[redacted]

REPORT

[redacted] Skin 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]

Table of Contents

Details of Sponsor and Test Facility 2
Compliance with Good Laboratory Practice 4
Quality Assurance Statement 5
Contributing Scientist 6
Summary 7
1. Introduction 8
2. Test Substance 9
3. Experimental Procedure 10
3.1 Animal management 10
3.2 Test substance preparation 10
3.3 Treatment procedure 10
3.4 Serial observations 11
3.4.1 Clinical signs 11
3.4.2 Dermal responses 11
3.4.3 Interpretation of responses 12
3.4.4 Termination 13
3.5 Archives 13
3.6 Deviations from protocol 13
4. Results 14
4.1 Clinical signs 14
4.2 Dermal responses 14
5. Conclusion 15

List of Tables

Table 1 Mean values for erythema and oedema 16
Table 2 Individual values for erythema and oedema 17

List of Annexes

Annex 1 Weight of evidence 18
Annex 2 GLP Compliance Statements 20

[redacted]

Compliance with Good Laboratory Practice

[redacted] Skin 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] Skin 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	09 Apr 2013	09 Apr 2013
Protocol Amendment No. 1	29 May 2013	29 May 2013
Report Audit	18 Jul 2013 – 22 Jul 2013	22 Jul 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 - dermal	06 Nov 2012	06 Nov 2012
Study management & Conduct	10 Jun 2013	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] **Skin Irritation to the Rabbit**

Study management

[redacted] (Hons) CBiol MSB
Study Director

Summary

A study was performed to assess the skin 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.4 Acute Toxicity: Dermal irritation/corrosion. 30 May 2008.
>
> OECD Guideline for Testing of Chemicals No.404 "Acute Dermal Irritation/Corrosion". Adopted 24 April 2002.
>
> EPA Health Effects Test Guidelines OPPTS 870.2500 Acute Dermal Irritation EPA 712-C-98-196. August 1998.
>
> Japanese Ministry of Agriculture, Forestry and Fisheries, Test Data for Registration of Agricultural Chemicals, Skin Irritation (2-1-4), 12 Nohsan No. 8147, Agricultural Production Bureau, November 24, 2000.

Three rabbits received a single four hour, semi-occlusive, dermal administration of approximately 0.5 g of the test substance as supplied and were observed for four days.

No dermal reaction was observed in any animal throughout the duration of the study.

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

Means of scores at approximately 24, 48 and 72 hours		
Animal number	**Erythema**	**Oedema**
20	0.0	0.0
21	0.0	0.0
98	0.0	0.0
EC trigger values*	≥2.3	≥2.3

*Classification according to regulation (EC) 1272/2008 is triggered if means of scores for either effect are ≥ 2.3 for two or three animals (or if effects persist to Day 14 in at least two animals).

The Primary Irritation Index was calculated to be 0.0; [redacted] was classified as 'non-irritant' according to the criteria of the ECETOC and did not require labelling in accordance with Commission Regulation 1272/2008.

1. Introduction

The study was designed to assess skin irritation potential of [redacted] following a single dermal application to rabbits. The test substance may come into contact with skin 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.4 Acute Toxicity: Dermal irritation/corrosion. 30 May 2008.
>
> OECD Guideline for Testing of Chemicals No.404 "Acute Dermal Irritation/Corrosion". Adopted 24 April 2002.
>
> EPA Health Effects Test Guidelines OPPTS 870.2500 Acute Dermal Irritation EPA 712-C-98-196. August 1998.
>
> Japanese Ministry of Agriculture, Forestry and Fisheries, Test Data for Registration of Agricultural Chemicals, Skin Irritation (2-1-4), 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 skin irritation studies and is the animal recommended in the test guidelines.

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

The protocol was approved by the Study Director and [redacted] Management on 5 April 2013 and by the Sponsor on 22 May 2013.

The experimental start date was 8 April 2013 and the experimental completion date was 3 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:	OF1211
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 3.06 to 4.13 kg and 33 to 48 weeks of age, prior to treatment (Day 1). All rabbits were acclimatised to the experimental environment for a period of 16 or 28 weeks prior to the start of the study.

The rabbits were housed individually in plastic cages with perforated floors. Each rabbit 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 was 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. The animals were given a dietary supplement of hay.

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

On the day before application of the test substance, hair was removed with clippers from the dorso-lumbar region of each rabbit exposing an appropriate sized area of skin.

The treatment site was ‘wetted’ with 0.5mL of reverse osmosis water and approximately 0.5 g of the test substance was applied under a 2-ply 25 mm x 25 mm porous gauze pad secured with ‘blenderm’ surgical tape to intact skin sites on three animals. An additional site was similarly treated with the exception of test substance and acted as a control.

A single animal (number 98) received three exposures of three minutes, one or four hours duration in a step-wise manner and acted as a preliminary screen. In the absence of a severe effect on removal of the dressings the next exposure was initiated. In the absence of a severe effect in this animal two further animals were committed to the study.

For exposures of one hour or more each treatment site was covered with cotton wool and "Tubigrip" elasticated bandage dressing for the duration of the exposure period. The animals were returned to their cages immediately after treatment.

At the end of the exposure period the semi-occlusive dressing and gauze pad were removed and the treatment site was washed with lukewarm water (30-40°C) to remove any residual test substance. The treated area was blotted dry with absorbent paper.

3.4 Serial observations

3.4.1 Clinical signs

All animals were observed daily for signs of ill health or toxicity.

3.4.2 Dermal responses

Examination of the treated skin was made on removal of the dressings (for 3 minute or one hour exposures) and approximately 1, 24, 48 and 72 hours later. Only the data for the four hour exposure are reported, the data from the three minute and one hour exposures are held in the archives. Additional observations were made on Day 8 for the sentinel animal; these data are not reported and are held in the archive.

Local dermal irritation was assessed using the prescribed numerical system:

Erythema and eschar formation:

No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) or eschar formation (injuries in depth) preventing grading of erythema	4

Oedema formation:

No oedema	0
Very slight oedema (barely perceptible)	1
Slight oedema (edges of area well-defined by definite raising)	2
Moderate oedema (raised approximately 1 millimetre)	3
Severe oedema (raised more than 1 millimetre and extending beyond the area of exposure)	4

3.4.3 Interpretation of responses

Primary Irritation Index

A primary irritation index (PII) was calculated from the erythema and oedema scores according to the following formula as described in Technical Report No. 66 "Skin irritation and Corrosion: Reference chemicals data bank" (March 1995) ECETOC, Brussels.

$$PII = \frac{\sum \text{Erythema at 24/48/72 hours} + \sum \text{Oedema at 24/48/72 hours}}{3 \times \text{number of animals}}$$

The maximum possible score was 8.0. The PII was then used to classify the test substance as follows:

Primary Irritation Index	Classification
0	non-irritant
>0 - 2.0	mildly irritating
2.1 - 5.0	moderate irritant
5.1 - 6.0	moderate to severe irritant
> 6.0	severe irritant

This classification system is a modification based on "Appraisal of the Safety of Chemicals in Foods Drugs and Cosmetics" published by the Association of Food and Drug Officials of the United States, 1959.

EU Classification

The Official Journal of the European Communities (Regulation 1272/2008) contains the following criteria for classification of irritants and corrosives to skin.

Corrosion: a corrosive substance is a substance that produces destruction of skin tissue, namely, visible necrosis through the epidermis and into the dermis, in at least one tested animal after exposure up to four hours duration. Corrosive reactions are typified by ulcers, bleeding, bloody scabs and, by the end of observation at 14 days, by discolouration due to blanching of the skin, complete areas of alopecia and scars. Corrosive substances are assigned to Category 1; three subcategories are provided within the corrosive category:

Subcategory 1A: where responses are noted following up to 3 minutes exposure and up to 1 hour observation

Subcategory 1B: where responses are described following exposure between 3 minutes and 1 hour and observations up to 14 days

Subcategory 1C: where responses occur after exposures between 1 hour and 4 hours and observations up to 14 days

Irritation: an irritant substance is a substance that produces reversible damage to the skin following an exposure of up to four hours. Irritant substances are assigned to Category 2; the following thresholds apply:

> Mean value of ≥2.3 - ≤4.0 for erythema/eschar or for oedema in at least 2 of 3 tested animals from gradings at 24, 48 and 72 hours after patch removal or, if reactions are delayed, from grades on 3 consecutive days after the onset of skin reactions.
>
> Inflammation that persists to the end of the observation period normally 14 days in at least 2 animals, particularly taking in to account alopecia (limited area), hyperkeratosis, hyperplasia, and scaling.
>
> In some cases where there is pronounced variability of response among animals, with very definite positive effects related to chemical exposure in a single animal but less that the criteria above.

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

The study protocol indicated that the animals were to be 12 to 40 weeks of age on commencement of the study. One of the animals was 48 weeks old when they were committed to the study. Since the animal was healthy and the skin was in good condition immediately prior to administration, this increase in age is considered to have no impact on the study outcome.

There was no other 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 Dermal responses

No dermal reaction was observed in any animal throughout the duration of the study.

5. Conclusion

The Primary Irritation Index was calculated to be 0.0; [redacted] classified as 'non-irritant' according to the criteria of the ECETOC and did not require labelling in accordance with Commission Regulation 1272/2008.

Table 1 Mean values for erythema and oedema

24, 48 and 72 hours after removal of dressings *

Animal number and sex	Erythema		Oedema	
	Test	Control	Test	Control
20 F	0.0	0.0	0.0	0.0
21 F	0.0	0.0	0.0	0.0
98 F	0.0	0.0	0.0	0.0

* 4 hour exposure
F Female

Table 2 Individual values for erythema and oedema

Test site: **0.5 g** [redacted]
Control site: No treatment
} semi occluded for four hours

Animal number and sex	Type of Response	Score after removal of dressings							
		1 hour		24 hours		48 hours		72 hours	
		Test site	Control site	Test site	Control site	Test site	Control site	Test site	Control site
20 F	Erythema	0	0	0	0	0	0	0	0
	Oedema	0	0	0	0	0	0	0	0
21 F	Erythema	0	0	0	0	0	0	0	0
	Oedema	0	0	0	0	0	0	0	0
98 F#	Erythema	0	0	0	0	0	0	0	0
	Oedema	0	0	0	0	0	0	0	0

F Female
Sentinel animal

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]

[redacted]

MW: [redacted]

Melting point: 176-190°C (HLS Study CVJ0166)

Boiling point: more than 210°C (HLS Study 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.

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] (Study [redacted] - 5.8.
pH (1% w/v dispersion as measured at [redacted] ([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 skin irritation study.

Annex 2 GLP Compliance Statements

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
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).

14/9/12

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

MHRA