to a complete necropsy examination, which will include evaluation of the carcass and musculoskeletal system; all external surfaces and orifices; cranial cavity and external surfaces of the brain; and thoracic, abdominal, and pelvic cavities with their associated organs and tissues.

Necropsy procedures will be performed by qualified personnel with appropriate training and experience in animal anatomy and gross pathology.

Images may be generated for illustration of or consultation on gross observations. Generation of such images will be documented. Images and associated documentation will be retained and archived.

Appendix 1

17.4. Tissue Collection and Preservation

Representative samples of the tissues identified in the Tissue Collection and Preservation table in Attachment A will be collected from all animals and preserved in 10% neutral buffered formalin, unless otherwise indicated. Additional tissue samples may be collected to elucidate abnormal findings.

18. COMPUTERIZED SYSTEMS

The following critical computerized systems may be used in the study. The actual critical computerized systems used will be specified in the Final Report.

Data for parameters not required by protocol, which are automatically generated by analytical devices used will be retained on file but not reported. Statistical analysis results that are generated by the program but are not required by protocol and/or are not scientifically relevant will be retained on file but will not be included in the tabulations.

Critical Computerized Systems

System Name	Description of Data Collected and/or Analyzed
Compaq Alpha DS10 Computer using the Toxicology Analysis System Customized, General Toxicology Module or Provantis	In-life; clinical pathology; postmortem
Bayer Advia 120® Automated Hematology Analyzer	hematology analysis
Olympus AU640e	clinical chemistry analysis
Stago STA Compact Analyzer	coagulation analysis
Instem Life Science Systems, DISPENSE	Test material receipt, accountability and/or formulation activities
Systems 600 Apogee Insight System	temperature and/or humidity (animal rooms, refrigerators, freezers, and compound storage)

19. CONSTRUCTED VARIABLES

Body Weight Changes: calculated between at least each scheduled interval

20. STATISTICAL ANALYSIS

Data will be presented as individual values by animal. The individual data tables will also include the calculated means and standard deviations for each group.

21. AMENDMENTS AND DEVIATIONS

Changes to the approved protocol shall be made in the form of an amendment, which will be signed and dated by the Study Director. Every reasonable effort will be made to discuss any necessary protocol changes in advance with the Sponsor.

Appendix 1

All protocol and SOP deviations will be documented in the study records. Deviations from the protocol and/or SOP related to the phase(s) of the study conducted at a Test Site shall be documented, acknowledged by the PI/IS, and reported to the Study Director for authorization/acknowledgement. The Study Director will notify the Sponsor of deviations that may result in a significant impact on the study as soon as possible.

22. RETENTION OF RECORDS, SAMPLES, AND SPECIMENS

All study-specific raw data, electronic data, documentation, protocol, retained samples and specimens, and interim (if applicable) and final reports will be archived by no later than the date of final report issue. All materials generated by Charles River from this study will be transferred to a Charles River archive. One year after issue of the draft report, the Sponsor will be contacted to determine the disposition of materials associated with the study.

23. REPORTING

A comprehensive Draft Report will be prepared following completion of the study and will be finalized following consultation with the Sponsor. The report will include all information necessary to provide a complete and accurate description of the experimental methods and results and any circumstances that may have affected the quality or integrity of the study.

The Sponsor will receive an electronic version of the Draft and Final Report provided in Adobe Acrobat PDF format (hyperlinked and searchable) along with a Microsoft Word version of the text. The PDF document will be created from native electronic files to the extent possible, including text and tables generated by the Testing Facility. Report components not available in native electronic files and/or original signature pages will be scanned and converted to PDF image files for incorporation. An original copy of the report with the Testing Facility's handwritten signatures will be retained.

Reports should be finalized within 6 months of issue of the Draft Report. If the Sponsor has not provided comments to the report within 6 months of draft issue, the report will be finalized by the Testing Facility unless other arrangements are made by the Sponsor.

24. ANIMAL WELFARE

This study will comply with all applicable sections of the Final Rules of the Animal Welfare Act regulations (Code of Federal Regulations, Title 9), the *Public Health Service Policy on Humane Care and Use of Laboratory Animals* from the Office of Laboratory Animal Welfare, and the *Guide for the Care and Use of Laboratory Animals* from the National Research Council.[1,2] The protocol and any amendments or procedures involving the care or use of animals in this study will be reviewed and approved by the Testing Facility Institutional Animal Care and Use Committee before the initiation of such procedures.

If an animal is determined to be in overt pain/distress, or appears moribund and is beyond the point where recovery appears reasonable, the animal will be euthanized for humane reasons in

Appendix 1

accordance with the *American Veterinary Medical Association (AVMA) Guidelines on Euthanasia* and with the procedures outlined in the protocol.[3]

By approving this protocol, the Sponsor affirms that there are no acceptable non-animal alternatives for this study, that this study is required by a relevant government regulatory agency(ies) and that it does not unnecessarily duplicate any previous experiments.

Appendix 1

25. REFERENCES

1. Office of Laboratory Animal Welfare. *Public Health Services Policy on Humane Care and Use of Laboratory Animals*. Bethesda, MD: National Institutes of Health. August 2002.

2. National Research Council. *Guide for the Care and Use of Laboratory Animals*. 8th edition. Washington, DC: National Academy Press. 2011.

3. American Veterinary Medical Association. *AVMA Guidelines on Euthanasia*. February 2013.

Appendix 1

26. TESTING FACILITY APPROVAL

The signature below indicates that Testing Facility Management approves the Study Director identified in this protocol.

Mark A. Morse Date: 15OCT2015

Mark A. Morse, PhD, DABT
Testing Facility Management

The signature below indicates that the Study Director approves the study protocol.

Alene T. McCoy Date: 15 OCT 2015

Alene T. McCoy, PhD
Study Director

Appendix 1

27. SPONSOR APPROVAL

The protocol was approved by the Sponsor by email on 15 Oct 2015. The signature below confirms the approval of the protocol by the Sponsor Representative.

Digitally signed by Erica Bruce
Reason: I am approving this document
Date: 2015.10.15 15:09:53 -05'00'
Adobe Acrobat DC version: 2015.006.30094

_____Date:_____

Erica Bruce, PhD
Sponsor Representative

Appendix 1

28. ATTACHMENT A

Tissue Collection and Preservation

Tissue	Weigh	Collect	Microscopic Evaluation	Comment
Animal identification	-	X	-	-
Artery, aorta	-	X	-	-
Body cavity, nasal	-	X	-	-
Bone marrow smear	-	X	-	Bone marrow smears will be collected from the femur at scheduled and unscheduled necropsies (for possible examination). Smears will not be collected from animals that are found dead or from animals that were euthanized moribund and then stored in the refrigerator prior to necropsy. Bone marrow smears are allowed to air dry and are not fixed in formalin.
Bone marrow	-	X	-	-
Bone, femur	-	X	-	-
Bone, sternum	-	X	-	-
Brain	-	X	-	-
Cervix	-	X	-	-
Epididymis	-	X	-	-
Esophagus	-	X	-	-
Eye	-	X	-	Preserve in Davidson's fixative.
Gland, adrenal	-	X	-	-
Gland, harderian	-	X	-	-
Gland, mammary	-	X	-	-
Gland, parathyroid	-	X	-	-
Gland, pituitary	-	X	-	-
Gland, prostate	-	X	-	-
Gland, salivary	-	X	-	-
Gland, seminal vesicle	-	X	-	-
Gland, thyroid	-	X	-	-
Gross lesions/masses	-	X	-	-
Gut-associated lymphoid tissue	-	X	-	-
Heart	-	X	-	-
Kidney	-	X	-	-
Large intestine, cecum	-	X	-	-
Large intestine, colon	-	X	-	-
Large intestine, rectum	-	X	-	-
Larynx	-	X	-	-
Liver	-	X	-	-
Lung	-	X	-	-
Lymph node, mandibular	-	X	-	-
Lymph node, mesenteric	-	X	-	-
Muscle, skeletal	-	X	-	-
Nerve, optic	-	X	-	Preserve in Davidson's fixative.
Nerve, sciatic	-	X	-	-

Appendix 1

Tissue	Weigh	Collect	Microscopic Evaluation	Comment
Ovary	-	X	-	-
Pancreas	-	X	-	-
Skin	-	X	-	-
Small intestine, duodenum	-	X	-	-
Small intestine, ileum	-	X	-	-
Small intestine, jejunum	-	X	-	-
Spinal cord	-	X	-	-
Spleen	-	X	-	-
Stomach	-	X	-	-
Testis	-	X	-	Preserve in Modified Davidson's fixative.
Thymus	-	X	-	-
Tongue	-	X	-	-
Trachea	-	X	-	-
Urinary bladder	-	X	-	-
Uterus	-	X	-	-
Vagina	-	X	-	-

X = Procedure to be conducted; - = Not applicable.

Appendix 1

PROTOCOL AMENDMENT NO. 1

Testing Facility Study No. 20086532

A Rising-dose and Multiple-dose Tolerance Study of OX-66 by Oral Gavage in Rats

SPONSOR:
Baylor University
One Bear Place
Waco, TX 76798
United States

TESTING FACILITY:
Charles River Laboratories, Inc.
640 N. Elizabeth Street
Spencerville, OH 45887
United States

Appendix 1

SUMMARY OF CHANGES AND JUSTIFICATIONS

Study Protocol effective date: 15-Oct-2015

Note: When applicable, additions are indicated in bold underlined text and deletions are indicated in bold strikethrough text in the affected sections of the document.

Item or Section(s)	Justification
Amendment 1	**Effective Date: 09-Nov-2015**
12. Experimental Design	To include the dose level for the multiple dose phase.

Appendix 1

TABLE OF CONTENTS

Appendix 1

Appendix 1

1. OBJECTIVE(S)

The objective of this study is to determine the potential toxicity of OX-66, an oxygenating therapeutic, when given orally as a single dose to rats followed by a minimum 24-hour observation period repeated for up to 4 cycles. In addition, the potential toxicity of OX-66 will be determined when given orally for 10 days to rats.

1.1. Study Classification

Study Category:	Toxicology
Study Type:	Repeat Dose Toxicity
Study Design:	Parallel
Primary Treatment CAS Registry Number:	Not Available
Primary Treatment Unique Ingredient ID:	Aluminum hydroxyl
Class of Compound:	Not Available

2. PROPOSED STUDY SCHEDULE

Proposed study dates are listed below. Actual applicable dates will be included in the Final Report.

Animal Arrival/Transfer:	20 Oct 2015 (Rising Dose Phase) 03 Nov 2015 (Multiple Dose Phase)
Initiation of Dosing (Rising-dose Study):	26 Oct 2015 (Group 1) 28 Oct 2015 (Group 2) 30 Oct 2015 (Group 3) 02 Nov 2015 (Group 4)
Completion of In-life (Rising-dose Study):	09 Nov 2015 (Group 1) 11 Nov 2015 (Group 2) 13 Nov 2015 (Group 3) 16 Nov 2015 (Group 4) (Last date of scheduled euthanasia)
Initiation of Dosing (Multiple-dose Study):	10 Nov 2015
Completion of In-life (Multiple-dose Study):	20 Nov 2015 (Last date of necropsy)
Draft Report:	22 Jan 2016

3. GUIDELINES FOR STUDY DESIGN

The design of this study was based on the study objective(s), the overall product development strategy for the test article, and the following study design guidelines:

Appendix 1

- Committee for Human Medicinal Products (CHMP). *Guideline on Repeated Dose Toxicity.* CPMP/SWP/1042/99 Rev 1 Corr.
- ICH Harmonised Tripartite Guideline M3 (R2). *Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals.*

4. REGULATORY COMPLIANCE

This study is not within the scope of regulations governing the conduct of nonclinical laboratory studies and is not intended to comply with such regulations.

5. SPONSOR

Sponsor Representative

Erica Bruce, PhD
Baylor University
Baylor Sciences BLDG
BSB A456R-Bruce Lab
101 Bagby Avenue
Waco, TX 76798
Tel: 254.710.4877
Fax: 254.710.3409
E-mail: erica_bruce@baylor.edu

6. RESPONSIBLE PERSONNEL

Study Director

Alene T. McCoy, PhD
Address as cited for Testing Facility
Tel: 419.647.4196
Fax: 419.647.6560
E-mail: alene.mccoy@crl.com

Management Contact

Mark A. Morse, PhD, DABT
Address as cited for Testing Facility
Tel: 419.647.4196
Fax: 419.647.6560
E-mail: mark.morse@crl.com

Appendix 1

7. TEST AND CONTROL ARTICLES

7.1. Test Article(s)

Identification: OX-66

Batch (Lot) Number: OX66-082015

Expiration Date: Concomitant assessment, ongoing

Physical Description: White powder

Correction Factors:

Name	Base/Salt Conversion	Purity	Hygroscopic Water	Total Correction (base/salt×purity×hygroscopic water)
OX-66	N/A	N/A[a]	N/A	100% (assumed for calculation purposes)

[a] Dose calculations will not be corrected for purity.

Storage Conditions: Kept in a controlled room temperature area

7.2. Control Article(s)

Identification: 0.9% Sodium Chloride, Injection

Supplier: To be included in the Final Report

Batch (Lot) Number: To be included in the Final Report

Expiration Date: To be included in the Final Report

Physical Description: Liquid

Storage Conditions: Kept in a room temperature area

7.3. Test Article Characterization

The Sponsor will provide to the Testing Facility documentation of the identity, strength, purity, composition, and stability for the test article, if available. A Certificate of Analysis or equivalent documentation will be provided for inclusion in the Final Report, if available.

The Sponsor has appropriate documentation on file concerning the method of synthesis, fabrication or derivation of the test article, and this information is available should it be requested.

7.4. Analysis of Test Article

The stability of the bulk test article will not be determined during the course of this study. Information to support the stability of each lot of the bulk test article will be provided by the Sponsor, if available.

Appendix 1

7.5. Test Article Inventory and Disposition

Records of the receipt, distribution, storage, and disposition of test article (including empty containers) will be maintained. All unused Sponsor-supplied bulk test article will be returned to the Sponsor (after issue of the Final Reports of all studies using these materials, unless otherwise instructed by the Sponsor). All empty containers will be maintained for the duration of the study.

Shipping Contact
Erica Bruce, PhD
Baylor University
Baylor Sciences BLDG
BSB A456R-Bruce Lab
101 Bagby Avenue
Waco, TX 76798
Tel: 254.710.4877
Fax: 254.710.3409
E-mail: erica_bruce@baylor.edu

8. SAFETY

The following safety instructions apply to this study:

Standard laboratory safety procedures will be employed for handling the test and control article(s). Specifically, laboratory gloves, laboratory coat, and eye protection will be worn. Safety information on the test article will be provided by the Sponsor in the form of a Material Safety Data Sheet or equivalent, if available.

9. DOSE FORMULATION AND ANALYSIS

9.1. Preparation of Test Article

Test article dosing formulations will be prepared at appropriate concentrations to meet dose level requirements. For the rising dose phase, the dosing formulations will be prepared on the day of dosing. For the multiple dose phase, the dosing formulations will be prepared daily. The dosing formulation will also be stirred continuously during dosing.

Any residual volumes will be discarded unless otherwise requested by the Study Director.

9.2. Sample Collection and Analysis

Samples for dose formulation analysis will not be collected by the Testing Facility.

10. TEST SYSTEM

Species:	Rat
Strain:	Crl:CD(SD) Sprague-Dawley rat

Appendix 1

Source:	Charles River Laboratories
Number of Males Ordered:	16 (rising dose phase) 6 (multiple dose phase)
Number of Females Ordered:	16 (rising dose phase) 6 (multiple dose phase)
Target Age at the Initiation of Dosing:	At least 8 weeks
Target Weight at the Initiation of Dosing:	200 to 300 g (males)/150 to 250 g (females)

The actual age, weight, and number of animals received will be listed in the Final Report.

10.1. Justification of Test System and Number of Animals

The Sprague Dawley rat was chosen as the animal model for this study as it is an accepted rodent species for preclinical toxicity testing by regulatory agencies.

The total number of animals to be used in this study is considered to be the minimum required to properly characterize the effects of the test article. This study has been designed such that it does not require an unnecessary number of animals to accomplish its objectives.

At this time, studies in laboratory animals provide the best available basis for extrapolation to humans and are required to support regulatory submissions. Acceptable models which do not use live animals currently do not exist.

10.2. Animal Identification

At study assignment, each animal will be identified using a metal ear tag. If required, animals may be temporarily identified using an approved identification method such as indelible ink.

Each animal will be identified by a cage card and metal ear tag after randomization.

10.3. Environmental Acclimation

The animals will be acclimated to their designated housing for at least 6 days before the first day of dosing.

10.4. Selection, Assignment, Replacement, and Disposition of Animals

Animals will be assigned to groups by a stratified randomization scheme designed to achieve similar group mean body weights. Males and females will be randomized separately. Animals in poor health or at extremes of body weight range will not be assigned to groups.

Before the initiation of dosing, any assigned animals considered unsuitable for use in the study will be replaced by alternate animals obtained from the same shipment and maintained under the same environmental conditions.

Appendix 1

After initiation of dosing, study animals may be replaced during the replacement period with alternate animals in the event of accidental injury, non-test article-related health issues, or similar circumstances.

The alternate animals may be used as replacements on the study within 3 days.

The disposition of all animals will be documented in the study records.

11. HUSBANDRY

11.1. Housing

On arrival, animals will be individually housed until randomization. Following randomization, animals will be group housed (up to 3 animals of the same sex and same dosing group together) in polycarbonate cages containing appropriate bedding equipped with an automatic watering valve. These housing conditions will be maintained unless deemed inappropriate by the Study Director and/or Clinical Veterinarian. The room(s) in which the animals will be kept will be documented in the study records.

Animals will be separated during designated procedures/activities. Each cage will be clearly labeled with a color-coded cage card indicating study, group, animal number(s), and sex. Cages will be arranged on the racks in group order. Where possible, control group animals will be housed on a separate rack from the test article treated animals.

11.2. Environmental Conditions

The targeted conditions for animal room environment will be as follows:

Temperature:	68°F to 79°F (20°C to 26°C)
Humidity:	30% to 70%
Light Cycle:	12 hours light and 12 hours dark (except during designated procedures)
Ventilation:	10 or more air changes per hour

11.3. Food

PMI Nutrition International Certified Rodent Chow No. 5CR4 (14% protein) will be provided ad libitum throughout the study, except during designated procedures. The same diet in meal form may be provided to individual animals as warranted by clinical signs (e.g., broken/damaged incisors or other health changes).

The feed is analyzed by the supplier for nutritional components and environmental contaminants. Results of the analysis are provided by the supplier and are on file at the Testing Facility.

It is considered that there are no known contaminants in the feed that would interfere with the objectives of the study.

Appendix 1

11.4. Water

Municipal tap water after treatment by reverse osmosis and ultraviolet irradiation will be freely available to each animal via an automatic watering system (except during designated procedures). Water bottles and/or supplemental water gel can be provided, if required.

Periodic analysis of the water is performed, and results of these analyses are on file at the Testing Facility.

It is considered that there are no known contaminants in the water that could interfere with the outcome of the study.

11.5. Animal Enrichment

Animals will be socially housed for psychological/environmental enrichment and will be provided with items such as a hiding device and/or a chewing object, except when interrupted by study procedures/activities.

11.6. Veterinary Care

Veterinary care will be available throughout the course of the study and animals will be examined by the veterinary staff as warranted by clinical signs or other changes. All veterinary examinations and recommended therapeutic treatments, if any, will be documented in the study records.

In the event that animals show signs of illness or distress, the responsible veterinarian may make initial recommendations about treatment of the animal(s) and/or alteration of study procedures, which must be approved by the Study Director. All such actions will be properly documented in the study records and, when appropriate, by protocol amendment. Treatment of the animal(s) for minor injuries or ailments may be approved without prior consultation with the Sponsor representative when such treatment does not impact fulfillment of the study objectives. If the condition of the animal(s) warrants significant therapeutic intervention or alterations in study procedures, the Sponsor representative will be contacted, when possible, to discuss appropriate action. If the condition of the animal(s) is such that emergency measures must be taken, the Study Director and/or attending veterinarian will attempt to consult with the Sponsor representative prior to responding to the medical crisis, but the Study Director and/or veterinarian has authority to act immediately at his/her discretion to alleviate suffering. The Sponsor representative will be fully informed of any such events.

Appendix 1

12. EXPERIMENTAL DESIGN

Experimental Design for the Rising-dose Study

Group No.	Test Material	Dose Level (mg/kg/day)	Dose Volume (mL/kg)	Dose Concentration (mg/mL)	Number of Animals Rising-dose Study	
					Males	Females
1	OX-66	250	5	50	3	3
2	OX-66	500	5	100	3	3
3	OX-66	750	5	150	3	3
4	OX-66	1000	5	200	3	3

Experimental Design for the Multiple-dose Study

Group No.	Test Material	Dose Level (mg/kg/day)	Dose Volume (mL/kg)	Dose Concentration (mg/mL)	Number of Animals Multiple-dose Study	
					Males	Females
1	OX-66	~~TBD~~ 1000	~~TBD~~ 5	~~TBD~~ 200	5	5

~~TBD = To be determined based on the results of the Rising-dose Study.~~

12.1. Administration of Test Article

For the rising-dose study, the test article will be administered to the appropriate animals by oral gavage. A minimum 24-hour observation period will be maintained before proceeding to the next dose level. The dose volume for each animal will be based on the most recent body weight measurement. The doses will be given using a syringe with attached gavage cannula. The first day of dosing for each group will be designated as Study Day 1.

For the multiple-dose study, the test article will be administered to the appropriate animals by once daily oral gavage from Days 1 to 10. The dose volume for each animal will be based on the most recent body weight measurement. The doses will be given using a syringe with attached gavage cannula. The first day of dosing will be designated as Study Day 1 (exception: alternate animals used for replacement after Day 1 will assume the day of the animal being replaced).

The dosing formulations will be stirred continuously during dose administration.

12.2. Justification of Route and Dose Levels

The oral route of exposure was selected because this is the intended route of human exposure.

For the rising-dose study, the dose levels were selected based on information provided by the Sponsor. The dose levels will match a PK study that will be performed by the Sponsor. In a previous rat study, there were no adverse effects noted in animals dosed orally for up to 7 days at 100 mg/kg/day.

For the multiple-dose study, the dose levels will be selected based on the results of the rising-dose study.

Appendix 1

13. IN-LIFE PROCEDURES, OBSERVATIONS, AND MEASUREMENTS– RISING-DOSE STUDY

13.1. Mortality/Moribundity Checks

Frequency:	Twice daily, once in the morning and once in the afternoon, throughout the study.
Procedure:	Animals will be observed for general health/mortality and moribundity. Animals will not be removed from cage during observation, unless necessary for identification or confirmation of possible findings.

13.2. Clinical Observations

13.2.1. Cage Side Observations

Frequency:	Once daily, beginning Week -1 and throughout the dosing period; 1 to 3 hours postdose on the days of dosing. Cage side observations are not required on the days of detailed clinical observations during the pretest (prior to Day 1) period.
Procedure:	Animals will not be removed from cage during observation, unless necessary for identification or confirmation of possible findings.

13.2.2. Detailed Clinical Observations

Frequency:	Day of randomization and at least once weekly beginning on Day 1
Procedure:	Animals removed from the cage for examination.

13.3. Body Weights

Frequency:	Day of randomization, at least once weekly beginning on Day 1, Day 15
Procedure:	Animals will be individually weighed. Terminal body weights will not be collected from animals found dead or euthanized moribund.

14. TERMINAL PROCEDURES-RISING-DOSE STUDY

14.1. Unscheduled Deaths

If a rising-dose study animal dies on study, a necropsy will be conducted. If necessary, the animal will be refrigerated to minimize autolysis.

Rising-dose study animals may be euthanized for humane reasons as per Testing Facility SOPs. These animals will undergo necropsy. If necessary, the animal will be refrigerated to minimize autolysis.

Appendix 1

14.2. Scheduled Euthanasia

Rising-dose study animals surviving until scheduled euthanasia will be euthanized by carbon dioxide inhalation on Day 15 and discarded. Animals may be anesthetized with isoflurane prior to euthanasia by carbon dioxide inhalation.

14.3. Necropsy

Rising-dose study animals that are found dead or euthanized moribund will be subjected to a complete necropsy examination, which will include evaluation of the carcass and musculoskeletal system; all external surfaces and orifices; cranial cavity and external surfaces of the brain; and thoracic, abdominal, and pelvic cavities with their associated organs and tissues. No tissues will be retained.

Necropsy procedures will be performed by qualified personnel with appropriate training and experience in animal anatomy and gross pathology.

Images may be generated for illustration of or consultation on gross observations. Generation of such images will be documented. Images and associated documentation will be retained and archived.

15. IN-LIFE PROCEDURES, OBSERVATIONS, AND MEASUREMENTS-MULTIPLE-DOSE STUDY

15.1. Mortality/Moribundity Checks

Frequency:	Twice daily, once in the morning and once in the afternoon, throughout the study.
Procedure:	Animals will be observed for general health/mortality and moribundity. Animals will not be removed from cage during observation, unless necessary for identification or confirmation of possible findings.

15.2. Clinical Observations

15.2.1. Cage Side Observations

Frequency:	Once daily, beginning Week -1 and throughout the dosing period; 1 to 3 hours postdose during the dosing period. Cage side observations are not required on the days of detailed clinical observations during the pretest (prior to Day 1) period.
Procedure:	Animals will not be removed from cage during observation, unless necessary for identification or confirmation of possible findings.

Appendix 1

15.2.2. Detailed Clinical Observations

Frequency: Day of randomization and Days 2, 7, and 10

Procedure: Animals removed from the cage for examination.

15.3. Body Weights

Frequency: Day of randomization and Days 1, 4, 7, and 10

Procedure: Animals will be individually weighed. A fasted weight will be recorded on the day of necropsy. Terminal body weights will not be collected from animals found dead or euthanized moribund.

16. LABORATORY EVALUATIONS-MULTIPLE-DOSE STUDY

16.1. Clinical Pathology

16.1.1. Sample Collection

Blood will be collected from the vena cava (under isoflurane anesthesia at gross necropsy). Blood for unscheduled euthanasia animals may be collected under isoflurane anesthesia from the jugular vein or orbital plexus. Additional blood samples may be obtained (e.g., due to clotting of non-serum samples) if permissible sampling frequency and blood volume are not exceeded. After collection, samples will be transferred to the appropriate laboratory for processing.

Multiple-dose study animals will be fasted overnight before scheduled clinical pathology sample collections (fasting of the animals is not required for hematology determinations), but will have access to water ad libitum. Samples will be collected according to the following table:

Samples for Clinical Pathology Evaluation

Group Nos.	Time Point	Hematology	Coagulation	Clinical Chemistry
1	Day 11	X	X	X
Unscheduled euthanasia (when possible)	Before euthanasia	X	X	X

X = sample to be collected; - = not applicable.

Any residual/retained clinical pathology samples will be discarded before issue of the Final Report.

16.1.2. Hematology

Target Volume: 0.5 mL

Anticoagulant: K_2EDTA

Appendix 1

Hematology Parameters

Red blood cell count	White blood cell count
Hemoglobin concentration	Neutrophil count (absolute)
Hematocrit	Lymphocyte count (absolute)
Mean corpuscular volume	Monocyte count (absolute)
Red Blood Cell Distribution Width	Eosinophil count (absolute)
Mean corpuscular hemoglobin concentration	Basophil count (absolute)
Mean corpuscular hemoglobin	Large unstained cells
Reticulocyte count (absolute)	Other cells (as appropriate)
Platelet count	

One blood smear will be prepared from each hematology sample. The slide will be labeled, stained, and archived. Slide review will only be performed on samples that meet flagging criteria in order to confirm accurate hematology analyzer results. If additional examination of blood smears is deemed necessary, the smears may be subsequently evaluated at additional cost by protocol amendment.

16.1.3. Coagulation

Target Volume: 1.8 mL

Anticoagulant: Sodium citrate

Processing: To plasma

Coagulation Parameters

Activated partial thromboplastin time	Prothrombin time
Fibrinogen	

16.1.4. Clinical Chemistry

Target Volume: 2 mL

Anticoagulant: None

Processing: To serum

Clinical Chemistry Parameters

Alanine aminotransferase	Total protein
Aspartate aminotransferase	Albumin
Alkaline phosphatase	Globulin
Gamma-glutamyltransferase	Albumin/globulin ratio
Creatine Kinase	Glucose
Total bilirubin[a]	Cholesterol
Urea nitrogen	Triglycerides
Creatinine	Sodium
Calcium	Potassium
Phosphorus	Chloride

[a] When total bilirubin is > 0.5 mg/dL, direct bilirubin will be measured and indirect bilirubin will be calculated.

Appendix 1

17. TERMINAL PROCEDURES-MULTIPLE-DOSE STUDY

Terminal procedures are summarized in the following table:

Terminal Procedures for Multiple-dose Study Animals

Group No.	Number of Animals		Scheduled Euthanasia Day	Necropsy Procedures			Histology	Histopathology
	M	F		Necropsy	Tissue Collection	Organ Weights		
1	5	5	11	X	X	-	-	-
Unscheduled Deaths				X	X	-	-	-
Replaced animals (prestudy)				X	-	-	-	-
Replaced animals (after dosing start)				X	X	-	-	-

X = procedure to be conducted; - = not applicable.
[a] See Tissue Collection and Preservation table for listing of tissues.

17.1. Unscheduled Deaths

If a multiple-dose study animal dies on study, a necropsy will be conducted and specified tissues will be saved. If necessary, the animal will be refrigerated to minimize autolysis.

Multiple-dose study animals may be euthanized for humane reasons as per Testing Facility SOPs. Samples for evaluation of clinical pathology parameters will be obtained if possible as specified in Section 16 (priority is hematology > clinical chemistry > coagulation). These animals will undergo necropsy, and specified tissues will be retained. If necessary, the animal will be refrigerated to minimize autolysis.

17.2. Scheduled Euthanasia

Multiple-dose study animals surviving until scheduled euthanasia will have a terminal body weight recorded; samples collected for evaluation of clinical pathology parameters as specified in Section 16; and the animals will be euthanized by isoflurane inhalation, followed by exsanguination. Animals will be fasted (overnight) before their scheduled necropsy.

17.3. Necropsy

Multiple-dose study animals will be subjected to a complete necropsy examination, which will include evaluation of the carcass and musculoskeletal system; all external surfaces and orifices; cranial cavity and external surfaces of the brain; and thoracic, abdominal, and pelvic cavities with their associated organs and tissues.

Necropsy procedures will be performed by qualified personnel with appropriate training and experience in animal anatomy and gross pathology.

Images may be generated for illustration of or consultation on gross observations. Generation of such images will be documented. Images and associated documentation will be retained and archived.

Appendix 1

17.4. Tissue Collection and Preservation

Representative samples of the tissues identified in the Tissue Collection and Preservation table in Attachment A will be collected from all animals and preserved in 10% neutral buffered formalin, unless otherwise indicated. Additional tissue samples may be collected to elucidate abnormal findings.

18. COMPUTERIZED SYSTEMS

The following critical computerized systems may be used in the study. The actual critical computerized systems used will be specified in the Final Report.

Data for parameters not required by protocol, which are automatically generated by analytical devices used will be retained on file but not reported. Statistical analysis results that are generated by the program but are not required by protocol and/or are not scientifically relevant will be retained on file but will not be included in the tabulations.

Critical Computerized Systems

System Name	Description of Data Collected and/or Analyzed
Compaq Alpha DS10 Computer using the Toxicology Analysis System Customized, General Toxicology Module or Provantis	In-life; clinical pathology; postmortem
Bayer Advia 120® Automated Hematology Analyzer	hematology analysis
Olympus AU640e	clinical chemistry analysis
Stago STA Compact Analyzer	coagulation analysis
Instem Life Science Systems, DISPENSE	Test material receipt, accountability and/or formulation activities
Systems 600 Apogee Insight System	temperature and/or humidity (animal rooms, refrigerators, freezers, and compound storage)

19. CONSTRUCTED VARIABLES

Body Weight Changes: calculated between at least each scheduled interval

20. STATISTICAL ANALYSIS

Data will be presented as individual values by animal. The individual data tables will also include the calculated means and standard deviations for each group.

21. AMENDMENTS AND DEVIATIONS

Changes to the approved protocol shall be made in the form of an amendment, which will be signed and dated by the Study Director. Every reasonable effort will be made to discuss any necessary protocol changes in advance with the Sponsor.

Appendix 1

All protocol and SOP deviations will be documented in the study records. Deviations from the protocol and/or SOP related to the phase(s) of the study conducted at a Test Site shall be documented, acknowledged by the PI/IS, and reported to the Study Director for authorization/acknowledgement. The Study Director will notify the Sponsor of deviations that may result in a significant impact on the study as soon as possible.

22. RETENTION OF RECORDS, SAMPLES, AND SPECIMENS

All study-specific raw data, electronic data, documentation, protocol, retained samples and specimens, and interim (if applicable) and final reports will be archived by no later than the date of final report issue. All materials generated by Charles River from this study will be transferred to a Charles River archive. One year after issue of the draft report, the Sponsor will be contacted to determine the disposition of materials associated with the study.

23. REPORTING

A comprehensive Draft Report will be prepared following completion of the study and will be finalized following consultation with the Sponsor. The report will include all information necessary to provide a complete and accurate description of the experimental methods and results and any circumstances that may have affected the quality or integrity of the study.

The Sponsor will receive an electronic version of the Draft and Final Report provided in Adobe Acrobat PDF format (hyperlinked and searchable) along with a Microsoft Word version of the text. The PDF document will be created from native electronic files to the extent possible, including text and tables generated by the Testing Facility. Report components not available in native electronic files and/or original signature pages will be scanned and converted to PDF image files for incorporation. An original copy of the report with the Testing Facility's handwritten signatures will be retained.

Reports should be finalized within 6 months of issue of the Draft Report. If the Sponsor has not provided comments to the report within 6 months of draft issue, the report will be finalized by the Testing Facility unless other arrangements are made by the Sponsor.

24. ANIMAL WELFARE

This study will comply with all applicable sections of the Final Rules of the Animal Welfare Act regulations (Code of Federal Regulations, Title 9), the *Public Health Service Policy on Humane Care and Use of Laboratory Animals* from the Office of Laboratory Animal Welfare, and the *Guide for the Care and Use of Laboratory Animals* from the National Research Council.[1,2] The protocol and any amendments or procedures involving the care or use of animals in this study will be reviewed and approved by the Testing Facility Institutional Animal Care and Use Committee before the initiation of such procedures.

If an animal is determined to be in overt pain/distress, or appears moribund and is beyond the point where recovery appears reasonable, the animal will be euthanized for humane reasons in

Appendix 1

accordance with the *American Veterinary Medical Association (AVMA) Guidelines on Euthanasia* and with the procedures outlined in the protocol.[3]

By approving this protocol, the Sponsor affirms that there are no acceptable non-animal alternatives for this study, that this study is required by a relevant government regulatory agency(ies) and that it does not unnecessarily duplicate any previous experiments.

Appendix 1

25. REFERENCES

1. Office of Laboratory Animal Welfare. *Public Health Services Policy on Humane Care and Use of Laboratory Animals*. Bethesda, MD: National Institutes of Health. August 2002.

2. National Research Council. *Guide for the Care and Use of Laboratory Animals*. 8th edition. Washington, DC: National Academy Press. 2011.

3. American Veterinary Medical Association. *AVMA Guidelines on Euthanasia*. February 2013.

Appendix 1

26. AMENDMENT APPROVAL

Date: 09NOV 2015

Alene T. McCoy, PhD
Study Director

Appendix 1

27. ATTACHMENT A

Tissue Collection and Preservation

Tissue	Weigh	Collect	Microscopic Evaluation	Comment
Animal identification	-	X	-	-
Artery, aorta	-	X	-	-
Body cavity, nasal	-	X	-	-
Bone marrow smear	-	X	-	Bone marrow smears will be collected from the femur at scheduled and unscheduled necropsies (for possible examination). Smears will not be collected from animals that are found dead or from animals that were euthanized moribund and then stored in the refrigerator prior to necropsy. Bone marrow smears are allowed to air dry and are not fixed in formalin.
Bone marrow	-	X	-	-
Bone, femur	-	X	-	-
Bone, sternum	-	X	-	-
Brain	-	X	-	-
Cervix	-	X	-	-
Epididymis	-	X	-	-
Esophagus	-	X	-	-
Eye	-	X	-	Preserve in Davidson's fixative.
Gland, adrenal	-	X	-	-
Gland, harderian	-	X	-	-
Gland, mammary	-	X	-	-
Gland, parathyroid	-	X	-	-
Gland, pituitary	-	X	-	-
Gland, prostate	-	X	-	-
Gland, salivary	-	X	-	-
Gland, seminal vesicle	-	X	-	-
Gland, thyroid	-	X	-	-
Gross lesions/masses	-	X	-	-
Gut-associated lymphoid tissue	-	X	-	-
Heart	-	X	-	-
Kidney	-	X	-	-
Large intestine, cecum	-	X	-	-
Large intestine, colon	-	X	-	-
Large intestine, rectum	-	X	-	-
Larynx	-	X	-	-
Liver	-	X	-	-
Lung	-	X	-	-
Lymph node, mandibular	-	X	-	-
Lymph node, mesenteric	-	X	-	-
Muscle, skeletal	-	X	-	-
Nerve, optic	-	X	-	Preserve in Davidson's fixative.
Nerve, sciatic	-	X	-	-

Appendix 1

Tissue	Weigh	Collect	Microscopic Evaluation	Comment
Ovary	-	X	-	-
Pancreas	-	X	-	-
Skin	-	X	-	-
Small intestine, duodenum	-	X	-	-
Small intestine, ileum	-	X	-	-
Small intestine, jejunum	-	X	-	-
Spinal cord	-	X	-	-
Spleen	-	X	-	-
Stomach	-	X	-	-
Testis	-	X	-	Preserve in Modified Davidson's fixative.
Thymus	-	X	-	-
Tongue	-	X	-	-
Trachea	-	X	-	-
Urinary bladder	-	X	-	-
Uterus	-	X	-	-
Vagina	-	X	-	-

X = Procedure to be conducted; - = Not applicable.

Appendix 1

DEVIATIONS

All deviations that occurred during the study have been authorized/acknowledged by the Study Director, assessed for impact, and documented in the study records. All protocol deviations and those SOP deviations that could have impacted the quality or integrity of the study are listed below. Minor SOP deviations that did not impact the quality or integrity of the study have been included at the discretion of the Study Director.

None of the deviations were considered to have impacted the overall integrity of the study or the interpretation of the study results and conclusions.

In-life Observations, Measurements, and Evaluations

- During the Rising-dose Study, there was no documentation of a date that the animals were randomized or that metal ear tags were applied. This deviation had no impact on the study because it was considered a documentation error.
- On Day -5 of the Rising-dose Study, an A.M. mortality check was not performed. All animals were normal at the P.M. mortality check. This deviation had no impact on the study since all animals were normal at the P.M. mortality check.
- On Days 1 and 8 of the Multiple-dose Study, detailed observations were performed when not required by protocol. This deviation had no study impact as this was considered additional information.
- On Day 8 of the Multiple-dose Study, Animal Nos. 9567 and 9568 were missing eartags. Due to the animals being socially housed, technicians were unable to identify the animals. Animals were identified according to previous body weight comparisons. When the animals were given replacement ear tags, the incorrect form was used for documentation and, therefore, there was no documentation of which animal received what ear tag. The possibility that Animal Nos. 9567 and 9568 received the wrong replacement ear tags was mitigated by the comparison of each to its prior body weight. Additionally, both animals were in the same dose group.

Postmortem and Pathology

- Group 1 animals in the Rising-dose Study have no documentation of having been euthanized via carbon dioxide inhalation. This deviation had no impact on the study because it was a minor documentation error.

Appendix 2

Materials Safety Data Sheet

Section 1 - Product Identification
Trade Name

Ox-66

Section 2 - Hazardous Ingredients
The material has no hazardous ingredients.

Section 3 -Physical Data
A non-flammable -water soluble slightly base white to light blue/white powder

Non-vapor producing. May produce a very light dust they may dry the skin and nasal passages. Application of water remedies the condition and no side effects are suggested

Appearance: white to slightly blue/white powder with mass but very little weight. One gallon weighs less than 4.5 ounces.

Section 4 - Fire and Explosion Hazard Data
Material is non-flammable and is stable in temperatures over 3000 degrees F.

Section 5 Health Hazard Data
Material is benign for all handling and use.

Used as directed the material indicates no actual risk to skin or clothing. The material will react vigorously when brought into contact with acids or other low PH materials.

Ingestion: No adverse effects seen.

Eye contact: Flush eyes with running water if needed. If any irritation persists, obtain additional medical attention.

Section 6 - Spill or Leak Procedure
Spills can be handled routinely. The material is water soluble and can be dispersed by using fresh water. The resulting washed material is not damaging to the environment and will readily disperse.

Section 7 - Special Protection Information
The material requires no special care in handling. Standard eye protection is satisfactory. If handling in volume respiratory protection is suggested for dust

Appendix 2

exposure but not necessary since no fumes or gases are present only the risk from drying of membranes from exposure to the dust.

Section 8 - Regulatory Information
D.O.T. proper shipping and labeling name is: “aluminum hydroxyl” or "aluminum hydroxy-polyhydrate" and requires no special permits to ship and/or handle. This material has not been listed as a cancer suspect agent.

Important
All information above has been obtained from sources believed to be reliable. It is presented without guarantees or obligations for the accuracy or sufficiency thereof for the user's consideration and verification. Information herein is for the product stated and may not be valid when the product is combined with other materials.

Appendix 2

FYI DATA:

AL12/H42/O36 (determined by atomic wt. and valence calculations)

The wet chemical analysis is as follows:

%AL	26.81%
%Cl	0.53%
%No3	0.01%
%C	0.40%
%H2*O*-(calculated)	5.40%
%O*2*	66.20%

Free oxygen determinations indicate availability of O2 as high as 25% in aqueous solutions. The material is not crystalline but is instead a true clatherate (lattice like) structure with very large areas within the structure to capture and hold oxygen and other gases. Hyper oxygenation of the material seems likely but has not been fully studied. Tests with Nitrous oxide and other nitrogen gases show significant absorption potentials. The material seems to blend with numerous non-acidic materials without losing any efficacy.

Appendix 3

Individual Mortality Explanation Page

Abbreviation	Description	Abbreviation	Description
AM SIRT	Mortality/moribundity check in the morning	PM SIRT	Mortality/moribundity check in the afternoon
DE	Detailed examination	CSO	Cage side observation
PreRx	Observation predosing	Post Rx	Observation post dosing
TE	Terminal Euthanasia	TERM	Terminal Euthanasia
UE	Unscheduled Euthanasia	UNSC	Unscheduled Euthanasia
FD	Found Dead	REC	Recovery Euthanasia
INTM	Interim Euthanasia		

Note: This is a comprehensive list of abbreviations. All of the abbreviations listed may not be applicable to this report.

Appendix 3

Individual Mortality: Rising-dose Study

20086532

Group	Dose Level	Sex	Animal	Cage	Removal Day	Removal Week	Removal Date	Removal Time	Time Slot	Removal Symptom	Pathology Reason
1	250 mg/kg/day	Male	9564	1	15	3	09NOV2015	8:42	.	TE	.
			9565	1	15	3	09NOV2015	8:42	.	TE	.
			9566	1	15	3	09NOV2015	8:42	.	TE	.
1	250 mg/kg/day	Female	9567	2	15	3	09NOV2015	8:42	.	TE	.
			9568	2	15	3	09NOV2015	8:42	.	TE	.
			9569	2	15	3	09NOV2015	8:42	.	TE	.
2	500 mg/kg/day	Male	9570	3	15	3	11NOV2015	8:18	.	TE	.
			9571	3	15	3	11NOV2015	8:18	.	TE	.
			9572	3	15	3	11NOV2015	8:19	.	TE	.
2	500 mg/kg/day	Female	9573	4	15	3	11NOV2015	8:19	.	TE	.
			9574	4	15	3	11NOV2015	8:19	.	TE	.
			9575	4	15	3	11NOV2015	8:19	.	TE	.
3	750 mg/kg/day	Male	9576	5	15	3	13NOV2015	7:57	.	TE	.
			9577	5	15	3	13NOV2015	7:57	.	TE	.
			9578	5	15	3	13NOV2015	7:57	.	TE	.
3	750 mg/kg/day	Female	9579	6	15	3	13NOV2015	7:57	.	TE	.
			9580	6	15	3	13NOV2015	7:57	.	TE	.
			9581	6	15	3	13NOV2015	7:57	.	TE	.
4	1000 mg/kg/day	Male	9582	7	15	3	16NOV2015	9:11	.	TE	.
			9583	7	15	3	16NOV2015	9:11	.	TE	.
			9584	7	15	3	16NOV2015	9:11	.	TE	.
4	1000 mg/kg/day	Female	9585	8	15	3	16NOV2015	9:11	.	TE	.
			9586	8	15	3	16NOV2015	9:11	.	TE	.
			9587	8	15	3	16NOV2015	9:11	.	TE	.

Appendix 4

Individual Clinical Observations: Rising-dose Study

20086532

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	15
1	m	9564	Terminal Euthanasia		X
		9565	Terminal Euthanasia		X
		9566	Terminal Euthanasia		X

Severity Codes: X = Present

Group 1 - 250 mg/kg/day Group 2 - 500 mg/kg/day Group 3 - 750 mg/kg/day Group 4 - 1000 mg/kg/day

Appendix 4

Individual Clinical Observations: Rising-dose Study

20086532

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	15
2	m	9570	Terminal Euthanasia		X
		9571	Terminal Euthanasia		X
		9572	Terminal Euthanasia		X

Severity Codes: X = Present

Group 1 - 250 mg/kg/day Group 2 - 500 mg/kg/day Group 3 - 750 mg/kg/day Group 4 - 1000 mg/kg/day

Appendix 4

Individual Clinical Observations: Rising-dose Study

20086532

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	15
3	m	9576	Terminal Euthanasia		X
		9577	Terminal Euthanasia		X
		9578	Terminal Euthanasia		X

Severity Codes: X = Present

Group 1 - 250 mg/kg/day Group 2 - 500 mg/kg/day Group 3 - 750 mg/kg/day Group 4 - 1000 mg/kg/day

Appendix 4

Individual Clinical Observations: Rising-dose Study

20086532

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	15
4	m	9582	Terminal Euthanasia		X
		9583	Terminal Euthanasia		X
		9584	Terminal Euthanasia		X

Severity Codes: X = Present

Group 1 - 250 mg/kg/day Group 2 - 500 mg/kg/day Group 3 - 750 mg/kg/day Group 4 - 1000 mg/kg/day

Appendix 4

Individual Clinical Observations: Rising-dose Study

20086532

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	15
1	f	9567	Terminal Euthanasia		X
		9568	Terminal Euthanasia		X
		9569	Terminal Euthanasia		X

Severity Codes: X = Present

Group 1 - 250 mg/kg/day Group 2 - 500 mg/kg/day Group 3 - 750 mg/kg/day Group 4 - 1000 mg/kg/day

Appendix 4

Individual Clinical Observations: Rising-dose Study

20086532

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	15
2	f	9573	Terminal Euthanasia		X
		9574	Terminal Euthanasia		X
		9575	Terminal Euthanasia		X

Severity Codes: X = Present

Group 1 - 250 mg/kg/day Group 2 - 500 mg/kg/day Group 3 - 750 mg/kg/day Group 4 - 1000 mg/kg/day

Appendix 4

Individual Clinical Observations: Rising-dose Study

20086532

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	15
3	f	9579	Terminal Euthanasia		X
		9580	Terminal Euthanasia		X
		9581	Terminal Euthanasia		X

Severity Codes: X = Present

Group 1 - 250 mg/kg/day Group 2 - 500 mg/kg/day Group 3 - 750 mg/kg/day Group 4 - 1000 mg/kg/day

Appendix 4

Individual Clinical Observations: Rising-dose Study

20086532

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	15
4	f	9585	Terminal Euthanasia		X
		9586	Terminal Euthanasia		X
		9587	Terminal Euthanasia		X

Severity Codes: X = Present

Group 1 - 250 mg/kg/day Group 2 - 500 mg/kg/day Group 3 - 750 mg/kg/day Group 4 - 1000 mg/kg/day

Appendix 5

Individual Body Weights (g): Rising-dose Study

Group 1 - OX-66 250 mg/kg/day
Group 2 - OX-66 500 mg/kg/day
Group 3 - OX-66 750 mg/kg/day
Group 4 - OX-66 1000 mg/kg/day

Group / Sex	Animal No.	Day -10	-9	-7	-5	-3	1	8	15
1M	9564	--	--	--	185.0	205.0	224.0	278.0	314.0
	9565	--	--	--	202.0	220.0	250.0	313.0	366.0
	9566	--	--	--	190.0	215.0	243.0	309.0	359.0
1M	Mean	--	--	--	192.3	213.3	239.0	300.0	346.3
	SD	--	--	--	8.7	7.6	13.5	19.2	28.2
2M	9570	--	--	192.0	221.0	--	270.0	330.0	380.0
	9571	--	--	184.0	201.0	--	234.0	277.0	312.0
	9572	--	--	187.0	211.0	--	253.0	312.0	333.0
2M	Mean	--	--	187.7	211.0	--	252.3	306.3	341.7
	SD	--	--	4.0	10.0	--	18.0	27.0	34.8
3M	9576	--	184.0	203.0	--	--	259.0	305.0	345.0
	9577	--	199.0	221.0	--	--	288.0	347.0	404.0
	9578	--	194.0	216.0	--	--	270.0	321.0	373.0
3M	Mean	--	192.3	213.3	--	--	272.3	324.3	374.0
	SD	--	7.6	9.3	--	--	14.6	21.2	29.5

Appendix 5

Individual Body Weights (g): Rising-dose Study

Group 1 - OX-66 250 mg/kg/day
Group 2 - OX-66 500 mg/kg/day
Group 3 - OX-66 750 mg/kg/day
Group 4 - OX-66 1000 mg/kg/day

Group / Sex	Animal No.	Day -10	-9	-7	-5	-3	1	8	15
4M	9582	218.0	--	--	--	--	293.0	332.0	364.0
	9583	220.0	--	--	--	--	317.0	375.0	418.0
	9584	209.0	--	--	--	--	290.0	337.0	376.0
4M	Mean	215.7	--	--	--	--	300.0	348.0	386.0
	SD	5.9	--	--	--	--	14.8	23.5	28.4

Appendix 5

Individual Body Weights (g): Rising-dose Study

Group 1 - OX-66 250 mg/kg/day
Group 2 - OX-66 500 mg/kg/day
Group 3 - OX-66 750 mg/kg/day
Group 4 - OX-66 1000 mg/kg/day

Group / Sex	Animal No.	Day -10	-9	-7	-5	-3	1	8	15
1F	9567	--	--	--	173.0	189.0	197.0	212.0	219.0
	9568	--	--	--	165.0	180.0	187.0	207.0	220.0
	9569	--	--	--	166.0	173.0	188.0	206.0	218.0
1F	Mean	--	--	--	168.0	180.7	190.7	208.3	219.0
	SD	--	--	--	4.4	8.0	5.5	3.2	1.0
2F	9573	--	--	166.0	171.0	--	191.0	204.0	195.0
	9574	--	--	170.0	179.0	--	192.0	219.0	228.0
	9575	--	--	174.0	189.0	--	206.0	220.0	233.0
2F	Mean	--	--	170.0	179.7	--	196.3	214.3	218.7
	SD	--	--	4.0	9.0	--	8.4	9.0	20.6
3F	9579	--	166.0	176.0	--	--	191.0	201.0	214.0
	9580	--	174.0	184.0	--	--	208.0	224.0	229.0
	9581	--	171.0	180.0	--	--	192.0	211.0	228.0
3F	Mean	--	170.3	180.0	--	--	197.0	212.0	223.7
	SD	--	4.0	4.0	--	--	9.5	11.5	8.4

Appendix 5

Individual Body Weights (g): Rising-dose Study

Group 1 - OX-66 250 mg/kg/day
Group 2 - OX-66 500 mg/kg/day
Group 3 - OX-66 750 mg/kg/day
Group 4 - OX-66 1000 mg/kg/day

Group / Sex	Animal No.	Day -10	-9	-7	-5	-3	1	8	15
4F	9585	178.0	--	--	--	--	201.0	202.0	221.0
	9586	174.0	--	--	--	--	198.0	208.0	217.0
	9587	185.0	--	--	--	--	211.0	230.0	243.0
4F	Mean	179.0	--	--	--	--	203.3	213.3	227.0
	SD	5.6	--	--	--	--	6.8	14.7	14.0

Appendix 6

Individual Body Weight Gains (g): Rising-dose Study

Group 1 - OX-66 250 mg/kg/day
Group 2 - OX-66 500 mg/kg/day
Group 3 - OX-66 750 mg/kg/day
Group 4 - OX-66 1000 mg/kg/day

Group / Sex	Animal No.	Day Change 1 - 8	Day Change 8 - 15	Change 1 - 15
1M	9564	54.0	36.0	90.0
	9565	63.0	53.0	116.0
	9566	66.0	50.0	116.0
1M	Mean	61.0	46.3	107.3
	SD	6.2	9.1	15.0
2M	9570	60.0	50.0	110.0
	9571	43.0	35.0	78.0
	9572	59.0	21.0	80.0
2M	Mean	54.0	35.3	89.3
	SD	9.5	14.5	17.9
3M	9576	46.0	40.0	86.0
	9577	59.0	57.0	116.0
	9578	51.0	52.0	103.0
3M	Mean	52.0	49.7	101.7
	SD	6.6	8.7	15.0

Appendix 6

Individual Body Weight Gains (g): Rising-dose Study

Group 1 - OX-66 250 mg/kg/day
Group 2 - OX-66 500 mg/kg/day
Group 3 - OX-66 750 mg/kg/day
Group 4 - OX-66 1000 mg/kg/day

Group / Sex	Animal No.	Day Change 1 - 8	Day Change 8 - 15	Day Change 1 - 15
4M	9582	39.0	32.0	71.0
	9583	58.0	43.0	101.0
	9584	47.0	39.0	86.0
4M	Mean	48.0	38.0	86.0
	SD	9.5	5.6	15.0

Appendix 6

Individual Body Weight Gains (g): Rising-dose Study

Group 1 - OX-66 250 mg/kg/day
Group 2 - OX-66 500 mg/kg/day
Group 3 - OX-66 750 mg/kg/day
Group 4 - OX-66 1000 mg/kg/day

Group / Sex	Animal No.	Day Change 1 - 8	Day Change 8 - 15	Change 1 - 15
1F	9567	15.0	7.0	22.0
	9568	20.0	13.0	33.0
	9569	18.0	12.0	30.0
1F	Mean	17.7	10.7	28.3
	SD	2.5	3.2	5.7
2F	9573	13.0	-9.0	4.0
	9574	27.0	9.0	36.0
	9575	14.0	13.0	27.0
2F	Mean	18.0	4.3	22.3
	SD	7.8	11.7	16.5
3F	9579	10.0	13.0	23.0
	9580	16.0	5.0	21.0
	9581	19.0	17.0	36.0
3F	Mean	15.0	11.7	26.7
	SD	4.6	6.1	8.1

Appendix 6

Individual Body Weight Gains (g): Rising-dose Study

Group 1 - OX-66 250 mg/kg/day
Group 2 - OX-66 500 mg/kg/day
Group 3 - OX-66 750 mg/kg/day
Group 4 - OX-66 1000 mg/kg/day

Group / Sex	Animal No.	Day Change 1 - 8	Day Change 8 - 15	Day Change 1 - 15
4F	9585	1.0	19.0	20.0
	9586	10.0	9.0	19.0
	9587	19.0	13.0	32.0
4F	Mean	10.0	13.7	23.7
	SD	9.0	5.0	7.2

Appendix 7

Individual Mortality Explanation Page

Abbreviation	Description	Abbreviation	Description
AM SIRT	Mortality/moribundity check in the morning	PM SIRT	Mortality/moribundity check in the afternoon
DE	Detailed examination	CSO	Cage side observation
PreRx	Observation predosing	Post Rx	Observation post dosing
TE	Terminal Euthanasia	TERM	Terminal Euthanasia
UE	Unscheduled Euthanasia	UNSC	Unscheduled Euthanasia
FD	Found Dead	REC	Recovery Euthanasia
INTM	Interim Euthanasia		

Note: This is a comprehensive list of abbreviations. All of the abbreviations listed may not be applicable to this report.

Appendix 7

Individual Mortality: Multiple-dose Study

20086532

Group	Dose Level	Sex	Animal	Cage	Removal Day	Removal Week	Removal Date	Removal Time	Time Slot	Removal Symptom	Pathology Reason
1	1000 mg/kg/day	Male	9596	13	11	2	20NOV2015	12:23	.	TE	TERM
			9597	13	11	2	20NOV2015	12:23	.	TE	TERM
			9598	14	11	2	20NOV2015	12:24	.	TE	TERM
			9599	14	11	2	20NOV2015	12:24	.	TE	TERM
			9600	14	11	2	20NOV2015	12:24	.	TE	TERM
1	1000 mg/kg/day	Female	9601	15	11	2	20NOV2015	12:24	.	TE	TERM
			9602	15	11	2	20NOV2015	12:24	.	TE	TERM
			9603	16	11	2	20NOV2015	12:24	.	TE	TERM
			9604	16	11	2	20NOV2015	12:24	.	TE	TERM
			9605	16	11	2	20NOV2015	12:24	.	TE	TERM

Appendix 8

Individual Clinical Observations: Multiple-dose Study

20086532

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	11
1	m	9596	Terminal Euthanasia		X
		9597	Terminal Euthanasia		X
		9598	Terminal Euthanasia		X
		9599	Terminal Euthanasia		X
		9600	Terminal Euthanasia		X

Severity Codes: X = Present

Group 1 - 1000 mg/kg/day

Appendix 8

Individual Clinical Observations: Multiple-dose Study

20086532

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	11
1	f	9601	Terminal Euthanasia		X
		9602	Terminal Euthanasia		X
		9603	Terminal Euthanasia		X
		9604	Terminal Euthanasia		X
		9605	Terminal Euthanasia		X

Severity Codes: X = Present

Group 1 - 1000 mg/kg/day

Appendix 9

Individual Body Weights (g): Multiple-dose Study

Group 1 - OX-66 1000 mg/kg/day

Group / Sex	Animal No.	Day -1	1	4	7	10
1M	9596	249.0	249.0	270.0	284.0	298.0
	9597	247.0	248.0	275.0	289.0	307.0
	9598	231.0	232.0	252.0	262.0	276.0
	9599	243.0	250.0	273.0	289.0	308.0
	9600	248.0	256.0	281.0	309.0	338.0
1M	Mean	243.6	247.0	270.2	286.6	305.4
	SD	7.4	8.9	10.9	16.8	22.3

Appendix 9

Individual Body Weights (g): Multiple-dose Study

Group 1 - OX-66 1000 mg/kg/day

Group / Sex	Animal No.	Day -1	Day 1	Day 4	Day 7	Day 10
1F	9601	189.0	185.0	205.0	218.0	230.0
	9602	180.0	180.0	195.0	208.0	216.0
	9603	193.0	191.0	208.0	210.0	220.0
	9604	182.0	182.0	195.0	202.0	212.0
	9605	177.0	185.0	192.0	208.0	223.0
1F	Mean	184.2	184.6	199.0	209.2	220.2
	SD	6.6	4.2	7.0	5.8	6.9

Appendix 10

Individual Body Weight Gains (g): Multiple-dose Study

Group 1 - OX-66 1000 mg/kg/day

Group / Sex	Animal No.	Day Change 1 - 4	Change 4 - 7	Change 7 - 10	Change 1 - 10
1M	9596	21.0	14.0	14.0	49.0
	9597	27.0	14.0	18.0	59.0
	9598	20.0	10.0	14.0	44.0
	9599	23.0	16.0	19.0	58.0
	9600	25.0	28.0	29.0	82.0
1M	Mean	23.2	16.4	18.8	58.4
	SD	2.9	6.8	6.1	14.6

Appendix 10

Individual Body Weight Gains (g): Multiple-dose Study

Group 1 - OX-66 1000 mg/kg/day

Group / Sex	Animal No.	Day Change 1 - 4	Change 4 - 7	Change 7 - 10	Change 1 - 10
1F	9601	20.0	13.0	12.0	45.0
	9602	15.0	13.0	8.0	36.0
	9603	17.0	2.0	10.0	29.0
	9604	13.0	7.0	10.0	30.0
	9605	7.0	16.0	15.0	38.0
1F	Mean	14.4	10.2	11.0	35.6
	SD	4.9	5.6	2.6	6.5

Appendix 11

Individual Hematology Values Explanation Page

Bayer Advia 120 Analyzer

Analyzed Parameter Descriptions

Parameter	Abbreviation	Units	Methodology
Erythrocytes Distribution Width	RDW	%	Calculated
Hematocrit	HCT	%	Calculated
Hemoglobin	HGB	g/dL	Colorimetric
Mean Corpuscular Hemoglobin	MCH	pg	Calculated
Mean Corpuscular Hemoglobin Concentration	MCHC	g/dL	Calculated
Mean Corpuscular Volume	MCV	fL	
Mean Platelet Volume	MPV	fL	
Platelet Count	PLT	$x10^3/\mu L$	Flow cytometry
Red Blood Cell Count	RBC	$x10^6/\mu L$	Flow cytometry
Reticulocytes	RETIC	$x10^9/L$	Flow cytometry
Reticulocytes Percent	RETIC	%	Flow cytometry
White Blood Cell Count	WBC	$x10^3/\mu L$	Flow cytometry
White Blood Cell Differential Count			
Neutrophils Percent	NEUT	%	Flow cytometry
Lymphocytes Percent	LYMPH	%	Flow cytometry
Monocytes Percent	MONO	%	Flow cytometry
Eosinophils Percent	EOS	%	Flow cytometry
Basophils Percent	BASO	%	Flow cytometry
Large Unstained Cells Percent	LUC	%	Flow cytometry
Neutrophils	NEUT	$x10^3/\mu L$	Flow cytometry
Lymphocytes	LYMPH	$x10^3/\mu L$	Flow cytometry
Monocytes	MONO	$x10^3/\mu L$	Flow cytometry
Eosinophils	EOS	$x10^3/\mu L$	Flow cytometry
Basophils	BASO	$x10^3/\mu L$	Flow cytometry
Large Unstained Cells	LUC	$x10^3/\mu L$	Flow cytometry

Appendix 11

Manual and Visual

Analyzed Parameter Descriptions

Parameter	Abbreviation	Units / Key to Results (Code)	Methodology
CELL MORPHOLOGY			
- Cytoplasmic Basophilia Neutrophil	CYTO BASO NEUT	1+ (Minimal) 2+ (Mild)	Microscopic Examination
- Polychromasia	POLY	3+ (Moderate)	
- Anisocytosis	ANISO	4+ (Marked)	
- Hypochromasia	HYPO		
- Reactive Lymphocytes	REACTIVE LYMPH		
- Megakaryocytes	MEGAK		
- Smudge Cells	SMUDGE CELL		
- Microcytes	MICROCYTES		
- Macrocytes	MACROCYTES		
- Poikilocytosis	POIK		
- Rouleaux Formation	ROULEAUX		
- Agglutination	AGGL		
- Acanthocytes	ACAN		
- Codocytes	TARGET CELL		
- Dacryocytes	DACR		
- Platelet Clumps	PLATELET CLUMPS		
- Eccentrocytes	ECCENTCY		
- Schistocytes	SCHZ		
- Spherocytes	SPHR		
- Stomatocytes	STOM		
- Howell Jolly Bodies	HJB		
- Basophilic Stippling	BASO STIP		
- Echinocytes	ECHINO		
- Vacuolated Neutrophils	VAC NEUT		
- Vacuolated Lymphocyted	VAC LYM		
- Döhle Bodies	DOHLE BODY		
- Degenerated Cells	DEG CELL		
- Ovalocytes	OVAL		
- Large Platelets Alpha	LARGE PLATELETS		
- Immature Neutrophils Morphology	IMM NEUT MORPH		
- Heinz Bodies	HEINZ BODY		
- Plasmodium	PLASMOD		
- Kurloff Cell	KURL		
- Burr Cells	BURR		
- Neutrophils Band Form Morphology	NEUT BAND MORPH		
- Nuclear Swelling	NUC SWELL NEUT		
- Red Blood Cell Morphology	RBC MORPH		
- White Blood Cell Morphology	WBC MORPH		
- Toxic Granulation	TOXG		
- Platelet Morphology	PLT MORPH		

Appendix 11

Manual and Visual

Analyzed Parameter Descriptions

Parameter	Abbreviation	Units	Methodology
Bone Marrow Stain		None	Manual, Wright-Giemsa stain
Bone Marrow Slide Fixation		None	Manual, Fixative

Other Abbreviations

Abbreviation	Description	Abbreviation	Description
ADQ	Adequate	AVS	Suspected aberrant value
CLOT	Sample clotted	COMM	Comment added
DEC	Decreased	INC	Increased
IRPS	Presence of abundant plasmodium species	L100	Less than 100 cells used to perform differential
MDIFF	Manual differential	NA	Not applicable
NAF	No abnormal findings	NC	Not calculable
NRBC	WBC corrected for presence of nucleated RBC	NSCH	Not scheduled to be performed
OA	Omitted activity	OOS	Sample analysed outside of established stability
QNS	Quantity not sufficient	SAMU	Large number of smudge cells
SNC	Sample not collected	SNR	Sample not received
TNP	Test not performed	UDPC	Results not confirmed by smear review
UNCR	Results are not reproducible	UNEX	Unscheduled data excluded from statistics
UPTD	Unable to perform due to technical difficulty	UTC	Unable to collect
UTD	Unable to determine	VARR	Assigned value above reportable range
VBRR	Assigned value below reportable range	X	Excluded from mean
RBCNUCLE	Nucleated RBCs	--	Not required for veterinary monitoring

Note: This is a comprehensive list of parameters and abbreviations. All of the parameters and abbreviations listed may not be applicable to this report.

Appendix 11

Individual Coagulation Values Explanation Page

STA-Compact Stago Analyzer

Analyzed Parameter Descriptions

Parameter	Abbreviation	Units	Methodology
Activated Partial Thromboplastin Time	APTT	sec	Mechanical Viscosity
Fibrinogen	FIB	mg/dL	Mechanical Viscosity
Prothrombin Time	PT	sec	Mechanical Viscosity

Plasma Appearance (Reported as SAMQ PLASMA)

Analyzed Parameter Descriptions

Parameter	Abbreviation	Degree is graded as	Methodology
Normal sample	N	Normal	Manual and visual
Hemolyzed sample	H	+ = slight (pale/light red) ++ = moderate (red) +++ = severe (dark red)	Manual and visual
Lipemic sample	L	+ = slight (cloudy) ++ = moderate (turbid) +++ = severe (lactescent)	Manual and visual
Icterus sample	I	+ = slight (dark yellow) ++ = moderate (very dark yellow) +++ = severe (dark yellow-green)	Manual and visual
Atypical sample	A	Color is identified	Manual and visual

Appendix 11

Other Abbreviations

Abbreviation	Description	Abbreviation	Description
APSP or ANSP	Agglutination confirm by saline preparation	AVS	Aberrant value suspected
CLOT	Sample clotted	COMM	Comment added
FD	Fibrin detected	NA	Not applicable
NC	Not calculable	NSCH	Not scheduled to be performed
OA	Omitted activity	OOS	Sample analysed outside of established stability
QNS	Quantity not sufficient	SNC	Sample not collected
SNR	Sample not received	SPNA or ANSP	Saline preparation negative for agglutination
TNP	Test not performed	UNCR	Results are not reproducible
UNEX	Unscheduled data excluded from statistics	UPTD	Unable to perform due to technical difficulty
USP	Unable to perform saline preparation for agglutination	UTC	Unable to collect
UTD	Unable to determine	VARR	Assigned value above reportable range
VBRR	Assigned value below reportable range	X	Excluded from mean
--	Not required for veterinary monitoring		

Note: This is a comprehensive list of parameters and abbreviations. All of the parameters and abbreviations listed may not be applicable to this report.

Appendix 11

Individual Hematology and Coagulation Values: Multiple-dose Study: Day 11

Group 1 - OX-66 1000 mg/kg/day

Group / Sex	Animal No.	WBC $10^3/\mu L$	NEUT $10^3/\mu L$	LYMPH $10^3/\mu L$	MONO $10^3/\mu L$	EOS $10^3/\mu L$	BASO $10^3/\mu L$	LUC $10^3/\mu L$
1M	9596	7.86	1.56	5.72	0.21	0.21	0.13	0.04
	9597	9.97	1.41	8.01	0.25	0.11	0.13	0.06
	9598	7.54	1.71	5.24	0.22	0.23	0.08	0.06
	9599	6.23	1.16	4.62	0.21	0.09	0.09	0.05
	9600	10.54	1.35	8.68	0.25	0.04	0.12	0.09
1M	Mean	8.43	1.44	6.45	0.23	0.14	0.11	0.06
	SD	1.79	0.21	1.79	0.02	0.08	0.02	0.02

Appendix 11

Individual Hematology and Coagulation Values: Multiple-dose Study: Day 11

Group 1 - OX-66 1000 mg/kg/day

Group / Sex	Animal No.	RBC $10^6/\mu L$	HGB g/dL	HCT %	MCV fL(μm^3)	MCH pg	MCHC g/dL	RDW %
1M	9596	7.08	13.2	40.9	57.8	18.7	32.4	13.4
	9597	6.79	12.4	39.9	58.8	18.3	31.1	13.5
	9598	6.58	12.2	37.5	57.0	18.5	32.4	13.4
	9599	6.10	11.2	36.3	59.6	18.4	30.9	12.9
	9600	5.89	12.0	37.9	64.4	20.4	31.6	14.8
1M	Mean	6.49	12.2	38.5	59.5	18.9	31.7	13.6
	SD	0.49	0.7	1.9	2.9	0.9	0.7	0.7

Appendix 11

Individual Hematology and Coagulation Values: Multiple-dose Study: Day 11

Group 1 - OX-66 1000 mg/kg/day

Group / Sex	Animal No.	PLT $10^3/\mu L$	RETIC $10^9/L$	PT sec	APTT sec	FIB mg/dL	SAMQ PLASMA
1M	9596	555	258.5	15.8	8.6	326	N
	9597	1132	393.0	18.9	13.5	306	N
	9598	1253	275.7	18.3	13.3	328	N
	9599	1066	315.6	18.6	13.0	238	N
	9600	1106	414.8	16.6	13.6	301	N
1M	Mean	1022	331.5	17.6	12.4	300	--
	SD	270	69.7	1.4	2.1	37	--

Appendix 11

Individual Hematology and Coagulation Values: Multiple-dose Study: Day 11

Group 1 - OX-66 1000 mg/kg/day

Group / Sex	Animal No.	WBC $10^3/\mu L$	NEUT $10^3/\mu L$	LYMPH $10^3/\mu L$	MONO $10^3/\mu L$	EOS $10^3/\mu L$	BASO $10^3/\mu L$	LUC $10^3/\mu L$
1F	9601	7.99	0.75	6.76	0.20	0.07	0.14	0.07
	9602	4.72	0.75	3.66	0.16	0.05	0.05	0.04
	9603	7.07	0.66	6.07	0.10	0.10	0.08	0.06
	9604	4.82	0.79	3.76	0.12	0.06	0.05	0.03
	9605	6.83	0.88	5.61	0.14	0.07	0.07	0.07
1F	Mean	6.29	0.77	5.17	0.14	0.07	0.08	0.05
	SD	1.45	0.08	1.40	0.04	0.02	0.04	0.02

Appendix 11

Individual Hematology and Coagulation Values: Multiple-dose Study: Day 11

Group 1 - OX-66 1000 mg/kg/day

Group / Sex	Animal No.	RBC $10^6/\mu L$	HGB g/dL	HCT %	MCV fL(μm^3)	MCH pg	MCHC g/dL	RDW %
1F	9601	6.41	12.9	39.8	62.0	20.1	32.3	10.9
	9602	6.39	12.6	38.2	59.8	19.7	32.9	11.2
	9603	6.41	12.5	38.5	60.0	19.4	32.4	10.9
	9604	6.44	12.3	38.0	58.9	19.1	32.5	11.4
	9605	6.40	12.1	37.0	57.8	18.9	32.7	11.1
1F	Mean	6.41	12.5	38.3	59.7	19.4	32.6	11.1
	SD	0.02	0.3	1.0	1.6	0.5	0.2	0.2

Appendix 11

Individual Hematology and Coagulation Values: Multiple-dose Study: Day 11

Group 1 - OX-66 1000 mg/kg/day

Group / Sex	Animal No.	PLT $10^3/\mu L$	RETIC $10^9/L$	PT sec	APTT sec	FIB mg/dL	SAMQ PLASMA
1F	9601	990	205.0	18.9	13.5	269	N
	9602	1172	294.1	17.3	13.4	278	N
	9603	1136	145.9	17.5	13.7	244	N
	9604	976	207.3	17.7	11.5	255	N
	9605	1013	201.2	17.6	8.1	287	N
1F	Mean	1057	210.7	17.8	12.0	267	--
	SD	90	53.1	0.6	2.4	17	--

Appendix 12

Individual Clinical Chemistry Values Explanation Page

Olympus AU640e

Analyzed Parameter Descriptions

Parameter	Abbreviation	Units	Methodology
Alanine Aminotransferase	ALT	U/L	Kinetic
Albumin	ALB	g/dL	Dye binding-BCG
Alkaline Phosphatase	ALP	U/L	Kinetic
Aspartate Aminotransferase	AST	U/L	Kinetic
Calcium	CA	mg/dL	Arsenazo III Dye
Cholesterol	CHOL	mg/dL	Endpoint-Cholesterol Esterase
Creatinine	CREAT	mg/dL	Kinetic-Alk. Picrate
Creatine Kinase	CK	U/L	Kinetic
Direct Bilirubin	DBIL	mg/dL	Diazonium Salt/Ion w/BL
GAMMA-Glutamyl Transferase	GGT	U/L	Kinetic
Glucose	GLUC	mg/dL	Hexokinase, UV
Phosphorus	PHOS	mg/dL	Endpoint
Sodium	NA	mmol/L	Ion Selectivity
Potassium	K	mmol/L	Ion Selectivity
Chloride	CL	mmol/L	Ion Selectivity
Total Bilirubin	TBIL	mg/dL	Diazonium Salt/Ion w/BL
Total Protein	TPROT	g/dL	Biuret
Triglycerides	TRIG	mg/dL	Enz Color without GB with SB
Urea Nitrogen	UREAN	mg/dL	Urease with GLDH

Calculations

Analyzed Parameter Descriptions

Parameter	Abbreviation	Units	Methodology
Albumin/Globulin ratio	A/G	None	Calculated
Globulin	GLOB	g/dL	Calculated
Indirect Bilirubin	IBIL	mg/dL	Calculated

Serum and Plasma Appearance (Reported as SAMQ SERUM)

Analyzed Parameter Descriptions

Parameter	Abbreviation	Degree is graded as	Methodology
Normal sample	N	Normal	Manual and visual
Hemolyzed sample	H	+ = slight (pale/light red) ++ = moderate (red) +++ = severe (dark red)	Manual and visual
Lipemic sample	L	+ = slight (cloudy) ++ = moderate (turbid) +++ = severe (lactescent)	Manual and visual
Icterus sample	I	+ = slight (dark yellow) ++ = moderate (very dark yellow) +++ = severe (dark yellow-green)	Manual and visual
Atypical sample	A	Color is identified	Manual and visual

Appendix 12

Other Abbreviations

Abbreviation	Description	Abbreviation	Description
AVS	Suspected aberrant value	COMM	Comment added
ISH	Result invalid due to severe hemolysis	ISL	Result invalid due to severe lipemia
LLOD/LLD	Less than lower limit of detection	LLOQ/LLQ	Less than lower limit of quantitation
NA	Not applicable	NC	Not calculable
NSCH	Not scheduled to be performed	OA	Omitted activity
OOS	Sample analysed outside of established stability	QNS	Quantity not sufficient
SNC	Sample not collected	SNR	Sample not received
TNP	Test not performed	TNR	Test not reported
UNCR	Results are not reproducible	UNEX	Unscheduled data excluded from statistics
UPTD	Unable to perform due to technical difficulty	UTC	Unable to collect
UTD	Unable to determine	VARR	Assigned value above reportable range
VBRR	Assigned value below reportable range	X	Excluded from mean
--	Not required for veterinary monitoring		

Note: This is a comprehensive list of parameters and abbreviations. All of the parameters and abbreviations listed may not be applicable to this report.

Appendix 12

Individual Clinical Chemistry Values: Multiple-dose Study: Day 11

Group 1 - OX-66 1000 mg/kg/day

Group / Sex	Animal No.	AST U/L	ALT U/L	ALP U/L	GGT U/L	CK U/L	TBIL mg/dL	UREAN mg/dL
1M	9596	66	39	217	0	205	0.11	12
	9597	65	48	374	0	216	0.16	13
	9598	69	53	308	0	184	0.11	15
	9599	57	45	283	0	120	0.11	15
	9600	62	42	434	0	113	0.12	15
1M	Mean	64	45	323	0	168	0.12	14
	SD	5	5	84	0	48	0.02	1

Appendix 12

Individual Clinical Chemistry Values: Multiple-dose Study: Day 11

Group 1 - OX-66 1000 mg/kg/day

Group / Sex	Animal No.	CREAT mg/dL	GLUC mg/dL	CHOL mg/dL	TRIG mg/dL	TPROT g/dL	ALB g/dL	GLOB g/dL
1M	9596	0.3	167	75	43	5.1	2.9	2.2
	9597	0.3	236	52	53	5.1	2.8	2.3
	9598	0.3	242	67	87	5.3	2.8	2.5
	9599	0.3	258	47	39	4.7	2.5	2.1
	9600	0.3	169	56	60	5.1	2.9	2.2
1M	Mean	0.3	214	59	56	5.1	2.8	2.3
	SD	0.0	43	11	19	0.2	0.2	0.2

Appendix 12

Individual Clinical Chemistry Values: Multiple-dose Study: Day 11

Group 1 - OX-66 1000 mg/kg/day

Group / Sex	Animal No.	A/G ratio	CA mg/dL	PHOS mg/dL	NA mEq/L	K mEq/L	CL mEq/L	SAMQ SERUM
1M	9596	1.3	9.6	9.0	141	3.9	102	N
	9597	1.2	10.2	8.5	137	4.4	99	N
	9598	1.2	9.8	9.8	140	4.4	100	N
	9599	1.2	9.9	9.5	140	4.5	104	N
	9600	1.3	10.7	8.4	144	4.4	105	N
1M	Mean	1.2	10.0	9.0	140	4.3	102	--
	SD	0.1	0.4	0.6	3	0.2	3	--

Appendix 12

Individual Clinical Chemistry Values: Multiple-dose Study: Day 11

Group 1 - OX-66 1000 mg/kg/day

Group / Sex	Animal No.	AST U/L	ALT U/L	ALP U/L	GGT U/L	CK U/L	TBIL mg/dL	UREAN mg/dL
1F	9601	62	30	156	0	196	0.10	13
	9602	62	36	212	0	105	0.11	12
	9603	50	32	157	0	100	0.08	15
	9604	58	35	195	0	82	0.11	17
	9605	74	37	153	0	217	0.11	10
1F	Mean	61	34	175	0	140	0.10	13
	SD	9	3	27	0	62	0.01	3

Appendix 12

Individual Clinical Chemistry Values: Multiple-dose Study: Day 11

Group 1 - OX-66 1000 mg/kg/day

Group / Sex	Animal No.	CREAT mg/dL	GLUC mg/dL	CHOL mg/dL	TRIG mg/dL	TPROT g/dL	ALB g/dL	GLOB g/dL
1F	9601	0.3	185	77	31	5.3	3.0	2.3
	9602	0.3	163	72	26	5.3	3.0	2.3
	9603	0.3	192	84	25	5.7	3.3	2.4
	9604	0.3	201	67	35	5.2	2.8	2.4
	9605	0.3	198	64	25	5.4	3.0	2.4
1F	Mean	0.3	188	73	28	5.4	3.0	2.4
	SD	0.0	15	8	4	0.2	0.2	0.1

Appendix 12

Individual Clinical Chemistry Values: Multiple-dose Study: Day 11

Group 1 - OX-66 1000 mg/kg/day

Group / Sex	Animal No.	A/G ratio	CA mg/dL	PHOS mg/dL	NA mEq/L	K mEq/L	CL mEq/L	SAMQ SERUM
1F	9601	1.4	10.4	8.9	141	4.1	105	N
	9602	1.3	9.7	7.6	141	4.0	105	N
	9603	1.4	10.4	8.8	141	3.8	105	N
	9604	1.2	10.3	8.3	142	3.9	106	N
	9605	1.3	10.2	9.1	140	4.1	105	N
1F	Mean	1.3	10.2	8.5	141	4.0	105	--
	SD	0.1	0.3	0.6	1	0.1	0	--

Appendix 13

SPECIES RAT — HISTORICAL CONTROL DATA — DATES:09-MAY-09 09-MAY-14
STRAIN CRL:CD(SD) — HEMATOLOGY DATA
SUPPLIER CHARLES RIVER

MALES 5 TO 12 WEEKS

		NO. OF TESTS	NO. OF ANIMALS	MEAN	RANGE OF ACTUAL VALUES		95 % 2.5%	SPREAD 97.5%
ERYTHROCYTES	10*6/CMM	731	689	7.491	4.780	9.550	6.240	8.600
HEMOGLOBIN	G/DL	731	689	14.52	9.20	17.80	12.70	16.10
HEMATOCRIT	%	731	689	43.66	28.10	53.50	38.50	48.40
MCH	PG	731	689	19.44	17.00	23.50	17.70	21.50
MCHC	G/DL	731	689	33.28	30.30	36.40	31.10	35.40
MCV	FL	731	689	58.48	51.20	71.60	52.70	66.10
RETICULOCYTES	10*9/L	731	689	302.01	88.20	1124.00	145.00	678.60
RETICULOCYTES	% RBC	15	15	3.00	2.19	4.22	2.19	4.22
PLATELETS	10*3/CMM	731	689	1172.4	496.0	1800.0	841.0	1548.0
ACTIVATED PTT	SCNDS	547	525	15.88	8.70	20.90	11.10	18.70
PROTHROMBIN TIME	SCNDS	513	505	16.71	13.60	20.50	15.10	19.00
FIBRINOGEN	MG/DL	203	203	310.8	242.0	489.0	258.0	395.0
LEUKOCYTES	10*3/CMM	731	689	9.26	1.42	19.95	4.89	15.39
LYMPHOCYTES	10*3/CMM	731	689	7.30	1.05	16.04	3.82	12.55

Appendix 13

SPECIES RAT — HISTORICAL CONTROL DATA — DATES:09-MAY-09 09-MAY-14
STRAIN CRL:CD(SD) — HEMATOLOGY DATA
SUPPLIER CHARLES RIVER

MALES 5 TO 12 WEEKS

		NO. OF TESTS	NO. OF ANIMALS	MEAN	RANGE OF ACTUAL VALUES		95 % 2.5%	SPREAD 97.5%
MONOCYTES	10*3/CMM	731	689	0.27	0.04	1.10	0.09	0.53
SEGD NEUTROPHILS	10*3/CMM	731	689	1.51	0.33	8.02	0.58	3.74
EOSINOPHILS	10*3/CMM	731	689	0.08	0.00	0.49	0.02	0.19
BASOPHILS	10*3/CMM	731	689	0.03	0.00	0.21	0.01	0.08
LG UNSTAIN CELL	10*3/CMM	730	688	0.075	0.000	0.370	0.010	0.210
SEGD NEUTROPHILS	% WBC	731	689	16.2	5.2	55.1	7.4	32.7
LYMPHOCYTES	% WBC	731	689	78.8	39.5	90.7	62.8	88.7
MONOCYTES	% WBC	731	689	2.9	0.9	7.3	1.4	5.2
BASOPHILS	% WBC	731	689	0.3	0.0	1.2	0.1	0.7
EOSINOPHILS	% WBC	731	689	0.9	0.0	3.9	0.3	2.0
LG UNSTAIN CELL	% WBC	730	688	0.793	0.000	5.100	0.200	2.000
MPV	FL	45	30	8.02	7.20	9.10	7.30	8.80
RED DIST WIDTH	%	469	442	12.83	10.90	16.40	11.40	14.80
HGB DIST WIDTH	G/DL	10	10	2.51	2.21	2.67	2.21	2.67
PLT DIST WIDTH	%	10	10	48.70	45.80	53.40	45.80	53.40

Appendix 13

SPECIES RAT — HISTORICAL CONTROL DATA — DATES:09-MAY-09 09-MAY-14
STRAIN CRL:CD(SD) — HEMATOLOGY DATA
SUPPLIER CHARLES RIVER

FEMALES 5 TO 12 WEEKS

		NO. OF TESTS	NO. OF ANIMALS	MEAN	RANGE OF ACTUAL VALUES		95 % 2.5%	SPREAD 97.5%
ERYTHROCYTES	10*6/CMM	666	626	7.353	3.430	9.270	6.160	8.340
HEMOGLOBIN	G/DL	666	626	14.20	6.50	17.40	12.00	15.80
HEMATOCRIT	%	666	626	41.60	17.60	51.70	35.20	46.90
MCH	PG	666	626	19.36	17.00	23.50	17.80	21.30
MCHC	G/DL	666	626	34.18	30.90	37.70	31.60	36.70
MCV	FL	666	626	56.71	49.50	73.00	51.60	65.50
RETICULOCYTES	10*9/L	666	626	225.20	69.60	1404.00	110.90	533.10
RETICULOCYTES	% RBC	15	15	2.86	1.75	3.73	1.75	3.73
PLATELETS	10*3/CMM	666	626	1223.8	457.0	1968.0	858.0	1606.0
ACTIVATED PTT	SCNDS	491	473	13.62	8.10	27.40	9.40	16.60
PROTHROMBIN TIME	SCNDS	457	453	16.43	13.00	30.80	14.60	18.30
FIBRINOGEN	MG/DL	168	168	255.2	109.0	529.0	203.0	314.0
LEUKOCYTES	10*3/CMM	666	626	7.65	2.58	22.07	3.85	13.92
LYMPHOCYTES	10*3/CMM	666	626	6.21	2.12	16.28	2.85	11.60
MONOCYTES	10*3/CMM	666	626	0.20	0.04	0.95	0.07	0.46

Appendix 13

SPECIES RAT — HISTORICAL CONTROL DATA — DATES:09-MAY-09 09-MAY-14
STRAIN CRL:CD(SD) — HEMATOLOGY DATA
SUPPLIER CHARLES RIVER

FEMALES 5 TO 12 WEEKS

		NO. OF TESTS	NO. OF ANIMALS	MEAN	RANGE OF ACTUAL VALUES		95 % 2.5%	SPREAD 97.5%
SEGD NEUTROPHILS	10*3/CMM	666	626	1.06	0.29	5.56	0.39	2.60
EOSINOPHILS	10*3/CMM	666	626	0.09	0.01	0.61	0.03	0.20
BASOPHILS	10*3/CMM	666	626	0.02	0.00	0.13	0.00	0.07
LG UNSTAIN CELL	10*3/CMM	666	626	0.060	0.000	0.530	0.010	0.180
SEGD NEUTROPHILS	% WBC	666	626	14.0	3.2	50.8	5.7	29.6
LYMPHOCYTES	% WBC	666	626	81.1	46.1	91.9	64.9	89.8
MONOCYTES	% WBC	666	626	2.7	0.9	8.9	1.3	5.7
BASOPHILS	% WBC	666	626	0.3	0.0	1.3	0.1	0.7
EOSINOPHILS	% WBC	666	626	1.3	0.1	8.0	0.5	2.8
LG UNSTAIN CELL	% WBC	666	626	0.761	0.100	7.500	0.200	1.800
MPV	FL	45	30	7.63	6.70	8.80	6.80	8.70
RED DIST WIDTH	%	455	429	11.77	10.20	14.30	10.70	13.70
HGB DIST WIDTH	G/DL	10	10	2.53	2.30	2.84	2.30	2.84
PLT DIST WIDTH	%	10	10	45.32	42.60	48.80	42.60	48.80

Appendix 13

SPECIES RAT — HISTORICAL CONTROL DATA — DATES:09-MAY-09 09-MAY-14
STRAIN CRL:CD(SD) — CLINICAL CHEMISTRY DATA
SUPPLIER CHARLES RIVER

MALES 5 TO 12 WEEKS

		NO. OF TESTS	NO. OF ANIMALS	MEAN	RANGE OF ACTUAL VALUES		95 % 2.5%	SPREAD 97.5%
AST	IU/L	732	703	81.7	39.0	191.0	51.0	122.0
ALT	IU/L	732	703	29.7	15.0	97.0	18.0	64.0
ALK PHOS'TASE	IU/L	732	703	188.8	73.0	598.0	93.0	397.0
GGT,SERUM	IU/L	725	693	0.085	0.000	2.070	0.000	0.780
TOTAL BILIRUBIN	MG/DL	732	703	0.124	0.050	0.410	0.070	0.200
CHOLESTEROL	MG/DL	735	703	63.8	19.0	149.0	32.0	113.0
TRIGLYCERIDE	MG/DL	735	703	54.9	15.0	163.0	22.0	114.0
TOTAL PROTEIN	G/DL	735	703	5.409	4.540	6.430	4.800	6.120
ALBUMIN	G/DL	735	703	3.006	2.310	3.590	2.670	3.340
GLOBULIN	G/DL	735	703	2.403	1.570	3.340	1.910	2.980
A/G RATIO	RATIO	735	703	1.267	0.810	1.980	1.010	1.600
GLUCOSE	MG/DL	785	703	135.0	46.0	264.0	74.0	205.0
UREA NITROGEN	MG/DL	745	713	12.6	5.0	30.0	7.0	18.0
CREATININE	MG/DL	745	713	0.248	0.090	0.670	0.130	0.350
CALCIUM	MG/DL	735	703	10.010	8.690	11.190	9.300	10.800

Appendix 13

SPECIES RAT — HISTORICAL CONTROL DATA — DATES:09-MAY-09 09-MAY-14
STRAIN CRL:CD(SD) — CLINICAL CHEMISTRY DATA
SUPPLIER CHARLES RIVER

MALES 5 TO 12 WEEKS

		NO. OF TESTS	NO. OF ANIMALS	MEAN	RANGE OF ACTUAL VALUES		95 % 2.5%	SPREAD 97.5%
SODIUM	MMOL/L	734	702	143.1	123.0	151.0	139.0	147.0
POTASSIUM	MMOL/L	734	702	4.775	3.790	6.810	4.130	5.380
CHLORIDE	MMOL/L	734	702	103.3	88.0	114.0	99.0	107.0
CREATINE KINASE	IU/L	186	167	334.7	86.0	1379.0	98.0	793.0
IRON	UG/DL	15	15	143.5	70.0	320.0	70.0	320.0
PHOSPHORUS	MG/DL	735	703	8.718	6.670	12.250	7.030	10.920

Appendix 13

SPECIES RAT — HISTORICAL CONTROL DATA — DATES:09-MAY-09 09-MAY-14
STRAIN CRL:CD(SD) — CLINICAL CHEMISTRY DATA
SUPPLIER CHARLES RIVER

FEMALES 5 TO 12 WEEKS

		NO. OF TESTS	NO. OF ANIMALS	MEAN	RANGE OF ACTUAL VALUES		95 % 2.5%	SPREAD 97.5%
AST	IU/L	665	641	90.6	42.0	6611.0	49.0	128.0
ALT	IU/L	665	641	32.8	9.0	5573.0	14.0	46.0
ALK PHOS'TASE	IU/L	665	641	107.3	38.0	379.0	50.0	225.0
GGT,SERUM	IU/L	658	631	0.174	0.000	3.680	0.000	1.040
TOTAL BILIRUBIN	MG/DL	665	641	0.122	0.010	0.450	0.080	0.200
CHOLESTEROL	MG/DL	668	641	71.5	14.0	132.0	38.0	112.0
TRIGLYCERIDE	MG/DL	668	641	34.3	9.0	128.0	18.0	64.0
TOTAL PROTEIN	G/DL	668	641	5.736	4.370	7.250	4.940	6.710
ALBUMIN	G/DL	668	641	3.225	2.220	4.070	2.770	3.750
GLOBULIN	G/DL	668	641	2.512	1.660	3.540	1.930	3.130
A/G RATIO	RATIO	668	641	1.300	0.780	1.770	1.000	1.630
GLUCOSE	MG/DL	718	641	134.7	68.0	245.0	83.0	209.0
UREA NITROGEN	MG/DL	678	651	14.8	7.0	110.0	9.0	21.0
CREATININE	MG/DL	678	651	0.291	0.110	0.880	0.170	0.420
CALCIUM	MG/DL	668	641	10.106	8.760	12.320	9.260	11.050

Appendix 13

SPECIES RAT — HISTORICAL CONTROL DATA — DATES:09-MAY-09 09-MAY-14
STRAIN CRL:CD(SD) — CLINICAL CHEMISTRY DATA
SUPPLIER CHARLES RIVER

FEMALES 5 TO 12 WEEKS

		NO. OF TESTS	NO. OF ANIMALS	MEAN	RANGE OF ACTUAL VALUES		95 % 2.5%	SPREAD 97.5%
SODIUM	MMOL/L	667	640	141.4	125.0	148.0	137.0	146.0
POTASSIUM	MMOL/L	667	640	4.547	3.460	5.940	3.870	5.260
CHLORIDE	MMOL/L	667	640	103.7	92.0	122.0	99.0	109.0
CREATINE KINASE	IU/L	175	156	384.7	54.0	1363.0	69.0	960.0
IRON	UG/DL	15	15	240.2	158.0	364.0	158.0	364.0
PHOSPHORUS	MG/DL	668	641	7.772	4.900	11.970	5.580	9.720

Appendix 14

20086532 - Individual Animal Data Gross Pathology Findings

Animal:	9596	Group:	1	Sex: Male
Species:	Rat	Strain:	Sprague-Dawley	
		Dose:	1000 mg/kg/day	
		Removal Reason:	Terminal Euthanasia	
		Day (Week) of Death:	11 (2)	

Gross Pathology Animal Details:

Comments: Tissues submitted Into 10% neutral buffered formalin except eyes and optic nerves submitted in Davidson`s and testes in modified Davidson`s Fixative.

Animal Notes: EUTHANASIA VIA ANESTHESIA AND EXSANGUINATION

Gross Pathology Observations:

No observations found

Any remaining protocol required tissues, which have been examined, have no visible lesions

Gross Pathology - The following Tissues were Not Examined:

None

Appendix 14

20086532 - Individual Animal Data Gross Pathology Findings

Animal:	9597	Group:	1	Sex: Male
Species:	Rat	Strain:	Sprague-Dawley	
		Dose:	1000 mg/kg/day	
		Removal Reason:	Terminal Euthanasia	
		Day (Week) of Death:	11 (2)	

Gross Pathology Animal Details:

Comments: Tissues submitted Into 10% neutral buffered formalin except eyes and optic nerves submitted in Davidson`s and testes in modified Davidson`s Fixative.

Animal Notes: EUTHANASIA VIA ANESTHESIA AND EXSANGUINATION

Gross Pathology Observations:

No observations found

Any remaining protocol required tissues, which have been examined, have no visible lesions

Gross Pathology - The following Tissues were Not Examined:

None

Appendix 14

20086532 - Individual Animal Data Gross Pathology Findings

Animal:	9598	Group:	1	Sex: Male
Species:	Rat	Strain:	Sprague-Dawley	
		Dose:	1000 mg/kg/day	
		Removal Reason:	Terminal Euthanasia	
		Day (Week) of Death:	11 (2)	

Gross Pathology Animal Details:

Comments: Tissues submitted Into 10% neutral buffered formalin except eyes and optic nerves submitted in Davidson`s and testes in modified Davidson`s Fixative.

Animal Notes: EUTHANASIA VIA ANESTHESIA AND EXSANGUINATION

Gross Pathology Observations:

No observations found

Any remaining protocol required tissues, which have been examined, have no visible lesions

Gross Pathology - The following Tissues were Not Examined:

None

Appendix 14

20086532 - Individual Animal Data Gross Pathology Findings

Animal:	9599	Group:	1	Sex: Male
Species:	Rat	Strain:	Sprague-Dawley	
		Dose:	1000 mg/kg/day	
		Removal Reason:	Terminal Euthanasia	
		Day (Week) of Death:	11 (2)	

Gross Pathology Animal Details:

Comments: Tissues submitted Into 10% neutral buffered formalin except eyes and optic nerves submitted in Davidson`s and testes in modified Davidson`s Fixative.

Animal Notes: EUTHANASIA VIA ANESTHESIA AND EXSANGUINATION

Gross Pathology Observations:

No observations found

Any remaining protocol required tissues, which have been examined, have no visible lesions

Gross Pathology - The following Tissues were Not Examined:

None

Appendix 14

20086532 - Individual Animal Data Gross Pathology Findings

Animal:	9600	Group:	1	Sex: Male
Species:	Rat	Strain:	Sprague-Dawley	
		Dose:	1000 mg/kg/day	
		Removal Reason:	Terminal Euthanasia	
		Day (Week) of Death:	11 (2)	

Gross Pathology Animal Details:

Comments: Tissues submitted Into 10% neutral buffered formalin except eyes and optic nerves submitted in Davidson`s and testes in modified Davidson`s Fixative.

Animal Notes: EUTHANASIA VIA ANESTHESIA AND EXSANGUINATION

Gross Pathology Observations:

LUNG : Focus; dark : left lobe, multiple, up to 0.1 cm diameter

Any remaining protocol required tissues, which have been examined, have no visible lesions

Gross Pathology - The following Tissues were Not Examined:

None

Appendix 14

20086532 - Individual Animal Data Gross Pathology Findings

Animal:	9601	Group:	1	Sex: Female
Species:	Rat	Strain:	Sprague-Dawley	
		Dose:	1000 mg/kg/day	
		Removal Reason:	Terminal Euthanasia	
		Day (Week) of Death:	11 (2)	

Gross Pathology Animal Details:

Comments: Tissues submitted in 10% neutral buffered formalin except eyes and optic nerves submitted in Davidson`s fixative

Animal Notes: EUTHANASIA VIA ANESTHESIA AND EXSANGUINATION

Gross Pathology Observations:

No observations found

Any remaining protocol required tissues, which have been examined, have no visible lesions

Gross Pathology - The following Tissues were Not Examined:

None

Appendix 14

20086532 - Individual Animal Data Gross Pathology Findings

Animal:	9602	Group:	1	Sex: Female
Species:	Rat	Strain:	Sprague-Dawley	
		Dose:	1000 mg/kg/day	
		Removal Reason:	Terminal Euthanasia	
		Day (Week) of Death:	11 (2)	

Gross Pathology Animal Details:

Comments: Tissues submitted in 10% neutral buffered formalin except eyes and optic nerves submitted in Davidson`s fixative

Animal Notes: EUTHANASIA VIA ANESTHESIA AND EXSANGUINATION

Gross Pathology Observations:

No observations found

Any remaining protocol required tissues, which have been examined, have no visible lesions

Gross Pathology - The following Tissues were Not Examined:

None

Appendix 14

20086532 - Individual Animal Data Gross Pathology Findings

Animal:	9603	Group:	1	Sex: Female
Species:	Rat	Strain:	Sprague-Dawley	
		Dose:	1000 mg/kg/day	
		Removal Reason:	Terminal Euthanasia	
		Day (Week) of Death:	11 (2)	

Gross Pathology Animal Details:

Comments: Tissues submitted in 10% neutral buffered formalin except eyes and optic nerves submitted in Davidson`s fixative

Animal Notes: EUTHANASIA VIA ANESTHESIA AND EXSANGUINATION

Gross Pathology Observations:

No observations found

Any remaining protocol required tissues, which have been examined, have no visible lesions

Gross Pathology - The following Tissues were Not Examined:

None

Appendix 14

20086532 - Individual Animal Data Gross Pathology Findings

Animal:	9604	Group:	1	Sex: Female
Species:	Rat	Strain:	Sprague-Dawley	
		Dose:	1000 mg/kg/day	
		Removal Reason:	Terminal Euthanasia	
		Day (Week) of Death:	11 (2)	

Gross Pathology Animal Details:

Comments: Tissues submitted in 10% neutral buffered formalin except eyes and optic nerves submitted in Davidson`s fixative

Animal Notes: EUTHANASIA VIA ANESTHESIA AND EXSANGUINATION

Gross Pathology Observations:

No observations found

Any remaining protocol required tissues, which have been examined, have no visible lesions

Gross Pathology - The following Tissues were Not Examined:

None

Appendix 14

20086532 - Individual Animal Data Gross Pathology Findings

Animal:	9605	Group:	1	Sex: Female
Species:	Rat	Strain:	Sprague-Dawley	
		Dose:	1000 mg/kg/day	
		Removal Reason:	Terminal Euthanasia	
		Day (Week) of Death:	11 (2)	

Gross Pathology Animal Details:

Comments: Tissues submitted Into 10% neutral buffered formalin except eyes and optic nerves submitted in Davidson`s

Animal Notes: EUTHANASIA VIA ANESTHESIA AND EXSANGUINATION

Gross Pathology Observations:

No observations found

Any remaining protocol required tissues, which have been examined, have no visible lesions

Gross Pathology - The following Tissues were Not Examined:

None