SafePharm Laboratories

13F-SFMA MONOMER:

ACUTE EYE IRRITATION IN THE RABBIT

SPL PROJECT NUMBER: 1458/0067

AUTHOR:

STUDY SPONSOR:

Daikin Industries, Ltd.
1-1, Nishi Hitotsuya, Settsu-shi
OSAKA 566-8585
JAPAN

TEST FACILITY:

Safepharm Laboratories Limited
Shardlow Business Park
Shardlow
Derbyshire
DE72 2GD
UK

Telephone: +44 (0) 1332 792896

Facsimile: +44 (0) 1332 799018

QUALITY ASSURANCE REPORT

This study type is classed as short-term. The standard test method for this study type ("General Study Plan" in OECD terminology) was reviewed for compliance once only on initial production. Inspection of the routine and repetitive procedures that constitute the study is carried out as a continuous process designed to encompass the major phases at or about the time this study was in progress.

This report has been audited by Safepharm Quality Assurance Unit, and is considered to be an accurate account of the data generated and of the procedures followed.

In each case, the outcome of QA evaluation is reported to the Study Director and Management on the day of evaluation. Audits of study documentation, and process inspections appropriate to the type and schedule of this study were as follows:

	31 January 2003	Standard Test Method Compliance Audit
	05 February 2007	Test Material Preparation
	05 February 2007	Animal Preparation
	05 February 2007	Dosing
	14 February 2007	Assessment of Response
§	21 March 2007	Draft Report Audit
§	Date of QA Signature	Final Report Audit

§ Evaluation specific to this study

..... DATE: 28 MAR 2007

For Safepharm Quality Assurance Unit*

***Authorised QA Signatures:**
Head of Department:
Deputy Head of Department:
Senior Audit Staff:

GLP COMPLIANCE STATEMENT

The work described 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 international standards are acceptable to the Regulatory agencies of the following countries: Australia, Austria, Belgium, Canada, the Czech Republic, Denmark, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Israel, Italy, Japan, Republic of Korea, Luxembourg, Mexico, The Netherlands, New Zealand, Norway, Poland, Portugal, Slovenia, South Africa, Spain, Sweden, Switzerland, Turkey, the United Kingdom, and the United States of America.

This report fully and accurately reflects the procedures used and data generated.

..... DATE: 28/3/07

Study Director

CONTENTS

13F-SFMA MONOMER:

ACUTE EYE IRRITATION IN THE RABBIT

SUMMARY

Introduction. The study was performed to assess the irritancy potential of the test material to the eye of the New Zealand White rabbit. The method was designed to meet the requirements of the following:

- OECD Guidelines for the Testing of Chemicals No. 405 "Acute Eye Irritation/Corrosion" (adopted 24 April 2002)
- Method B5 Acute Toxicity (Eye Irritation) of Commission Directive 2004/73/EC

Result. A single application of the test material to the non-irrigated eye of three rabbits produced minimal conjunctival irritation. One treated eye appeared normal throughout the study and the remaining two treated eyes appeared normal at the 24-hour observation.

Conclusion. The test material produced a maximum group mean score of 2.7 and was classified as a minimal irritant (Class 3 on a 1 to 8 scale) to the rabbit eye according to a modified Kay and Calandra classification system.

13F-SFMA MONOMER:

ACUTE EYE IRRITATION IN THE RABBIT

1. INTRODUCTION

The study was performed to assess the irritancy potential of the test material following a single application to the rabbit eye. The method was designed to meet the requirements of the following:

- OECD Guidelines for the Testing of Chemicals No. 405 "Acute Eye Irritation/Corrosion" (adopted 24 April 2002)
- Method B5 Acute Toxicity (Eye Irritation) of Commission Directive 2004/73/EC

The albino rabbit has been shown to be a suitable model for this type of study and is recommended in the test method. The results of the study are believed to be of value in predicting the likely eye irritancy potential of the test material to man.

The study was performed between 29 January 2007 and 08 February 2007.

2. TEST MATERIAL

2.1 Description, Identification and Storage Conditions

Sponsor's identification	:	13F-SFMA MONOMER
Description	:	clear colourless liquid
Batch number	:	061115
Date received	:	24 November 2006
Storage conditions	:	room temperature in the dark

The integrity of supplied data relating to the identity, purity and stability of the test material is the responsibility of the Sponsor.

2.2 Preparation of Test Material

For the purpose of the study the test material was used as supplied.

The absorption of the test material was not determined.

2.3 Measurement of pH

The pH of the test material was determined prior to commencement of the study and found to be as follows:

Preparation	pH Measurement	
	immediately	after 10 minutes
Undiluted as Supplied	8.4	not applicable
90% v/v aqueous preparation of the test material	8.4	8.6

3. METHODS

3.1 Animals and Animal Husbandry

Three New Zealand White rabbits were supplied by an accredited supplier. At the start of the study the animals were in the weight range of 2.0 to 3.5 kg and were twelve to twenty weeks old. After an acclimatisation period of at least five days each animal was given a number unique within the study which was written with a black indelible marker-pen on the inner surface of the ear and on the cage label.

The animals were individually housed in suspended cages. Free access to mains drinking water and food (Certified Rabbit Diet) was allowed throughout the study. The diet and drinking water were considered not to contain any contaminant of a level that might have affected the purpose or integrity of the study.

The temperature and relative humidity were set to achieve limits of 17 to 23°C and 30 to 70% respectively. Any occasional deviations from these targets were considered not to have affected the purpose or integrity of the study. 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.

3.2 Procedure

Immediately before the start of the test, both eyes of the provisionally selected test rabbits were examined for evidence of ocular irritation or defect with the aid of a light source from a standard ophthalmoscope. Only animals free of ocular damage were used.

Initially, a single rabbit was treated. A volume of 0.1 ml of the test material was placed into the conjunctival sac of the right eye, formed by gently pulling the lower lid away from the eyeball. The upper and lower eyelids were held together for about one second immediately after treatment, to prevent loss of the test material, and then released. The left eye remained untreated and was used for control purposes. Immediately after administration of the test material, an assessment of the initial pain reaction was made according to the six point scale shown in Appendix 1.

After consideration of the ocular responses produced in the first treated animal, two additional animals were treated.

Assessment of ocular damage/irritation was made approximately 1 hour and 24, 48 and 72 hours following treatment, according to the numerical evaluation given in Appendix 2, (from Draize J H (1977) "Dermal and Eye Toxicity Tests" In: Principles and Procedures for Evaluating the Toxicity of Household Substances, National Academy of Sciences, Washington DC p.48 to 49).

Any other ocular effects were also noted. Examination of the eye was facilitated by the use of the light source from a standard ophthalmoscope.

3.3 Interpretation of Results

The numerical values corresponding to each animal, tissue and observation time were recorded. The data relating to the conjunctivae were designated by the letters A (redness), B (chemosis) and C (discharge), those relating to the iris designated by the letter D and those relating to the cornea by the letters E (degree of opacity) and F (area of cornea involved). For each tissue the score was calculated as follows:

Score for conjunctivae	=	(A + B + C) x 2
Score for iris	=	D x 5
Score for cornea	=	(E x F) x 5

Using the numerical data obtained a modified version of the system described by Kay J H and Calandra J C (1962), J. Soc. Cosmet. Chem. **13**, 281-289 (see Appendix 3) was used to classify the ocular irritancy potential of the test material. This was achieved by adding together the scores for the cornea, iris and conjunctivae for each time point for each rabbit. The group means of the total scores for each observation were calculated. The highest of these group means (the maximum group mean score) together with the persistence of the reactions enabled classification of the eye irritancy potential of the test material.

If evidence of irreversible ocular damage is noted, the test material will be classified as corrosive to the eye.

4. ARCHIVES

Unless instructed otherwise by the Sponsor, all original data and the final report will be retained in the Safepharm archives for five years, after which instructions will be sought as to further retention or disposal.

5. RESULTS

Individual and group mean scores for ocular irritation are given in Table 1 and Table 2.

No corneal or iridial effects were noted during the study.

Minimal conjunctival irritation was noted in two treated eyes one hour after treatment.

One treated eye appeared normal throughout the study and the remaining two treated eyes appeared normal at the 24-hour observation.

6. CONCLUSION

The test material produced a maximum group mean score of 2.7 and was classified as a MINIMAL IRRITANT (CLASS 3 ON A 1 TO 8 SCALE) to the rabbit eye according to a modified Kay and Calandra classification system.

Table 1 Individual Scores and Individual Total Scores for Ocular Irritation

Rabbit Number and Sex	65700 Male				65726 Male				65727 Male			
	IPR = 2				IPR = 2				IPR = 2			
Time After Treatment	1 Hour	24 Hours	48 Hours	72 Hours	1 Hour	24 Hours	48 Hours	72 Hours	1 Hour	24 Hours	48 Hours	72 Hours
CORNEA												
E = Degree of Opacity	0	0	0	0	0	0	0	0	0	0	0	0
F = Area of Cornea Involved	0	0	0	0	0	0	0	0	0	0	0	0
Score (E x F) x 5	0	0	0	0	0	0	0	0	0	0	0	0
IRIS												
D	0	0	0	0	0	0	0	0	0	0	0	0
Score (D x 5)	0	0	0	0	0	0	0	0	0	0	0	0
CONJUNCTIVAE												
A = Redness	1	0	0	0	0	0	0	0	1	0	0	0
B = Chemosis	0	0	0	0	0	0	0	0	0	0	0	0
C = Discharge	1	0	0	0	0	0	0	0	1	0	0	0
Score (A + B + C) x 2	4	0	0	0	0	0	0	0	4	0	0	0
Total Score	4	0	0	0	0	0	0	0	4	0	0	0

IPR = Initial pain reaction

13F-SFMA MONOMER : ACUTE EYE IRRITATION IN THE RABBIT

Table 2 Individual Total Scores and Group Mean Scores for Ocular Irritation

Rabbit Number and Sex	Individual Total Scores At:			
	1 Hour	24 Hours	48 Hours	72 Hours
65700 Male	4	0	0	0
65726 Male	0	0	0	0
65727 Male	4	0	0	0
Group Total	8	0	0	0
Group Mean Score	2.7	0.0	0.0	0.0

13F-SFMA MONOMER : ACUTE EYE IRRITATION IN THE RABBIT

Appendix 1 Initial Pain Reaction

When the material is instilled in the eye there may be an initial local pain reaction. The reaction will be graded as follows:

Class	Reaction by Animal	Descriptive Rating
0	No response	No initial pain
1	A few blinks only, normal within one or two minutes	Practically no initial pain
2	Rabbit blinks and tries to open eye, but reflex closes it	Slight initial pain
3	Rabbit holds eye shut and puts pressure on lids, may rub eye with paw	Moderate initial pain
4	Rabbit holds eye shut vigorously, may squeal	Severe initial pain
5	Rabbit holds eye shut vigorously, may squeal, claw at eye, jump and try to escape	Very severe initial pain

There is often no correlation between the initial pain and the subsequent eye irritation.

13F-SFMA MONOMER : ACUTE EYE IRRITATION IN THE RABBIT

Appendix 2 Draize Scale for Scoring Ocular Irritation

1. CONJUNCTIVAE

(A) Redness (refers to palpebral and bulbar conjunctivae excluding cornea and iris)

Vessels normal	0
Vessels definitely injected above normal	1
More diffuse, deeper crimson red, individual vessels not easily discernible	2
Diffuse beefy red	3

(B) Chemosis

No swelling	0
Any swelling above normal (includes nictitating membrane)	1
Obvious swelling with partial eversion of lids	2
Swelling with lids about half closed	3
Swelling with lids half closed to completely closed	4

(C) Discharge

No discharge	0
Any amount different from normal (does not include small amounts observed in inner canthus of normal animals)	1
Discharge with moistening of the lids and hairs just adjacent to lids	2
Discharge with moistening of the lids and hairs a considerable area around the eye	3

THE TOTAL SCORE = (A + B + C) x 2 **MAXIMUM TOTAL = 20**

2. IRIS

(D) Values

Normal	0
Folds above normal, congestion, swelling, circumcorneal injection (any or all 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

THE TOTAL SCORE = D x 5 **MAXIMUM TOTAL = 10**

3. CORNEA

(E) Degree of Opacity (most dense area used)

No opacity	0
Scattered or diffuse areas, details of iris clearly visible	1
Easily discernible translucent areas, details of iris slightly obscured	2
Opalescent areas, no details of iris visible, size of pupil barely discernible	3
Opaque, iris not discernible through the opacity	4

(F) Area of Cornea Involved

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 TOTAL SCORE = (E x F) x 5 **MAXIMUM TOTAL = 80**

MAXIMUM TOTAL SCORE POSSIBLE = 110

13F-SFMA MONOMER : ACUTE EYE IRRITATION IN THE RABBIT

Appendix 3 Modified Kay and Calandra Interpretation of Eye Irritation Test

MAXIMUM MEAN SCORE		PERSISTENCE OF SCORE	DESCRIPTION RATING (AND CLASS)	
0.0 to 0.5	Group mean total score at 24 hours = 0 Group mean total score at 24 hours > 0		Non-irritant Practically non-irritant	(1) (2)
0.5 to 2.5	Group mean total score at 24 hours = 0 Group mean total score at 24 hours > 0		Practically non-irritant Minimal irritant	(2) (3)
2.5 to 15	Group mean total score at 48 hours = 0 Group mean total score at 48 hours > 0		Minimal irritant Mild irritant	(3) (4)
15 to 25	Group mean total score at 72 hours = 0 Group mean total score at 72 hours > 0		Mild irritant Moderate irritant	(4) (5)
25 to 50	Group mean total score at 7 days 20 or less	More than half of the individual total scores at 7 days 10 or less	Moderate irritant	(5)
		More than half of the individual total scores at 7 days > 10 but no individual total score at 7 days > 30	Moderate irritant	(5)
		More than half of the individual total scores at 7 days > 10 and any individual score at 7 days > 30	Severe irritant	(6)
	Group mean total score at 7 days > 20		Severe irritant	(6)
50 to 80	Group mean total score at 7 days 40 or less	More than half of the individual total scores at 7 days 30 or less	Severe irritant	(6)
		More than half of the individual total scores at 7 days > 30 but no individual total scores at 7 days > 60	Severe irritant	(6)
		More than half of the individual total scores at 7 days > 30 and individual total score at 7 days > 60	Very severe irritant	(7)
	Group mean total score at 7 days > 40		Very severe irritant	(7)
80 to 100	Group mean total score at 7 days 80 or less	More than half of the individual total scores at 7 days 60 or less	Very severe irritant	(7)
		More than half of the individual total scores at 7 days > 60 but no individual total score at 7 days > 100	Very severe irritant	(7)
		More than half of the individual total scores at 7 days > 60 and individual total score at 7 days > 100	Extremely severe irritant	(8)
	Group mean total score at 7 days > 80		Extremely severe irritant	(8)
100 to 110	Group mean total score at 7 days 80 or less		Very severe irritant	(7)
	Group mean total score at 7 days > 80		Extremely severe irritant	(8)

Appendix 4 Statement of GLP Compliance in Accordance with Directive 2004/9/EC

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

LABORATORY

SafePharm Laboratories Ltd.
Shardlow Business Park
London Road
Shardlow
Derby
DE72 2GD

TEST TYPE

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

DATE OF INSPECTION

30th August 2005

A general inspection for compliance with the Principles of Good Laboratory Practice was carried out at the above laboratory as part of the UK GLP Compliance Programme.

At the time of inspection no deviations were found of sufficient magnitude to affect the validity of non-clinical studies performed at these facilities.

105.

Head, UK GLP Monitoring Authority