REPORT

Gaussia Luciferase protein:
Acute Oral Toxicity in the Rat – Up and Down Procedure

Study Director:	A Pooles
Test Facility:	**Harlan Laboratories Ltd.** Shardlow Business Park Shardlow Derbyshire DE72 2GD UK
Sponsor:	**Prolume LTD** 6325 Old Mill Farm Drive Wendell NC 27591 UNITED STATES OF AMERICA
Harlan Study Number:	**41402551**
Study Completion Date:	22 October 2014

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STUDY DIRECTOR STATEMENT OF GLP COMPLIANCE

Harlan Laboratories Ltd., Shardlow Business Park, Shardlow, Derbyshire, DE72 2GD, UK

Harlan Study Number: 41402551
Study Title: Gaussia Luciferase protein:
Acute Oral Toxicity in the Rat – Up and Down Procedure

With the exception noted below this study was performed in compliance with UK GLP standards (Schedule 1, Good Laboratory Practice Regulations 1999 (SI 1999/3106 as amended by SI 2004/0994)). These Regulations are in accordance with GLP standards published as OECD Principles on Good Laboratory Practice (revised 1997, ENV/MC/CHEM(98)17); and are in accordance with, and implement, the requirements of Directives 2004/9/EC and 2004/10/EC.

These principles are compatible with Good Laboratory Practice regulations specified by regulatory authorities throughout the European Community, the United States (EPA and FDA), and Japan (MHLW, MAFF and METI).

No analysis was carried out to determine the homogeneity, concentration or stability of the test item formulation. The test item was formulated within two hours of it being applied to the test system; it is assumed that the formulation was stable for this duration. This exception is considered not to affect the purpose or integrity of the study.

This report fully and accurately reflects the procedures used and data generated. There were no circumstances considered to have affected the integrity of the study or the validity of the data.

Study Director: A Pooles

A. Poob

Date: 22|10|14

QUALITY ASSURANCE STATEMENT

Harlan Study Number: 41402551
Study Title: Gaussia Luciferase protein:
Acute Oral Toxicity in the Rat – Up and Down Procedure

The general facilities and activities are inspected at least once a year and the results are reported to the relevant responsible person and management.

Study-related procedures conducted at the test facility were audited and inspected. The details of these audits and inspections are given below.

Dates and Types of QA Inspections			**Reported to the relevant Study Director and Test Facility Management**
Date of Inspection	Type of Inspection	Phase Inspected	Report Date
17 July 2014	Study Plan Verification	N/A	17 July 2014
08, 28 July 2014	Process – based	Test Item Preparation	08, 28 July 2014
03 July 2014	Process – based	Test System Preparation and Application	03 July 2014
15 July 2014	Process – based	Assessment of Response	15 July 2014
10 July 2014	Process – based	Necropsy	10 July 2014
20 October 2014	Report Audit	N/A	20 October 2014

This statement confirms that this report reflects the raw data and the procedures followed.

Quality Assurance: N. TANK

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Date: 24 OCT 2014

SUMMARY

Introduction

The study was performed to assess the acute oral toxicity of the test item in the Wistar strain rat.

Methods

One fasted female animal was treated with the test item at a dose level of 5000 mg/kg body weight. The animal survived, therefore two additional females were treated sequentially at a dose level of 5000 mg/kg body weight so that a total of three animals were treated.

The test item was administered orally as a suspension in arachis oil BP. Clinical signs and body weight development were monitored during the study. All animals were subjected to gross necropsy.

Results

Mortality. There were no deaths.

Clinical Observations. There were no signs of systemic toxicity.

Body Weight. All animals showed expected gains in body weight.

Necropsy. No abnormalities were noted at necropsy.

Conclusion

The acute oral median lethal dose (LD_{50}) of the test item in the female Wistar strain rat was found to be greater than 5000 mg/kg body weight.

GENERAL INFORMATION

Schedule

Experimental Starting Date:	04 August 2014
Experimental Completion Date:	10 September 2014

Animal Welfare

The study was designed and conducted to cause the minimum suffering or distress to the animals consistent with the scientific objectives and in accordance with the Harlan Laboratories Ltd, Shardlow, UK policy on animal welfare and the requirements of the United Kingdom's Animals (Scientific Procedures) Act 1986 Amendment Regulations 2012. The conduct of the study may be reviewed, as part of the Harlan Laboratories Ltd, Shardlow, UK Ethical Review Process.

The study was conducted in accordance with the UK Home Office Guidance document on Regulatory Toxicology and Safety Evaluation Studies and the OECD guidance document on recognition, assessment and use of clinical signs as humane endpoints for experimental animals used in safety evaluation.

Deviations from Study Plan

There were no deviations (unplanned changes) from the study plan.

Archiving

Unless instructed otherwise by the Sponsor, the study plan (general study plan and study specific supplement), all raw data (paper and electronic) and the final report will be retained in the Harlan Laboratories Ltd., Shardlow, UK archives for five years after which instructions will be sought as to further retention or disposal. Further retention or return of the data will be chargeable to the Sponsor.

No data will be discarded without contacting the Sponsor to obtain their written consent.

1 INTRODUCTION AND PURPOSE

The study was performed to assess the acute oral toxicity of the test item in the Wistar strain rat.

1.1 Guidelines / Regulations

This study was designed to be compatible with the procedures indicated by the following internationally accepted guidelines and recommendations:

- OECD Guidelines for the Testing of Chemicals No. 425 "Acute Oral Toxicity - Up and Down Procedure (UDP)" (adopted 03 October 2008)

2 TEST ITEM

Information as provided by the Sponsor.

Identification:	Gaussia Luciferase protein
Batch:	T0613
Purity:	not supplied
Physical state / Appearance:	tan colored powder
Expiry date:	01 June 2023
Storage Conditions:	room temperature in the dark

3 MATERIALS AND METHODS

3.1 Test System

3.1.1 Animals and Animal Husbandry

Female Wistar (RccHan™:WIST) strain rats were supplied by Harlan Laboratories UK Ltd., Oxon, UK. On receipt the animals were randomly allocated to cages. The females were nulliparous and non-pregnant. After an acclimatization period of at least five days the animals were selected at random and given a number unique within the study which was written on a cage card. At the start of the study the animals were eight to twelve weeks of age. The body weight variation did not exceed ±20% of the body weight of the initially dosed animal.

The animals were individually housed in suspended solid-floor polypropylene cages furnished with woodflakes. With the exception of an overnight fast immediately before dosing and for approximately three to four hours after dosing, free access to mains drinking water and food (2014C Teklad Global Rodent diet supplied by Harlan Laboratories UK Ltd., Oxon, UK) was allowed throughout the study. The diet, drinking water and bedding were routinely analyzed and

were considered not to contain any contaminants that would reasonably be expected to affect the purpose or integrity of the study.

The temperature and relative humidity were set to achieve limits of 19 to 25 °C and 30 to 70% respectively. The rate of air exchange was at least fifteen changes per hour and the lighting was controlled by a time switch to give twelve hours continuous light (06:00 to 18:00) and twelve hours darkness.

The animals were provided with environmental enrichment items which were considered not to contain any contaminant of a level that might have affected the purpose or integrity of the study.

3.1.2 Justification

Rats are the preferred species of choice as historically used for safety evaluation studies and are specified in the appropriate test guidelines.

3.2 Test Item Formulation and Experimental Preparation

For the purpose of the study the test item was freshly prepared, as required, as a suspension in arachis oil BP. Arachis oil BP was used because the test item did not dissolve/suspend in distilled water.

The test item was formulated within two hours of being applied to the test system. It is assumed that the formulation was stable for this duration.

No analysis was conducted to determine the homogeneity, concentration or stability of the test item formulation. This is an exception with regard to GLP and has been reflected in the GLP compliance statement.

3.3 Procedure

Using available information on the toxicity of the test item, 5000 mg/kg was chosen as the starting dose.

Three individual fasted female animals were treated as follows:

Test Sequence (Animal number)	Dose Level mg/kg	Concentration (mg/mL)	Dose Volume (mL/kg)	Short-Term Result
1 (1-0)	5000	500	10	0
2 (2-0)	5000	500	10	0
3 (3-0)	5000	500	10	0

0 = Animal survived

The test was complete after the third animal had been dosed as the following stopping criterion was met:

- three consecutive animals survived at the maximum dose level (5000 mg/kg)

All animals were dosed once only by gavage, using a metal cannula attached to a graduated syringe. The volume administered to each animal was calculated according to the fasted bodyweight at the time of dosing. Treatment of animals was sequential. Sufficient time was allowed between each individual animal to confirm the survival of the previously dosed animals.

The animals were observed for deaths or overt signs of toxicity ½, 1, 2 and 4 hours after dosing and subsequently once daily for 14 days.

Individual bodyweights were recorded prior to dosing and seven and fourteen days after treatment.

At the end of the observation period the animals were killed by cervical dislocation. All animals were subjected to gross pathological examination. This consisted of an external examination and opening of the abdominal and thoracic cavities for examination of major organs. The appearance of any macroscopic abnormalities was recorded. No tissues were retained.

3.4 Evaluation of Data

The oral LD_{50} was calculated by the maximum likelihood method. Data evaluations also included the relationship, if any, between the exposure of the animal to the test item and the incidence and severity of all abnormalities including behavioral and clinical observations, gross lesions, body weight changes, mortality and any other toxicological effects.

Using the mortality data obtained, an estimate of the acute oral median lethal dose (LD_{50}) of the test item was made.

4 RESULTS

Individual clinical observations and mortality data are given in Table 1.

4.1 Mortality

There were no deaths.

4.2 Clinical Observations

No signs of systemic toxicity were noted during the observation period.

4.3 Body Weight

Individual body weights and body weight changes are given in Table 2.

All animals showed expected gains in body weight over the observation period.

4.4 Necropsy

Individual necropsy findings are given in Table 3.

No abnormalities were noted at necropsy.

5 CONCLUSION

The acute oral median lethal dose (LD_{50}) of the test item in the female Wistar strain rat was found to be greater than 5000 mg/kg body weight.

6 REFERENCES

ENVIRONMENT DIRECTORATE, ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT OECD (2000) *No. 19 Guidance Document on the Recognition, Assessment and use of Clinical Signs as Humane Endpoints for Experimental Animals Used in Safety Evaluation.* Paris: OECD Environmental Health and Safety Publications Series on Testing and Assessment.

The Animals (Scientific Procedures) Act 1986 Amendment Regulations 2012.

UK HOME OFFICE (2005) Guidance on the Conduct of Regulatory Toxicology and Safety Evaluation Studies.

TABLES

Table 1 Individual Clinical Observations and Mortality Data

Dose Level mg/kg	Animal Number and Sex	Effects Noted After Dosing (Hours)				Effects Noted During Period After Dosing (Days)													
		½	1	2	4	1	2	3	4	5	6	7	8	9	10	11	12	13	14
5000	1-0 Female	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2-0 F2male	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3-0 Female	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

0 = No signs of systemic toxicity

Table 2 Individual Body Weights and Body Weight Changes

Dose Level mg/kg	Animal Number and Sex	Body Weight (g) at Day			Body Weight Gain (g) During Week	
		0	7	14	1	2
5000	1-0 Female	147	171	184	24	13
	2-0 Female	144	179	195	35	16
	3-0 Female	154	174	180	20	6

Table 3 Individual Necropsy Findings

Dose Level mg/kg	Animal Number and Sex	Time of Death	Macroscopic Observations
5000	1-0 Female	Killed Day 14	No abnormalities detected
	2-0 Female	Killed Day 14	No abnormalities detected
	3-0 Female	Killed Day 14	No abnormalities detected

APPENDIX

Monitoring Authority Statement of GLP Compliance

DH Department of Health

THE DEPARTMENT OF HEALTH OF THE GOVERNMENT OF THE UNITED KINGDOM

GOOD LABORATORY PRACTICE

STATEMENT OF COMPLIANCE IN ACCORDANCE WITH DIRECTIVE 2004/9/EC

TEST FACILITY

Harlan Laboratories Limited
Shardlow Business Park
London Road
Shardlow
Derbyshire
DE72 2GD

TEST TYPE(S)

Analytical/Clinical Chemistry
Environmental Fate
Environmental Toxicity
Phys.Chem. Testing
Mutagenicity
Toxicology

DATE OF INSPECTION **12 to 14 March 2014**

An inspection for compliance with the Principles of Good Laboratory Practice was carried out at the above test facility as part of the UK Good Laboratory Practice Compliance Monitoring Programme.

This statement confirms that, on the date of issue, the UK Good Laboratory Practice Monitoring Authority were satisfied that the above test facility was operating in compliance with the OECD Principles of Good Laboratory Practice.

This statement constitutes a Good Laboratory Practice Instrument (as defined in the UK Good Laboratory Practice Regulations 1999).

12/5/14

Dr. Andrew J. Gray
Head, UK GLP Monitoring Authority

MHRA