[redacted]

REPORT

[redacted] Eye Irritation to the Rabbit

[redacted]	[redacted]
Version ID:	Final report
Issue date:	16 October 2013

Details of Sponsor and Test Facility

Sponsor [redacted]

Test facility [redacted]

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Compliance with Good Laboratory Practice

[redacted] Eye Irritation to the Rabbit

The study described in this report was conducted in compliance with the following Good Laboratory Practice standards and I consider the data generated to be valid.

> The UK Good Laboratory Practice Regulations (Statutory Instrument 1999 No. 3106, as amended by Statutory Instrument 2004 No. 994).
>
> OECD Principles of Good Laboratory Practice (as revised in 1997), ENV/MC/CHEM (98) 17.
>
> EC Commission Directive 2004/10/EC of 11 February 2004 (Official Journal No. L 50/44).

These principles of Good Laboratory Practice are accepted by the regulatory authorities of the United States of America and Japan on the basis of intergovernmental agreements.

[redacted]

16 October 2013
Date

[redacted]

Quality Assurance Statement

[redacted] Eye Irritation to the Rabbit

The following inspections and audits have been carried out in relation to this study:

Study Phase	Date(s) of Inspection	Date of Reporting to Study Director and Management
Protocol Audit	04 Jul 2013	04 Jul 2013
Report Audit	30 Aug 2013	30 Aug 2013

Process based inspections

At or about the time this study was in progress inspections of procedures employed on this type of study were carried out. These were conducted and reported to appropriate Company Management as indicated below:

Process Based Inspections	Date(s) of Inspection	Date of Reporting to Management
Dose administration & Observations – Ocular	04 Jun 2013	10 Jun 2013
Study Management and Conduct	10 Jun 2103	21 Jun 2013

In addition, an inspection of the facility where this study was conducted was carried out on an annual basis. These inspections were promptly reported to Company Management.

[redacted]

16 October 2013
Date

Contributing Scientist

[redacted] Eye Irritation to the Rabbit

Study management

[redacted] (Hons) CBiol MSB
Study Director

Summary

A study was performed to assess the eye irritation potential of [redacted] to the rabbit. The method followed was that described in:

> EEC Methods for the determination of toxicity and other health effects. Commission Regulation No. 440/2008. Part B, Method B.5 Acute Toxicity: Eye irritation/corrosion 30 May 2008.
>
> OECD Guideline for Testing of Chemicals No.405 "Acute Eye Irritation/Corrosion". Adopted 24 April 2002.
>
> EPA Health Effects Test Guidelines OPPTS 870.2400 Acute Eye Irritation EPA 712-C-98-195. August 1998.
>
> Japanese Ministry of Agriculture, Forestry and Fisheries, Test Data for Registration of Agricultural Chemicals, Eye Irritation (2-1-5), 12 Nohsan No. 8147, Agricultural Production Bureau, November 24, 2000.

Three rabbits were each administered a single ocular dose of a volume of 0.1 mL of the test substance weighing approximately 43 mg and observed for eight days after instillation.

A crimson-red conjunctival appearance with very-slight chemosis and, in one case, slight discharge, was evident in all animals one hour after instillation. In two animals injection of the conjunctival blood vessels persisted 24 or 48 hours after instillation after which the eyes were overtly normal. In the remaining animal a beefy-red conjunctival appearance with very slight chemosis, scattered or diffuse areas of opacity covering up to a quarter of the corneal surface and iritis were apparent 24 hours after instillation. The opacity, iritis and chemosis resolved within the next 24 hours by when a crimson-red conjunctival appearance was evident. Injection of the conjunctival blood vessels was apparent 72 hours after instillation, resolving by Day 8.

The treated eye of all animals was overtly normal by Day 8.

Instillation of the test substance gave rise to a moderate initial pain response in the sentinel animal; subsequent animals were treated with local (ocular) anaesthesia prior to instillation. The total mean scores according to the system of Kay and Calandra (1962) are summarised below:

	Time after instillation of [redacted]				
Area of eye	**1 hour**	**24 hours**	**48 hours**	**72 hours**	**Day 8**
Cornea	0.0	1.7	0.0	0.0	0.0
Iritis	0.0	1.7	0.0	0.0	0.0
Conjunctiva	6.7	4.0	2.0	0.7	0.0
Total mean score	6.7	7.3	2.0	0.7	0.0

The highest total mean score was 7.3 occurring at the 24-hour observation; accordingly under the criteria of Kay and Calandra (1962) [redacted] was classified as "minimally irritating" to the eye.

The means of scores for the ocular reactions at approximately 24, 48 and 72 hours after administration, calculated separately for each animal, are summarised below:

Animal number	Corneal opacity	Iridial lesions	Conjunctival	
			Redness	Chemosis
93	0.0	0.0	0.7	0.0
94	0.0	0.0	0.3	0.0
95	0.3	0.3	2.0	0.3
EC Category 1 trigger values*	≥3	>1.5		
EC Category 2 trigger values*	≥1	≥1	≥2	≥2

* Classification triggered if any value is attained by two or more animals.

[redacted] did not require labelling in accordance with European Commission regulation 1272/2008.

1. Introduction

The study was designed to assess eye irritation potential of [redacted] following a single instillation into the eye of the rabbit. The test substance may come into contact with the eye during handling or use.

The study was conducted in compliance with the following guidelines:

> EEC Methods for the determination of toxicity and other health effects. Commission Regulation No. 440/2008. Part B, Method B.5 Acute Toxicity: Eye irritation/corrosion 30 May 2008.
>
> OECD Guideline for Testing of Chemicals No.405 "Acute Eye Irritation/Corrosion". Adopted 24 April 2002.
>
> EPA Health Effects Test Guidelines OPPTS 870.2400 Acute Eye Irritation EPA 712-C-98-195. August 1998.
>
> Japanese Ministry of Agriculture, Forestry and Fisheries, Test Data for Registration of Agricultural Chemicals, Eye Irritation (2-1-5), 12 Nohsan No. 8147, Agricultural Production Bureau, November 24, 2000.

The albino rabbit was chosen as it has been shown to be a suitable model for eye irritation studies and is the animal recommended in the test guidelines.

The amount of test substance instilled was chosen in compliance with the guidelines.

The protocol was approved by the Study Director and [redacted] Management on 3 July 2013 and by the Sponsor on 24 July 2013.

The experimental start date was 5 July 2013 and the experimental completion date was 30 July 2013.

2. Test Substance

Identification:	[redacted]
Chemical name:	[redacted]
Intended use:	[redacted]
Description:	Pale yellow powder
Storage conditions:	Ca. 20°C in the dark
Batch number:	[redacted]
Date of receipt:	28th January 2013
Expiry date:	30th April 2014
Purity:	99.9%

3. Experimental Procedure

3.1 Animal management

Animals for this study were selected from a stock supply of healthy adult rabbits of the New Zealand White strain. They were in the weight range of 3.55 to 4.57 kg and 36 to 38 weeks of age, prior to treatment (Day 1). All rabbits were acclimatised to the experimental environment for a period of 18 weeks prior to the start of the study.

Each animal was housed individually in a plastic cage with perforated floors and was offered 125 g of a standard laboratory rabbit diet per day; drinking water was provided *ad libitum*. The batch of diet used for the study is analysed for nutrients, possible contaminants and micro-organisms likely to be present in the diet and which, if in excess of specified amounts, might have an undesirable effect on the test system. A dietary supplement of hay was offered during acclimatisation until two days prior to dose instillation, for the remainder of acclimatisation and throughout the study observation period wholemeal bread was offered.

During the acclimatisation and study period the animals were given small soft white untreated wood blocks for environmental enrichment.

Results of routine physical and chemical examination of drinking water, as conducted by the supplier are made available to [redacted]

Animal room environmental controls were set to maintain temperature within the range 16 to 20°C, and relative humidity within 40 to 70%. These environmental parameters were recorded and the permanent record archived with other departmental raw data. Lighting was controlled by means of a time switch to give 12 hours of artificial light (06:00 to 18:00 GMT) in each 24 hour period.

Each animal was identified by a numbered tag placed through the edge of one ear. This identification was unique within the Department throughout the duration of the study. Each cage was identified by a coloured label displaying the study number and animal number.

3.2 Test substance preparation

[redacted] was administered as supplied by the Sponsor.

The absorption of [redacted] was not determined.

The identity, strength and purity of the test substance received, its stability under the storage conditions and the conditions of administration were the responsibility of the Sponsor.

3.3 Treatment procedure

The eyes of each animal were examined prior to instillation of the test substance to ensure that there was no pre-existing corneal damage, iridial inflammation or conjunctival irritation. Each animal was gently restrained. The dose was instilled into the right eye by pulling the lower eyelid away from the eye ball to form a cup into which the test substance was dropped. The eyelids were then gently held together for one second before releasing; the left eye remained untreated.

A single animal (Number 93) was treated in advance; in the absence of a severe effect in this animal two further animals were committed to the study.

3.4 Serial observations

3.4.1 Clinical signs

The behaviour of each rabbit was observed immediately following instillation of the test substance to allow assessment of the initial pain response by the criteria detailed below. Since the sentinel animal showed a moderate initial pain response, the subsequent two animals were pre-treated with a local (ocular) anaesthesia prior to instillation of the test substance.

Criteria for assessment of pain response (not a component of the regulatory guidelines)

Reaction of animal to instillation of test substance	Class	Descriptive Rating
No Response	0	No initial pain
A few blinks only; normal within one or two minutes	1	Practically no initial pain
Rabbit blinks and tries to open eye but the reflexes close it	2	Slight initial pain
Rabbit holds eye shut and puts pressure on lids; may rub eye with paw, may vocalise	3	Moderate initial pain
Rabbit holds eye shut vigorously, may tilt head, may have reluctance to rub eye; may vocalise	4	Severe initial pain
Rabbit holds eye shut very firmly; may vocalise, claws at eye, jumps and tries to escape	5	Very severe initial pain

The animals were returned to their cages and checked at least twice during the first hour after dosing and at regular intervals throughout the day to ensure no severe injury passed unnoticed. Ocular reactions to treatment were assessed 1, 24, 48 and 72 hours and eight days after treatment, according to the criteria below. Reactions not included overleaf were described in detail.

3.4.2 Ocular responses

The untreated eye was used as a comparison with the treated eye during assessment of ocular lesions.

Ocular irritation was assessed using the prescribed numerical system:

Cornea Opacity: degree of density (area most dense taken for reading)	
No opacity	0
Scattered or diffuse areas of opacity (other than slight dulling of normal lustre), details of iris clearly visible	1
Easily discernible translucent areas, details of iris slightly obscured	2
Nacreous areas, no details of iris visible, size of pupil barely discernible	3
Opaque cornea, iris not discernible through the opacity	4
Area of cornea involved:	
None	0
One quarter (or less) but not zero	1
Greater than one quarter, but less than half	2
Greater than half, but less than three quarters	3
Greater than three quarters, up to whole area	4

The presence or absence of corneal ulceration or stippling was designated + (positive) or - (negative).

Iris:	
Normal	0
Markedly deepened rugae, congestion, swelling, moderate circumcorneal hyperaemia or injection, any of these or combination of any thereof, iris still reacting to light (sluggish reaction is positive)	1
No reaction to light, haemorrhage, gross destruction (any or all of these)	2
Conjunctivae Redness: (refers to the most severe reading of palpebral and bulbar conjunctivae, as compared to the control eye)	
Blood vessels normal	0
Some blood vessels definitely hyperaemic (injected)	1
Diffuse, crimson colour, individual vessels not easily discernible	2
Diffuse beefy red	3
Chemosis: (lids and/or nictating membranes)	
No swelling	0
Any swelling above normal (includes nictating membranes)	1
Obvious swelling with partial eversion of lids	2
Swelling with lids about half-closed	3
Swelling with lids more than half-closed	4

The presence or absence of ulceration or necrosis was designated + (positive) or - (negative).

Discharge:	
No discharge	0
Any amount different from normal	1
Discharge with moistening of lids and hairs adjacent to lids	2
Discharge with moistening of lids and hairs and considerable area around eye	3*
Extensive mucoid discharge	4

* Maximum score permissible under system of Kay and Calandra.

A pencil beam torch was available for use to facilitate inspection of the eyes.

3.4.3 Interpretation of responses

Kay and Calandra

The classification system of Kay and Calandra (1962)* was employed on this study. This system classifies ocular irritants thus:

Corneal scoring using the grade for degree of opacity density (A) and the grade for the area of cornea involved (B):

Corneal score = A x B x 5 (theoretical maximum = 80)

Iridial scoring using the grades for iritis (C):

Iridial score = C x 5 (theoretical maximum = 10)

Conjunctival score using the grade for conjunctival redness (D), chemosis grade (E) and grade for discharge (F):

Conjunctival score = (D + E + F) x2 (theoretical maximum = 20)

The scores for each area are meaned for each time point and the highest total meaned score is used for the classification thus:

Total mean score	Classification
0.0 – 0.5	Non-irritating
0.5 – 2.5	Practically non-irritating
2.5 – 15	Minimally irritating
15 – 25	Mildly irritating
25 – 50	Moderately irritating
50 – 80	Severely irritating
80 – 100	Extremely irritating
100 – 110	Maximally irritating

* Kay, JH and Calandra, JC (1962). Interpretation of eye irritation tests. Journal of the Society of Cosmetic Chemists.13: 281-289.

EU Classification

The Official Journal of the European Communities (Regulation 1272/2008) contains the following criteria for the classification of eye lesions:

Irreversible effects on the eye/serious damage to eyes: substances that have the potential to seriously damage the eyes are classified as Category 1. Substances are assigned this hazard category on the basis of results of animal testing. These observations include animals with Grade 4 corneal lesions and other severe reactions (such as destruction of the cornea) observed at any time during the test as well as persistent corneal opacity, discolouration of the cornea by a dye substance, adhesion, pannus and interference with the function of the iris or other effects that impair sight. Persistent observations are those which are not fully reversible within an observation period of 21 days; the following thresholds apply:

> At least in one animal, effects on the cornea, iris or conjunctiva that are not expected to reverse or have not fully reversed within an observation period of normally 21 days
>
> At least 2 of 3 animals tested a positive response of:
> - corneal opacity $\geq$3 and/or
> - iritis >1.5
>
> Calculated as the mean scores following grading at 24, 48 and 72 hours after instillation of the test material

Irritating to eyes: substances that have the potential to induce reversible eye irritation are classified as Category 2; the following thresholds apply:

> At least 2 of 3 animals tested a positive response of:
> - corneal opacity $\geq$1 and/or
> - iritis >1 and/or
> - conjunctival redness >2 and/or
> - conjunctival oedema (chemosis)
>
> Calculated as the mean scores following grading at 24, 48 and 72 hours after instillation of the test material and which fully reverses within an observation period of 21 days

The observed pain response following instillation of the test substance was reported separately.

3.4.4 Termination

Following completion of the observation period the animals were humanely killed by an intravenous injection of sodium pentobarbital.

3.5 Archives

All raw data arising from the performance of this study at [redacted] is the property of the Sponsor and will be lodged together with a copy of the final report in the [redacted] Archive.

Such records will be retained for a minimum of one year from the date on which the Study Director signs the final report. At the end of the retention period the Sponsor will be contacted and advice sought on the return, disposal or further retention of the records.

[redacted] will retain a copy of the final report indefinitely and all Quality Assurance inspection records for a period of 20 years.

3.6 Deviations from protocol

There was no deviation from protocol.

4. Results

4.1 Clinical signs

There was no sign of toxicity or ill health in any rabbit during the observation period.

4.2 Ocular responses

A crimson-red conjunctival appearance with very-slight chemosis and, in one case, slight discharge, was evident in all animals one hour after instillation. In two animals injection of the conjunctival blood vessels persisted 24 or 48 hours after instillation after which the eyes were overtly normal. In the remaining animal a beefy-red conjunctival appearance with very slight chemosis, scattered or diffuse areas of opacity covering up to a quarter of the corneal surface and iritis were apparent 24 hours after instillation. The opacity, iritis and chemosis resolved within the next 24 hours by when a crimson-red conjunctival appearance was evident. Injection of the conjunctival blood vessels was apparent 72 hours after instillation, resolving by Day 8.

The treated eye of all animals was overtly normal by Day 8.

Instillation of the test substance gave rise to a moderate initial pain response in the sentinel animal; subsequent animals were treated with local (ocular) anaesthesia prior to instillation.

5. Conclusion

The highest total mean score was 7.3 occurring at the 24-hour observation; accordingly under the criteria Kay and Calandra (1962) [redacted] was classified as "minimally irritating" to the eye. [redacted] did not require labelling in accordance with European Commission regulation 1272/2008.

Table 1 Mean values for ocular lesions for Kay and Calandra classification

Mean irritation scores after instillation of [redacted]

Area of eye	1 hour	24 hours	48 hours	72 hours	8 Days
Cornea	0.0	1.7	0.0	0.0	0.0
Iritis	0.0	1.7	0.0	0.0	0.0
Conjunctiva	6.7	4.0	2.0	0.7	0.0
Total mean score	6.7	7.3	2.0	0.7	0.0

Table 2 Mean values for ocular lesions for EC (Regulation 1272/2008) and GHS classification

24, 48 and 72 hours after instillation of [redacted]

Animal number and sex	Corneal Opacity	Iridial lesions	Redness of Conjunctiva	Chemosis
93F	0.0	0.0	0.7	0.0
94F	0.0	0.0	0.3	0.0
95F	0.3	0.3	2.0	0.3

F Female

Table 3 Grades for ocular irritation responses following instillation of [redacted]

Animal number and sex: 93F #		Pain evaluation response: 3				
Region of the eye	Response	Grade of response at time after instillation				
		Hours				Day
		1	24	48	72	8
Cornea	Opacity (A)	0	0	0	0	0
	Area (B)	0	0	0	0	0
	Ulceration	-	-	-	-	-
	Stippling	-	-	-	-	-
Corneal score (A x B x 5)		**0**	**0**	**0**	**0**	**0**
Iris	Value (C)	0	0	0	0	0
Iridial score (C x 5)		**0**	**0**	**0**	**0**	**0**
Conjunctiva	Redness (D)	2	1	1	0	0
	Chemosis (E)	1	0	0	0	0
	Discharge (F)	0	0	0	0	0
	Necrosis	-	-	-	-	-
	Ulceration	-	-	-	-	-
Conjunctival score ((D+E+F)x2)		**6**	**2**	**2**	**0**	**0**

#	Sentinel animal
F	Female

Table 3 cont. Grades for ocular irritation responses following instillation of [redacted]

Animal number and sex: 94F		Pain evaluation response: N/A				
Region of the eye	Response	Grade of response at time after instillation				
		Hours				Day
		1	24	48	72	8
Cornea	Opacity (A)	0	0	0	0	0
	Area (B)	0	0	0	0	0
	Ulceration	-	-	-	-	-
	Stippling	-	-	-	-	-
Corneal score (A x B x 5)		**0**	**0**	**0**	**0**	**0**
Iris	Value (C)	0	0	0	0	0
Iridial score (C x 5)		**0**	**0**	**0**	**0**	**0**
Conjunctiva	Redness (D)	2	1	0	0	0
	Chemosis (E)	1	0	0	0	0
	Discharge (F)	0	0	0	0	0
	Necrosis	-	-	-	-	-
	Ulceration	-	-	-	-	-
Conjunctival score ((D+E+F)x2)		**6**	**2**	**0**	**0**	**0**

F Female
N/A Not applicable – local anaesthesia used

Table 3 cont. Grades for ocular irritation responses following instillation of [redacted]

Animal number and sex: 95F		Pain evaluation response: N/A				
Region of the eye	Response	Grade of response at time after instillation				
		Hours				Day
		1	24	48	72	8
Cornea	Opacity (A)	0	$1F^+$	$0F^-$	0	0
	Area (B)	0	1	0	0	0
	Ulceration	-	-	-	-	-
	Stippling	-	-	-	-	-
Corneal score (A x B x 5)		**0**	**5**	**0**	**0**	**0**
Iris	Value (C)	0	1	0	0	0
Iridial score (C x 5)		**0**	**5**	**0**	**0**	**0**
Conjunctiva	Redness (D)	2	3	2	1	0
	Chemosis (E)	1	1	0	0	0
	Discharge (F)	1	0	0	0	0
	Necrosis	-	-	-	-	-
	Ulceration	-	-	-	-	-
Conjunctival score ((D+E+F)x2)		**8**	**8**	**4**	**2**	**0**

F	Female
N/A	Not applicable – local anaesthesia used
F^+	Fluorescein positive
F^-	Fluorescein negative

[redacted]

Annex 1 Weight of evidence

Prior to undertaking in-vivo irritation testing the Study Director conducted a weight-of-the-evidence analysis to ensure that the *in-vivo* testing was sufficiently justified. The following factors have been taken into consideration:

1. Nature of test substance.

[redacted] is a pale yellow solid intended for use as a [redacted] The Sponsor advised the following information:

Chemical name:
[redacted]

Impurity:
[redacted]

Molecular formula: [redacted]

MW: [redacted]

Melting point: 176-190°C (Huntingdon Life Science Study Number CVJ0166)

Boiling point: more than 210°C (Huntingdon Life Science Study Number CVJ0166)

Structure

[redacted]

2. Evaluation of existing human and animal data (including dermal toxicity)

Dermal toxicity study in the rat [redacted]): no dermal irritation was evident at any time during the observation period.

Dermal irritation study in the rabbit ([redacted] no dermal irritation was evident at any time during the observation period.

Searches of the internet and databases did not yield skin or eye irritation data.

3. Analysis of structure activity relationships (SAR).
None available.

4. Physicochemical properties.
pH (aqueous 10% preparation as measured at [redacted]
5.8.
pH (1% w/v dispersion as measured at [redacted] - 6.2.

5. Results from *in-vitro* or *ex-vivo* tests.
None available.

The Study Director was satisfied that, in the absence of obtainable data, there was sufficient justification to conduct an *in-vivo* rabbit eye irritation study.

Annex 2 GLP Compliance Statements

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

[redacted]

TEST TYPE(S)

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

DATE OF INSPECTION
18th – 20th June 2012

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).

14/4/12

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

MHRA

[redacted]

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

[redacted]

TEST TYPE(S)

Analytical/Clinical Chemistry
Environmental Fate
Environmental Toxicity
Ecosystems
Toxicology
Residue Studies

Reissued February 2013 to include Residue Studies

DATE OF INSPECTION **20 to 22 November 2012**

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).

22/2/13

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

MHRA