Data

Polyglyceryl Fatty Acid Esters

CIR EXPERT PANEL MEETING

SEPTEMBER 26 - 27, 2016

INFORMATION INCLUDED IN THE DATA SUPPLEMENT

1. Information on Glyceryl/Polyglyceryl-6 Isostearate/Behenate Esters (*PGlyFE092016data_1*)
    a. 6 studies: dermal irritation in rabbits; clinical patch test; ocular irritation in rabbits; guinea pig maximization test; acute oral toxicity; Ames test

2. Information on Macadamia Seed Oil Polyglyceryl-6 Esters Behenate (*PGlyFE092016data_2*)
    a. 6 studies: acute oral toxicity; Ames test; clinical patch test; dermal irritation in rabbits; guinea pig maximization test; ocular irritation in rabbits

3. Information on Polyglyceryl-8 Decabehenate/Caprate (*PGlyFE092016data_3*)
    a. 6 studies: Ames test; dermal irritation in rabbits; clinical irritation study; ocular irritation in rabbits; guinea pig maximization test; acute oral toxicity

4. Information on Polyglyceryl-8 Decaerucate/Decaisostearate/Decaricinoleate (*PGlyFE092016data_4*)
    a. 5 studies: dermal irritation in rabbits; guinea pig maximization test; ocular irritation in rabbits; acute oral toxicity; Ames test

5. Information on Polyglyceryl-10 Decaethylhexanoate (*PGlyFE092016data_5*)
    a. 5 studies: bacterial reverse mutation assay; in vitro ocular irritation; clinical patch test; in vitro skin irritation; clinical sensitization test

6. Information on Polyglyceryl-10 Pentaisostearate (*PGlyFE092016data_6*)
    a. 5 studies: bacterial reverse mutation assay; in vitro ocular irritation; clinical patch test; in vitro skin irritation; clinical sensitization test

7. Information on Polyglyceryl-10 Nonaisostearate (*PGlyFE092016data_7; PGlyFE092016data_8*)
    a. 9 studies: ocular irritation in rabbits; acute oral toxicity; dermal irritation in rabbits; clinical irritation study; photosensitization in guinea pigs; dermal irritation in guinea pigs; LLNA; Ames test; phototoxicity in guinea pigs.

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Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: Lillian Gill, D.P.A.
Director - COSMETIC INGREDIENT REVIEW (CIR)

FROM: Beth A. Lange, Ph.D.
Industry Liaison to the CIR Expert Panel

DATE: March 25, 2016

SUBJECT: Information on Glyceryl/Polyglyceryl-6 Isostearate/Behenate Esters (SLR posted January 28, 2016)

SafePharm Laboratories. 2006. Acute dermal irritation in the rabbit (Glyceryl/Polyglyceryl-6 Isostearate/Behenate Esters).

Japan Hair Science Association. 2006. Patch test on the cosmetic ingredient Glyceryl/Polyglyceryl-6 Isostearate/Behenate Esters.

SafePharm Laboratories. 2006. Acute eye irritation in the rabbit Glyceryl/Polyglyceryl-6 Isostearate/Behenate Esters).

Phycher. 2006. Skin sensitization in the guinea pig- Magnusson and Kligman maximisation method (Glyceryl/Polyglyceryl-6 Isostearate/Behenate Esters).

SafePharm Laboratories. 2006. Acute oral toxicity in the rat - acute toxic class method (Glyceryl/Polyglyceryl-6 Isostearate/Behenate Esters).

SafePharm Laboratories. 2006. Reverse mutation assay "Ames test" using *Salmonella typhimurium* and *Escherichia coli* (Glyceryl/Polyglyceryl-6 Isostearate/Behenate Esters).

1620 L Street, N.W., Suite 1200 | Washington, D.C. 20036 | 202.331.1770 | 202.331.1969 (fax) | www.personalcarecouncil.org

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SafePharm Laboratories

S-Face VL-212:

Glyceryl / Polyglyceryl-6 Isostearate / Behenate Esters

ACUTE DERMAL IRRITATION IN THE RABBIT

SPL PROJECT NUMBER: 0432/0086

AUTHOR: A Pooles

STUDY SPONSOR:

Sakamoto Yakuhin Kogyo Co., Ltd.
1-20, Rinkaicho
Izumiotsu-city
OSAKA 595-01075
JAPAN

TEST FACILITY:

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

Telephone: +44 (0) 1332 792896

Facsimile: +44 (0) 1332 799018

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QUALITY ASSURANCE REPORT

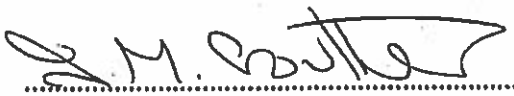
This study type is classed as short-term. The standard test method for this study type ("General Study Plan" in OECD terminology) was reviewed for compliance once only on initial production. Inspection of the routine and repetitive procedures that constitute the study is carried out as a continuous process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

	15 June 2004	Standard Test Method Compliance Audit
	03 October 2006	Test Material Preparation
	03 October 2006	Animal Preparation
	03 October 2006	Dosing
	25 October 2006	Assessment of Response
§	23 November 2006	Draft Report Audit
§	Date of QA Signature	Final Report Audit

§ Evaluation specific to this study



DATE: 20 DEC 2006

For Safepharm Quality Assurance Unit*

*Authorised QA Signatures:
Head of Department: JR Pateman CBiol MIBiol DipRQA AIQA FRQA
Deputy Head of Department: JM Crowther MIScT MRQA
Senior Audit Staff: JV Johnson BSc MRQA; G Wren ONC MRQA

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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.

A. Pools DATE: 8/12/06

A Pooles BA (Hons)
Study Director

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S-Face VL-212:

ACUTE DERMAL IRRITATION IN THE RABBIT

SUMMARY

Introduction. The study was performed to assess the irritancy potential of the test material to the skin 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. 404 "Acute Dermal Irritation/Corrosion" (adopted 24 April 2002)
- Method B4 Acute Toxicity (Skin Irritation) of Commission Directive 2004/73/EC

Results. A single 4-hour, semi-occluded application of the test material to the intact skin of three rabbits produced very slight erythema. One treated skin site appeared normal throughout the study and the two remaining treated skin sites appeared normal at the 48-hour observation.

3-minute and 1-hour semi-occluded applications of the test material to the intact skin of one rabbit produced no evidence of skin irritation.

Conclusion. The test material produced a primary irritation index of 0.3 and was classified as a mild irritant to rabbit skin according to the Draize classification scheme.

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S-Face VL-212:

ACUTE DERMAL IRRITATION IN THE RABBIT

1. INTRODUCTION

The study was performed to assess the irritancy potential of the test material following single, 3-minute, 1 and 4-hour, semi-occluded applications to the intact rabbit skin. The method was designed to meet the requirements of the following:

- OECD Guidelines for the Testing of Chemicals No. 404 "Acute Dermal Irritation/Corrosion" (adopted 24 April 2002)
- Method B4 Acute Toxicity (Skin 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 skin irritancy potential of the test material to man.

The study was performed between 19 September 2006 and 10 November 2006.

2. TEST MATERIAL

2.1 Description, Identification and Storage Conditions

Sponsor's identification	:	S-Face VL-212
Description	:	white waxy solid
Batch number	:	60209
Date received	:	05 June 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.

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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
10% w/w aqueous preparation of the test material	9.2 (approximately 7.8*)

* = after 10 and 20 minutes

3. METHODS

3.1 Animals and Animal Husbandry

One New Zealand White rabbit was supplied by Charles River (France), Charlaronne, France and two New Zealand White rabbits were supplied by Harlan UK Limited, Bicester, Oxon, UK. The animal supplier was different to that stated in the Standard Test Method. This deviation was considered not to affect the purpose or integrity of the study. 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 (Code 5322) supplied by BCM IPS Limited, London, UK) 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.

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3.2 Procedure

On the day before the test each rabbit was clipped free of fur from the dorsal/flank area using veterinary clippers. Only animals with a healthy intact epidermis by gross observation were selected for the study.

One rabbit was initially treated. Three suitable sites were selected on the back of the rabbit. At each test site a quantity of 0.5 ml of the test material was introduced under a 2.5 cm x 2.5 cm cotton gauze patch and placed in position on the shorn skin. Each patch was secured in position with a strip of surgical adhesive tape. To prevent the animal interfering with the patches, the trunk of the rabbit was wrapped in an elasticated corset and the animal was returned to its cage for the duration of the 1 and 4 hour exposure periods.

One patch was removed at each of three time points: 3 minutes, 1 hour and 4 hours after application. Any residual test material was removed by gentle swabbing with cotton wool soaked in distilled water.

After consideration of the skin reactions produced in the first animal, two additional animals were treated with 0.5ml of test material. One patch was applied to the back of each rabbit, and was allowed to remain in contact with the skin for a period of four hours.

Approximately one hour following the removal of the patches, and 24, 48 and 72 hours later, the test sites were examined for evidence of primary irritation and scored according to the following scale:

EVALUATION OF SKIN REACTIONS

Erythema and Eschar Formation	**Value**
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beef redness) to eschar formation preventing grading of erythema	4

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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

Any other skin reactions, if present, were also recorded.

3.3 Interpretation of Results

Calculation of Primary Irritation Index and Grading of Irritancy Potential Using the Draize Scheme

The scores for erythema and oedema at the 24 and 72-hour readings were totalled for the three test rabbits (12 values) and this total was divided by six to give the primary irritation index of the test material. The test material was classified according to the following scheme devised by Draize J H (1959) "Dermal Toxicity" In: Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics. Association of Food and Drug Officials of the United States, Austin, Texas, p.47:

Primary Irritation Index	Classification of Irritancy
0	Non-irritant
> 0 to 2	Mild irritant
> 2 to 5	Moderate irritant
> 5 to 8	Severe irritant

If irreversible alteration of the dermal tissue is noted in any rabbit, as judged by the Study Director, which include ulceration and clear necrosis or signs of scar tissue, the test material is classified as corrosive to rabbit skin. Classification according to Draize may, therefore, not be applicable.

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.

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5. RESULTS

5.1 4-Hour Exposure Period

The individual scores for erythema/eschar and oedema are given in Table 1.

Very slight erythema was noted at one treated skin site one hour after patch removal with very slight erythema noted at two treated skin sites at the 24-hour observation.

One treated skin site appeared normal throughout the study and the remaining two treated skin sites appeared normal at the 48-hour observation.

5.2 1-Hour Exposure Period

The individual scores for erythema/eschar and oedema are given in Appendix 1.

No evidence of skin irritation was noted during the study.

5.3 3-Minute Exposure Period

The individual scores for erythema/eschar and oedema are given in Appendix 1.

No evidence of skin irritation was noted during the study.

6. CONCLUSION

The test material produced a primary irritation index of 0.3 and was classified as a MILD IRRITANT to rabbit skin according to the Draize classification scheme. No corrosive effects were noted.

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S-Face VL-212 : ACUTE DERMAL IRRITATION IN THE RABBIT

Table 1 Individual Skin Reactions Following 4-Hour Exposure

Skin Reaction	Observation Time	Individual Scores – Rabbit Number and Sex			Total
		64941 Male	65088 Male	65089 Male	
Erythema/Eschar Formation	1 Hour	0	1	0	(1)
	24 Hours	0	1	1	2
	48 Hours	0	0	0	(0)
	72 Hours	0	0	0	0
Oedema Formation	1 Hour	0	0	0	(0)
	24 Hours	0	0	0	0
	48 Hours	0	0	0	(0)
	72 Hours	0	0	0	0
Sum of 24 and 72-hour Readings (S)		: 2			
Primary Irritation Index (S/6)		: 2/6 = 0.3			
Classification		: MILD IRRITANT			

( ) = Total values not used for calculation of primary irritation index

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S-Face VL-212 : ACUTE DERMAL IRRITATION IN THE RABBIT

Appendix 1 Individual Skin Reactions Following 1-Hour and 3-Minute Exposures

Skin Reaction	Observation Time	Individual Scores - Rabbit Number and Sex	
		64941 Male	
		1-Hour Exposure	3-Minute Exposure
Erythema/Eschar Formation	1 Hour	0	0
	24 Hours	0	0
	48 Hours	0	0
	72 Hours	0	0
Oedema Formation	1 Hour	0	0
	24 Hours	0	0
	48 Hours	0	0
	72 Hours	0	0

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Appendix 2 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	TEST TYPE
SafePharm Laboratories Ltd. **Shardlow Business Park** **London Road** **Shardlow** **Derby** **DE72 2GD**	**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.

Bryan J Wright 21/11/05.

Mr. Bryan J. Wright
Head, UK GLP Monitoring Authority

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STUDY REPORT

Patch test on the cosmetic ingredient

Japan Hair Science Association
(Incorporated Association)

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Nichimokyo №18018-(2)
5th August , 2006

To SAKAMOTO YAKUHIN KOGYO CO.,LTD.

This report presents the results of patch test on a cosmetic ingredient, performed by Japan Hair Science Association. It was sponsored by To SAKAMOTO YAKUHIN KOGYO CO.,LTD. on 2th June, 2006. This report is an English version of the original, which was written in Japanese. The undersigned hereby declare that this version faithfully reflects the original report to the best of my knowledge.

TEST FOR IRRITATION EFFECTS OF COSMETIC INGREDIENT ON HUMAN SKIN

1.Purpose of the test

This patch test was performed to assess possible irritation potential of a cosmetic ingredient on healthy human skin, sponsored by SAKAMOTO YAKUHIN KOGYO CO.,LTD. on 2th June, 2006.

2.Test sample

The supplied sample is the following cosmetic ingredient.

Sample : S-Face VL-212 Lot №60209 Glyceryl / Polyglyceryl-6 Isostearate / Behenate Esters

Control ①: Petrolatum(SUNWHITE P-1)

Control ②: Soap(0.5%)

3.Test procedure

(1)Volunteers

16 males and 29 females (45 people in total), age 20 to 65, were selected as volunteers.

(2)Applied amount

0.01g of test sample was applied to each volunteer.

(3)Method of patch test

0.01g of test sample was placed on FINN CHAMBER(EPITEST Ltd. Oy)and applied to the crooked side of upper arm of each volunteer for 24 hours in occlusive condition to for evaluation.

At the end of 24 hours exposure period, the test sample was removed. Skin reactions were evaluated 1 hour and 24 hours after removing the test sample.

(4)Criteria for evaluation

The reaction was evaluated using the following criteria.

−	: No reaction
±	: Very slight erythema
+	: Well-defined erythema
++	: Erythema with swelling or with papule
+++	: Blisters in addition to the above symptom
++++	: Large blister

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Takashi Nagashima, M.D., a Vice-chairmam of Directors of Japan Hair Science Association(Dermatologist) was in overall charge of the tests and observations reported herein.

4.Results

The results of this test on 45 volunteers were as shown in the Table. In all volunteers, no reaction was observed in 1 hour after removing the test sample (shown in Table as 24-hours Evaluation time). Similarly, no reaction was observed in 24 hours after removing the test sample (shown in Table as 48-hours Evaluation time) in all volunteers.

5.Conclusion

The result of patch test carried out based on the request from your company is as abovementioned.

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Table Results of patch test(1)

No.	Volunteer	Sex	Age	Evaluationtime(hrs.)	Sample	Control①	Control②	Note(anamncsis)
1	S. A.	F	48	24	–	–	–	Healthy
				48	–	–	–	
2	A. I.	F	46	24	–	–	–	Healthy
				48	–	–	–	
3	S. I.	F	20	24	–	–	–	Healthy
				48	–	–	–	
4	M. O.	F	21	24	–	–	–	Healthy
				48	–	–	–	
5	M. O.	F	20	24	–	–	–	Healthy
				48	–	–	–	
6	R. O.	F	33	24	–	–	–	Healthy
				48	–	–	–	
7	Y. K.	F	30	24	–	–	–	Healthy
				48	–	–	–	
8	S. K.	F	25	24	–	–	–	Healthy
				48	–	–	–	
9	A. K.	F	26	24	–	–	–	Healthy
				48	–	–	–	
10	K. K.	M	55	24	–	–	–	Healthy
				48	–	–	–	
11	J. K.	F	35	24	–	–	–	Healthy
				48	–	–	–	
12	A. K.	F	48	24	–	–	–	Healthy
				48	–	–	–	
13	I. S.	F	20	24	–	–	–	Healthy
				48	–	–	–	
14	M. S.	F	32	24	–	–	–	Healthy
				48	–	–	–	
15	I. S.	M	43	24	–	–	–	Healthy
				48	–	–	–	
16	Y. S.	M	20	24	–	–	–	Healthy
				48	–	–	–	
17	Y. S.	M	35	24	–	–	–	Healthy
				48	–	–	–	
18	T. S.	F	60	24	–	–	–	Healthy
				48	–	–	–	
19	M. T.	F	35	24	–	–	–	Healthy
				48	–	–	–	
20	K. T.	F	24	24	–	–	–	Healthy
				48	–	–	–	
21	S. T.	F	57	24	–	–	–	Healthy
				48	–	–	–	
22	J. T.	M	54	24	–	–	–	Healthy
				48	–	–	–	
23	K. T.	M	54	24	–	–	–	Healthy
				48	–	–	–	
24	T. T.	F	21	24	–	–	–	Healthy
				48	–	–	–	
25	K. T.	F	46	24	–	–	–	Healthy
				48	–	–	–	

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Table Results of patch test(2)

No.	Volunteer	Sex	Age	Evaluationtime(hrs.)	Sample	Control①	Control②	Note(anamnesis)
26	J. T.	F	43	24	–	–	–	Healthy
				48	–	–	–	
27	H. N.	M	23	24	–	–	–	Healthy
				48	–	–	–	
28	Y. N.	M	55	24	–	–	–	Healthy
				48	–	–	–	
29	E. N.	F	27	24	–	–	–	Healthy
				48	–	–	–	
30	T. H.	F	34	24	–	–	–	Healthy
				48	–	–	–	
31	N. B.	F	49	24	–	–	–	Healthy
				48	–	–	–	
32	M. H.	F	31	24	–	–	–	Healthy
				48	–	–	–	
33	R. F.	M	20	24	–	–	–	Healthy
				48	–	–	–	
34	S. F.	F	36	24	–	–	–	Healthy
				48	–	–	–	
35	K. H.	M	65	24	–	–	–	Healthy
				48	–	–	–	
36	S. M.	M	57	24	–	–	–	Healthy
				48	–	–	–	
37	N. M.	F	25	24	–	–	–	Healthy
				48	–	–	–	
38	T. M.	M	35	24	–	–	–	Healthy
				48	–	–	–	
39	M. Y.	F	38	24	–	–	–	Healthy
				48	–	–	–	
40	K. Y.	M	28	24	–	–	–	Healthy
				48	–	–	–	
41	K. Y.	F	30	24	–	–	–	Healthy
				48	–	–	–	
42	T. Y.	M	40	24	–	–	–	Healthy
				48	–	–	–	
43	S. Y.	M	60	24	–	–	–	Healthy
				48	–	–	–	
44	S. Y.	M	51	24	–	–	–	Healthy
				48	–	–	–	
45	A. Y.	F	43	24	–	–	–	Healthy
				48	–	–	–	

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SafePharm Laboratories

S-Face VL-212:
Glyceryl / Polyglyceryl-6 Isostearate / Behenate Esters
ACUTE EYE IRRITATION IN THE RABBIT

SPL PROJECT NUMBER: 0432/0087

AUTHOR: A Pooles

STUDY SPONSOR:

Sakamoto Yakuhin Kogyo Co., Ltd.
1-20, Rinkaicho
Izumiotsu-city
OSAKA 595-01075
JAPAN

TEST FACILITY:

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

Telephone: +44 (0) 1332 792896

Facsimile: +44 (0) 1332 799018

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QUALITY ASSURANCE REPORT

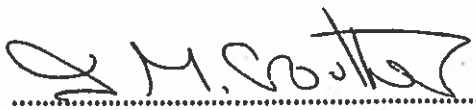
This study type is classed as short-term. The standard test method for this study type ("General Study Plan" in OECD terminology) was reviewed for compliance once only on initial production. Inspection of the routine and repetitive procedures that constitute the study is carried out as a continuous process 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
	03 October 2006	Test Material Preparation
	18, 21 November 2006	Animal Preparation
	21 November 2006	Dosing
	18, 20, 21 November 2006	Assessment of Response
§	05 December 2006	Draft Report Audit
§	Date of QA Signature	Final Report Audit

§ Evaluation specific to this study



DATE: 14 DEC 2006

For Safepharm Quality Assurance Unit*

*Authorised QA Signatures:

Head of Department:	JR Pateman CBiol MIBiol DipRQA AIQA FRQA
Deputy Head of Department:	JM Crowther MIScT MRQA
Senior Audit Staff:	JV Johnson BSc MRQA; G Wren ONC MRQA

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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.

A. Poob ..... DATE: 13/12/06

A Pooles BA (Hons)
Study Director

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S-Face VL-212:

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. Two treated eyes appeared normal at the 24-hour observation and the remaining treated eye appeared normal at the 48-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.

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S-Face VL-212:

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 13 November 2006 and 23 November 2006.

2. TEST MATERIAL

2.1 Description, Identification and Storage Conditions

Sponsor's identification	:	S-Face VL-212
Description	:	white waxy solid
Batch number	:	60209
Date received	:	05 June 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.

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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
10% w/w aqueous preparation of the test material	9.2 (7.8*)

* = Reading after 10 minutes

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.

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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	=	$(A + B + C) \times 2$
Score for iris	=	$D \times 5$
Score for cornea	=	$(E \times F) \times 5$

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.

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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.

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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 all treated eyes one hour after treatment and persisted in one treated eye at the 24-hour observation.

Two treated eyes appeared normal at the 24-hour observation and the remaining treated eye appeared normal at the 48-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	65134 Male				65169 Male				65170 Male			
	IPR = 1				IPR = 1				IPR = 1			
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	1	0	0	1	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	0	0	0	0
Score (A + B + C) x 2	4	2	0	0	2	0	0	0	2	0	0	0
Total Score	4	2	0	0	2	0	0	0	2	0	0	0

IPR = Initial pain reaction

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S-Face VL-212 : 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
65134 Male	4	2	0	0
65169 Male	2	0	0	0
65170 Male	2	0	0	0
Group Total	8	2	0	0
Group Mean Score	2.7	0.7	0.0	0.0

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S-Face VL-212 : 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.

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S-Face VL-212 : 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

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S-Face VL-212 : 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)

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

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.

Bryan J Wright 21/11/05.

Mr. Bryan J. Wright
Head, UK GLP Monitoring Authority

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Phycher
Bio développement

S-FACE VL-212
Glyceryl / Polyglyceryl -6 Isostearate / Behenate Esters
SKIN SENSITISATION IN THE GUINEA PIG -
MAGNUSSON AND KLIGMAN
MAXIMISATION METHOD

SPL PROJECT NUMBER: 0432/0088
Study n°: SMK-PH-06/0232

AUTHOR: F Richeux

STUDY SPONSOR:

Sakamoto Yakuhin Kogyo Co., Ltd.
1-20, Rinkai-cho
Izumiotsu-city
OSAKA 595-0075
JAPAN

STUDY MONITOR:

A. Pooles
Safepharm Laboratories Limited
Shardlow Business Park
Shardlow
Derbyshire
DE72 2GD
UK

TEST FACILITY:

Phycher Bio Développement
ZI de Toctoucau
18 Chemin de Lou Tribail
33611 CESTAS Cedex
FRANCE

Telephone: (33) 5 57 97 16 80

Facsimile: (33) 5 57 97 16 81

Siège social : 18, chemin de Lou Tribail ZI de Toctoucau 33611 CESTAS Cedex - ✆ 05 57 97 16 80 - Fax 05 57 97 16 81
info@phycher.com - www.phycher.com
S.A.R.L. AU CAPITAL DE 60 979,61 Euros - NAF 7312 - SIRET 402 524 088 000 30

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QUALITY ASSURANCE REPORT

The routine inspections of the toxicity tests performed at Phycher Bio Développement are carried out as a continuous process designed to encompass the major phases of at least one similar test at or about the time this study was in progress.

This report has been audited by Phycher Bio Développement 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 within twenty-four hours of evaluation. Audits of study documentation, and process inspections appropriate to the type and schedule of this study were as follows:

§	12 June 2006	Protocol Compliance Audit
	01 June 2006	Main study: skin reactions at the reading 48-hours after topical challenge
§	23 August 2006	Draft Report Audit
§	30 August 2006	Final Report Audit

§ Evaluation specific to this study

..... DATE: 30 August 2006

Bernard BENECH
Quality Assurance Manager

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GLP COMPLIANCE STATEMENT

I, the undersigned, François Richeux, Study director, certify that the study SMK-PH-06/0232 was performed on the premises of the Laboratory PHYCHER Bio Développement.

I, certify that the objectives laid down in the protocol were achieved and no undesirable event occurred to affect the quality or the integrity of the study. I consider the data generated to be valid. This report fully and accurately reflects the operating procedures used and data generated.

The entire work was devised and performed in compliance with the principles of the Good Laboratory Practice (G.L.P.), as defined in *the directive 2004/9/EC, the O.E.C.D. ruling relative to the mutual acceptance of data in the evaluation of chemical substances (C(81) 30 (final) Appendix 2 – May 12th , 1981; C (97) 186, November 26th, 1987),* and in the French decree n° 98-1312 dated December 31st, 1998 of the Journal Officiel de la République Française (see certificate of G.L.P. in appendix 7).

..... DATE: 30 August 2006

François Richeux
Study Director
Expert Toxicologist (PhD, ERT)

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S-FACE VL-212 :

SKIN SENSITISATION IN THE GUINEA PIG - MAGNUSSON AND KLIGMAN MAXIMISATION METHOD

SUMMARY

Introduction. The study was performed to assess the contact sensitisation potential of the test material in the albino guinea pig. The method was designed to meet the requirements of the following:

- OECD Guidelines for the Testing of Chemicals No. 406 "Skin Sensitisation" (adopted 17 July 1992)
- Method B6 Acute Toxicity (Skin Sensitisation) of Commission Directive 96/54/EC
- Japanese Ministry of Agriculture, Forestry and Fisheries (JMAFF), Testing Guidelines for Toxicology Studies, 12 NohSan No. 8147, Guideline No. 2-1-6, revised on 24 November 2004 and 13 SeiSan No. 3986 of October 10, 2001

Method. Ten test and five control animals were used for the main study. Two phases were involved in the main test; an induction of a response by intradermal injection and topical application and a topical challenge of that response.

Based on the results of sighting tests, the concentrations of test material for the topical induction and topical challenge phases were selected as:

Intradermal Induction	:	25% in olive oil
Topical Induction	:	100%
Topical Challenge	:	100% and 50% in liquid paraffin

Conclusion. Under the conditions of the test, the test material produced a 0% (0/10) sensitisation rate and was classified as a non-sensitiser to guinea pig skin. The test material did not meet the criteria for classification as a sensitiser according to EU labelling regulations Commission Directive 2001/59/EC. No symbol and risk phrase are required.

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S-FACE VL-212 :

SKIN SENSITISATION IN THE GUINEA PIG - MAGNUSSON AND KLIGMAN MAXIMISATION METHOD

1. INTRODUCTION

The study was performed to assess the contact sensitisation potential of the test material in the albino guinea pig. The method was designed to meet the requirements of the following:

- OECD Guidelines for the Testing of Chemicals No. 406 "Skin Sensitisation" (adopted 17 July 1992)
- Method B6 Acute Toxicity (Skin Sensitisation) of Commission Directive 96/54/EC
- Japanese Ministry of Agriculture, Forestry and Fisheries (JMAFF), Testing Guidelines for Toxicology Studies, 12 NohSan No. 8147, Guideline No. 2-1-6, revised on 24 November 2004 and 13 SeiSan No. 3986 of October 10, 2001

The albino guinea pig has been shown to be a suitable species for this type of study and is recommended in the test method. The strain used in these laboratories has been shown to produce satisfactory sensitisation responses using known positive sensitisers (see Appendix 6). The results of the study are believed to be of value in predicting the likely contact sensitisation potential of the test material to man.

The study was performed between 27 June 2006 and 03 August 2006.

2. TEST MATERIAL

2.1 Description, Identification and Storage Conditions

Sponsor's identification	:	S-FACE VL-212
Code number	:	PH-06/0232
Description	:	White solid
CAS number	:	-
Batch number	:	60209
Date received	:	16 June 2006
Storage conditions	:	room temperature

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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 this study the test material was freshly prepared in olive oil and paraffin oil. The concentrations used are stated in the procedure section.

To collect and liquefy the test material, it has been heated at 50°C.

The absorption of the test material was not determined.

Determination by analysis of the concentration, homogeneity and stability of the test material preparations was not appropriate because it was not specified in the Study Plan and is not a requirement of the Test Guideline.

3. METHODS

3.1 Animals and Animal Husbandry

Female albino Dunkin Hartley guinea pigs were supplied by Charles River (F-69592 L'Arbresle). Females were nulliparous and non-pregnant. After an acclimatisation period of at least five days, each animal was selected at random and identified individually by marking with picric acid and an ear tattoo. At the start of the main study the animals were in the weight range of 315 to 363 g.

The animals were housed in groups of two or three in makrolon cages furnished with woodflakes. Free access to mains tap water and food (Safe 91360 Villemoisson s/Orge, France) was allowed throughout the study. The diet, drinking water and bedding were routinely analysed and were considered not to contain any contaminant that could 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 58 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 ten changes per hour and the lighting was controlled by a time switch to give twelve hours continuous light (07:00 to 19:00) and twelve hours darkness.

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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

The method used for assessing the sensitising properties of the test material was based on the Guinea Pig Maximisation Test of Magnusson B & Kligman A M, (1969) J. Invest. Dermatol. 52: 268 - 276.

3.2.1 Selection of Concentrations for Main Study (Sighting Tests)

The concentrations of test material to be used at each stage of the main study were determined by 'sighting tests' in which groups of guinea pigs were treated with various concentrations of test material. The procedures were as follows:

3.2.1.1 Selection of Concentration for Intradermal Induction

Intradermal injections (0.1 ml/injection site) were made on the clipped shoulder of two guinea pigs, at the concentrations of 100%, 50%, 25%, 12.5%, 6.25% and 3.125% by successive dilution (v/v) in olive oil. A macroscopic evaluation of the injection sites was conducted approximately 24 hours after injection to determine whether these concentrations caused necrosis. Any evidence of systemic toxicity was also recorded. The highest concentration which did not cause necrosis was selected for the intradermal induction stage of the main study. The results are given in Appendix 1.

3.2.1.2 Selection of Concentration for Topical Induction

Two guinea pigs were treated with preparations of the test material at 100% and diluted at 50% 25% and 12.5% by successive dilution (v/v) in liquid paraffin. Applications were made to the clipped flanks under occlusive dressings for an exposure period of 24 hours. The degree of erythema and oedema was evaluated approximately 24 hours after dressing removal. The highest concentration producing only moderate dermal irritation was selected for the topical induction stage of the main study. The results are given in Appendix 2.

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3.2.1.3 Selection of Concentration for Topical Challenge

Four preparations of the test material at 100% and diluted at 50%, 25% and 12.5% by successive dilution (v/v) in liquid paraffin were applied to the clipped flanks of three guinea pigs under occlusive dressings for an exposure period of 24 hours. These guinea pigs did not form part of the main study but had been treated identically to the control animals of the main study, up to Day 14. The degree of erythema and oedema was evaluated approximately 24 hours after dressing removal. The highest non-irritant concentration of the test material and one lower concentration were selected for the topical challenge stage of the main study. The results are given in Appendix 3.

3.2.2 Main Study

A group of fifteen guinea pigs was used for the main study, ten test and five control. The bodyweight of each animal was recorded at the start and the end of the study and are presented in Appendix 4.

Two phases were involved in the main study; (a) an induction of a response and (b) a challenge of that response.

3.2.2.1 Induction

Induction of the Test Animals: Shortly before treatment on Day 0 the hair was removed from an area approximately 40 mm x 60 mm on the shoulder region of each animal with veterinary clippers. A row of three injections (0.1 ml each) was made on each side of the spine. The injections were:

a) Freund's Complete Adjuvant plus isotonic sodium chloride in the ratio 1:1

b) a 25% formulation of the test material in olive oil

c) a 25% formulation of the test material in a 1:1 preparation of Freund's Complete Adjuvant plus isotonic sodium chloride

On Day 6, the scapular region of all test and control animals was shaved.

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On Day 7 the same area on the shoulder region used previously for intradermal injections was clipped again and treated with a topical application of the test material formulation. A topical application of the test material at 100% under occlusive dressing was made. The occlusive dressing was kept in place for 48 hours.

Induction of the Control Animals: On Day 0, the intradermal induction was performed using an identical procedure to that used for the test animals except that the test material was omitted from the intradermal injections. Injection b) was therefore the vehicle alone, injection c) was a 50% formulation of the vehicle in a 1:1 preparation of Freund's Complete Adjuvant plus isotonic sodium chloride. Similarly, on Day 7, the topical induction procedure was identical to that used for the test animals except that the test material was omitted.

3.2.2.2 Challenge

The flanks of each animal were previously clipped free of hair with veterinary clippers.

The test material formulation at the maximum non-irritant concentration (100%) was applied to one side of the shorn flank of each animal under an occlusive dressing. To ensure that the maximum non-irritant concentration was used at challenge, the test material at a concentration of 50% (v/v) in liquid paraffin was similarly applied under an occlusive dressing to the opposite skin site on the shorn flank.

After 24 hours, the dressing was carefully removed and discarded. The topical challenge sites were cleaned if required.

Prior to the 24-hour observation the flanks were clipped to remove regrown hair.

Approximately 24 and 48 hours after challenge dressing removal, the degree of erythema and oedema was quantified using the scale shown in Appendix 5.

Any other reactions were also recorded.

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3.3 Interpretation of Results

The percentage of test animals that showed a more severe reaction at the test material challenge site than the most severe reaction seen in the control animals, was compared with the following scale:

PERCENTAGE OF ANIMALS SENSITISED	CLASSIFICATION OF SENSITISATION POTENTIAL
0	Non sensitiser
> 0 - 8	Weak sensitiser
> 8 - 28	Mild sensitiser
>28 – 64	Moderate sensitiser
>64 - 80	Strong sensitiser
>80 - 100	Extreme sensitiser

The results were evaluated according to Commission Directive 2001/59/EC for classification and labelling of dangerous substances.

4. ARCHIVES

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

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5. RESULTS

5.1 Preliminary Sighting Tests

5.1.1 Skin Reactions Observed After Intradermal Induction

Individual skin reactions at the intradermal induction sites of the preliminary sighting test animals are presented in Appendix 1.

Necrosis was noted at the intradermal induction sites of both preliminary sighting test animals following treatment with the test material at concentrations of 100% and 50%. A moderate erythema was noted at the intradermal induction sites of both preliminary sighting test animals following treatment with the test material at concentration of 25%. No skin reactions were noted at the intradermal induction sites of the preliminary sighting test animals following treatment with the test material at the concentrations of 12.5%, 6.25% and 3.125%.

The concentration chosen for use in the Intradermal Induction phase of the main test was 25% in olive oil (maximal non necrotising concentration (MNNC)).

5.1.2 Skin Reactions Observed After Topical Induction

Individual skin reactions at the topical induction sites of the preliminary sighting test animals are presented in Appendix 2.

No skin reactions were noted at the topical induction sites of the preliminary sighting test animals following treatment with the test material at concentrations of 100%, 50%, 25% and 12.5%.

The concentration chosen for use in the 2nd topical Induction of the main test was 100% and the 1st concentration chosen for the MNIC (maximal non irritant concentration) determination was 100%.

5.1.3 Skin Reactions Observed After Topical Challenge

Individual skin reactions at the topical challenge sites of the preliminary sighting test animals are presented in Appendix 3.

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No skin reactions were noted at the topical challenge sites of the preliminary sighting test animals following treatment with the test material at concentrations of 100%, 50%, 25% and 12.5%.

The concentrations chosen for use in the Topical Challenge phase of the main test were 100% (maximal non irritant concentration (MNIC)) and 50% (1/2 MNIC) in liquid paraffin.

5.2 Skin Reactions Observed After Topical Challenge

Individual skin reactions at the topical challenge sites of the test and control group animals are given in Table 1 and overall results are presented in Table 2.

No mortality occurred during this study.

100%

No skin reactions were noted at the topical challenge site of the test or control group animals at the 24 and 48-hour observations.

50% in liquid paraffin

No skin reactions were noted at the topical challenge site of the test or control group animals at the 24 and 48-hour observations.

6. CONCLUSION

Under the conditions of the test, the test material produced a 0% (0/10) sensitisation rate and was classified as a non-sensitiser to guinea pig skin

The test material did not meet the criteria for classification as a sensitiser according to EU labelling regulations Commission Directive 2001/59/EC. No symbol and risk phrase are required.

Table 1 Individual Skin Reactions at Topical Challenge

CHALLENGE CONCENTRATIONS: 100% and 50%

VEHICLE: liquid paraffin

Group	Animal Number	Skin Reactions (Hours after Removal of Dressings)											
		100%						50%					
		24 Hours			48 Hours			24 Hours			48 Hours		
		Er	Oe	Other	Er	Oe	Other	Er	Oe	Other	Er	Oe	Other
TEST	C5666	0	0	-	0	0	-	0	0	-	0	0	-
	C5667	0	0	-	0	0	-	0	0	-	0	0	-
	C5668	0	0	-	0	0	-	0	0	-	0	0	-
	C5669	0	0	-	0	0	-	0	0	-	0	0	-
	C5670	0	0	-	0	0	-	0	0	-	0	0	-
	C5671	0	0	-	0	0	-	0	0	-	0	0	-
	C5672	0	0	-	0	0	-	0	0	-	0	0	-
	C5673	0	0	-	0	0	-	0	0	-	0	0	-
	C5674	0	0	-	0	0	-	0	0	-	0	0	-
	C5675	0	0	-	0	0	-	0	0	-	0	0	-

Er = Erythema Oe = Oedema - = No other reactions noted

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Table 1 (continued) Individual Skin Reactions at Topical Challenge

CHALLENGE CONCENTRATIONS: **100% and 50%**

VEHICLE: **liquid paraffin**

Group	Animal Number	Skin Reactions (Hours after Removal of Dressings)											
		100%						50%					
		24 Hours			48 Hours			24 Hours			48 Hours		
		Er	Oe	Other	Er	Oe	Other	Er	Oe	Other	Er	Oe	Other
CONTROL	C5651	0	0	-	0	0	-	0	0	-	0	0	-
	C5652	0	0	-	0	0	-	0	0	-	0	0	-
	C5653	0	0	-	0	0	-	0	0	-	0	0	-
	C5654	0	0	-	0	0	-	0	0	-	0	0	-
	C5655	0	0	-	0	0	-	0	0	-	0	0	-

Er = Erythema Oe = Oedema - = No other reactions noted

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Table 2 **Assessment of the Sensitising Potential following Topical Challenge**

Group	Observation (Hours after Removal of Dressings)	Concentration	Number of Animals Showing Sensitisation Reactions				Positive Responses	Animals Sensitised
			0	1	2	3 or >		
Test	24	100%	10	0	0	0	0%	0%
	48	100%	10	0	0	0	0%	0%
	24	50%	10	0	0	0	0%	0%
	48	50%	10	0	0	0	0%	0%
Control	24	100%	5	0	0	0	0%	
	48	100%	5	0	0	0	0%	
	24	50%	5	0	0	0	0%	
	48	50%	5	0	0	0	0%	

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Appendix 1 Intradermal Sighting Test – Summary of Results

VEHICLE: olive oil

Animal Identification	Concentration of Test Material (%)	Time of Observation	Grade of Erythema at Injection Sites
C5558	100	24 Hours	Necrosis
	50	48 Hours	Necrosis
	25	48 Hours	2 (Er)
	12.5	48 Hours	0
	6.25	48 Hours	0
	3.125	48 Hours	0
C5559	100	24 Hours	Necrosis
	50	48 Hours	Necrosis
	25	48 Hours	2 (Er)
	12.5	48 Hours	0
	6.25	48 Hours	0
	3.125	48 Hours	0

The concentration of the test material selected for the intradermal induction test of the main test was 25% in olive oil

Er = Erythema

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Appendix 2 Topical Sighting Test for Topical Induction Application (24-Hour Exposure) – Individual Skin Reactions

VEHICLE: liquid paraffin

Animal Identification	Concentration of Test Material (%)	Skin Reactions (24 Hours After Removal of Patches)		
		Er	Oe	Other
C5558	100	0	0	-
	50	0	0	-
	25	0	0	-
	12.5	0	0	-
C5559	100	0	0	-
	50	0	0	-
	25	0	0	-
	12.5	0	0	-

The concentration of the test material selected for the main test topical induction was 100%

Er = Erythema Oe = Oedema - = No other reactions noted

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Appendix 3 Topical Sighting Test for Topical Challenge Application (24-Hour Exposure) – Individual Skin Reactions

VEHICLE: liquid paraffin

Animal Identification	Concentration of Test Material (%)	Skin Reactions (24 Hours After Removal of Patches)		
		Er	Oe	Other
C5560	100	0	0	*
	50	0	0	-
	25	0	0	-
	12.5	0	0	-
C5561	100	0	0	*
	50	0	0	-
	25	0	0	-
	12.5	0	0	-
C5562	100	0	0	*
	50	0	0	-
	25	0	0	-
	12.5	0	0	-

The concentrations of the test material selected for the main test topical challenge were 100% and 50% in liquid paraffin

Er = Erythema Oe = Oedema * = slight dryness - = No other reactions noted

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Appendix 4 Individual Bodyweights and Bodyweight Gains

Group	Animal Number	Bodyweight (g)		Bodyweight (g) Increase
		Day 0	Day 30	
TEST	C5666	349	508	159
	C5667	350	482	132
	C5668	363	531	168
	C5669	315	448	133
	C5670	333	489	156
	C5671	341	478	137
	C5672	330	461	131
	C5673	323	473	150
	C5674	319	494	175
	C5675	321	495	174

Bodyweight increases of the guinea pigs in the test group between Day 0 and Day 30 were comparable to those noted in the control group over the same period.

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Appendix 4 (continued) Individual Bodyweights and Bodyweight Gains

Group	Animal Number	Bodyweight (g)		Bodyweight (g) Increase
		Day 0	Day 30	
CONTROL	C5651	340	544	204
	C5652	347	521	174
	C5653	350	517	167
	C5654	334	507	173
	C5655	328	494	166

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S-FACE VL-212: SKIN SENSITISATION IN THE GUINEA PIG - MAGNUSSON AND KLIGMAN MAXIMISATION METHOD

Appendix 5 Scales for Evaluation of Skin Reactions

EVALUATION OF ERYTHEMA #	VALUE
No visible modification	0
Slight or patches of erythema	1
Moderate confluent erythema	2
Intense erythema and swelling	3

EVALUATION OF OEDEMA †	VALUE
No visible modification	0
Slight oedema	1
Moderate oedema	2
Severe oedema	3

From: Modified OECD Test Guideline 406, 1992 and Method B6 Skin Sensitisation of Commission Directive 96/54/EC.

† 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, p31.

Appendix 6 Summary of Positive Control Data for the Magnusson and Kligman Maximisation Study

Test Number	Date Start	Number of Animals Test	Positive Control Material	Challenge Concentration	Observation (Hours after Removal of Dressings)	Incidence of Sensitisation
10	06/09/2005	10	α-Hexylcinnamaldehyde CAS [101-86-0]	50%	24	100%
					48	70%
				25%	24	100%
					48	60%
11	31/01/2006	10	α-Hexylcinnamaldehyde CAS [101-86-0]	50%	24	70%
					48	70%
				25%	24	50%
					48	30%
12	07/02/2006	10	α-Hexylcinnamaldehyde CAS [101-86-0]	50%	24	100%
					48	90%
				25%	24	90%
					48	90%

Conclusion: Based on these results, under the conditions of the test, the Positive control material, α-Hexylcinnamaldehyde, was classified sensitisers according to EU labelling regulations Commission Directive 2001/59/EC. The symbol "Xi", the indication of danger 'irritant' and risk phrase R 43 "May Cause Sensitisation by Skin Contact" are therefore required.

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Appendix 7 Statement of GLP Compliance in Accordance with Directive 88/320/EEC

FRENCH REPUBLIC
INTERMINISTERIAL GROUP OF CHEMICALS (GIPC)

Paris, 13 December 2005

(Translated from the original version in French)

Subject: Evaluation of conformity to GLP according to the directive 88/320/EEC.

In the application of the 81-278 decree dated 25th March 1981 modified by the 90-206 decree dated 7th March 1990 and the 98-1312 decree dated 31 December 1998 concerning the Good Laboratory Practice, I confirm that, following the inspection visit made by the Comité Français d'Accréditation (COFRAC – Section Essais) in accordance with its usual procedures and upon your request, the GIPC was able to establish that the assays perform in your test facility comply with GLP.

Compliance with the GLP principles.
Areas of expertise
- **2 Toxicity studies**
- **3 Mutagenicity studies**

Inspection date: 03 October 2005

- **Periodical inspection (i.p.)**
- **Extension inspection (i.e.)**

Date of GIPC decision: 09 December 2005
Date of validity: 03 October 2005
Year of 1st validity: 1998
Period of validity: 18 months

PHYCHER BIO Developpement
ZI de Toctoucau
18, chemin de Lou Tribail
33611 CESTAS Cedex

President of the Interministerial
Group of Chemicals
Mr Pierre CREYSSEL

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SafePharm Laboratories

S-Face VL-212:
Glyceryl / Polyglyceryl-6 Isostearate / Behenate Esters
ACUTE ORAL TOXICITY IN THE RAT
- ACUTE TOXIC CLASS METHOD

SPL PROJECT NUMBER: 0432/0085

AUTHOR: A Pooles

STUDY SPONSOR:

Sakamoto Yakuhin Kogyo Co., Ltd.
1-20, Rinkaicho
Izumiotsu-city
OSAKA 595-01075
JAPAN

TEST FACILITY:

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

Telephone: +44 (0) 1332 792896

Facsimile: +44 (0) 1332 799018

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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:

	29 October 2004	Standard Test Method Compliance Audit
	08 August 2006	Test Material Preparation
	17 August 2006	Animal Preparation
	08, 15 August 2006	Dosing
	08, 12 August 2006	Assessment of Response
	17 August 2006	Necropsy
§	10 September 2006	Draft Report Audit
§	Date of QA Signature	Final Report Audit

§ Evaluation specific to this study

JWJohnson ..... DATE: 01 NOV 2006 .....

For Safepharm Quality Assurance Unit*

---

*Authorised QA Signatures:

Head of Department:	JR Pateman CBiol MIBiol DipRQA AIQA FRQA
Deputy Head of Department:	JM Crowther MIScT MRQA
Senior Audit Staff:	JV Johnson BSc MRQA; G Wren ONC MRQA

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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.

A. Poob ..... DATE: 31/10/06

A Pooles BA (Hons)
Study Director

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S-Face VL-212:

ACUTE ORAL TOXICITY IN THE RAT – ACUTE TOXIC CLASS METHOD

SUMMARY

Introduction. The study was performed to assess the acute oral toxicity of the test material following a single oral administration in the Sprague-Dawley CD strain rat. The method was designed to meet the requirements of the following:

- OECD Guidelines for the Testing of Chemicals No. 423 "Acute Oral Toxicity – Acute Toxic Class Method" (adopted 17 December 2001)
- Method B1 *tris* Acute Toxicity (Oral) of Commission Directive 2004/73/EC

Method. A group of three fasted females was treated with the test material at a dose level of 2000 mg/kg bodyweight. This was followed by a further group of three fasted females at the same dose level.

The test material was administered orally as a solution in arachis oil BP. Clinical signs and bodyweight development were monitored during the study. All animals were subjected to gross necropsy.

Mortality. There were no deaths.

Clinical Observations. There were no signs of systemic toxicity.

Bodyweight. All animals showed expected gains in bodyweight over the study period.

Necropsy. No abnormalities were noted at necropsy.

Conclusion. The acute oral median lethal dose (LD_{50}) of the test material in the female Sprague-Dawley CD strain rat was estimated to be greater than 2500 mg/kg bodyweight (Globally Harmonised Classification System – Unclassified).

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S-Face VL-212:

ACUTE ORAL TOXICITY IN THE RAT - ACUTE TOXIC CLASS METHOD

1. INTRODUCTION

The study was performed to assess the acute oral toxicity of the test material following a single oral administration in the Sprague-Dawley CD strain rat. The method was designed to meet the requirements of the following:

- OECD Guidelines for the Testing of Chemicals No. 423 "Acute Oral Toxicity – Acute Toxic Class Method" (adopted 17 December 2001)
- Method B1 *tris* Acute Toxicity (Oral) of Commission Directive 2004/73/EC

The rat was selected for this study as it is a readily available rodent species, historically used in safety evaluation studies, and is acceptable to appropriate regulatory authorities. The oral route was selected as the most appropriate route of exposure and the results are believed to be of value in predicting the likely toxicity of the test material to man.

The study was performed between 18 July 2006 and 10 August 2006.

2. TEST MATERIAL AND EXPERIMENTAL PREPARATION

2.1 Description, Identification and Storage Conditions

Sponsor's identification	:	S-Face VL-212
Description	:	white waxy solid
Batch number	:	60209
Date received	:	05 June 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.

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2.2 Preparation of Test Material

For the purpose of the study the test material was freshly prepared, as required, as a solution at the appropriate concentration in arachis oil BP. To aid preparation the test material formulation was heated at 50°C in a water bath until dissolved. Arachis oil BP was used because the test material did not dissolve/suspend in distilled water.

Determination by analysis of the concentration, homogeneity and stability of the test material preparations was not appropriate because it was not specified in the Study Plan and is not a requirement of the Test Guideline.

3. METHODS

3.1 Animals and Animal Husbandry

Female Sprague-Dawley CD (Crl: CD® (SD) IGS BR) strain rats were supplied by Charles River (UK) Ltd, Margate, Kent, UK. On receipt the animals were randomly allocated to cages. The animals were nulliparous and non-pregnant. After an acclimatisation period of at least five days the animals were selected at random and given a number unique within the study by indelible ink-marking on the tail and a number written on a cage card. At the start of the study the animals were eight to twelve weeks of age. The bodyweights fell within an interval of ± 20% of the mean initial bodyweight of the first treated group.

The animals were housed in groups of three 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 (Certified Rat and Mouse Diet (Code 5LF2) supplied by BCM IPS Limited, London, UK) was allowed throughout the study. The diet, drinking water and bedding were routinely analysed 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. 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.

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3.2 Procedure

Using available information on the toxicity of the test material, 2000 mg/kg was chosen as the starting dose.

Groups of fasted animals were treated as follows:

Dose Level (mg/kg)	Concentration (mg/ml)	Dose Volume (ml/kg)	Number of Rats
			Female
2000	200	10	3
2000	200	10	3

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 group 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 fourteen 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.

3.3 Evaluation of Data

Data evaluations included the relationship, if any, between the exposure of the animal to the test material and the incidence and severity of all abnormalities including behavioural and clinical observations, gross lesions, bodyweight 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 material was made as shown in the schematic diagram in Appendix 1.

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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.

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5. RESULTS

5.1 Mortality Data

Individual mortality data are given in Table 1.

There were no deaths.

5.2 Clinical Observations

Individual clinical observations are given in Table 2.

There were no signs of systemic toxicity.

5.3 Bodyweight

Individual bodyweights and weekly bodyweight changes are given in Table 3.

All animals showed expected gains in bodyweight over the study period.

5.4 Necropsy

Individual necropsy findings are given in Table 4.

No abnormalities were noted at necropsy.

6. CONCLUSION

The acute oral median lethal dose (LD_{50}) of the test material in the female Sprague-Dawley CD strain rat was estimated to be greater than 2500 mg/kg bodyweight (Globally Harmonised Classification System – Unclassified).

Table 1 Mortality Data

Dose Level mg/kg	Sex	Number of Animals Treated	Deaths During Day of Dosing (Hours)				Deaths During Period After Dosing (Days)								Deaths
			½	1	2	4	1	2	3	4	5	6	7	8-14	
2000	Female	3	0	0	0	0	0	0	0	0	0	0	0	0	0/3
	Female	3	0	0	0	0	0	0	0	0	0	0	0	0	0/3

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Table 2 Individual Clinical Observations

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
2000	1-0 Female	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	1-1 Female	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	1-2 Female	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2-0 Female	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2-1 Female	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2-2 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

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Table 3 Individual Bodyweights and Weekly Bodyweight Changes

Dose Level mg/kg	Animal Number and Sex	Bodyweight (g) at Day			Bodyweight Gain (g) During Week	
		0	7	14	1	2
2000	1-0 Female	233	268	273	35	5
	1-1 Female	207	236	259	29	23
	1-2 Female	215	245	261	30	16
	2-0 Female	207	233	250	26	17
	2-1 Female	198	225	250	27	25
	2-2 Female	193	215	253	22	38

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Table 4 Individual Necropsy Findings

Dose Level mg/kg	Animal Number and Sex	Time of Death	Macroscopic Observations
2000	1-0 Female	Killed Day 14	No abnormalities detected
	1-1 Female	Killed Day 14	No abnormalities detected
	1-2 Female	Killed Day 14	No abnormalities detected
	2-0 Female	Killed Day 14	No abnormalities detected
	2-1 Female	Killed Day 14	No abnormalities detected
	2-2 Female	Killed Day 14	No abnormalities detected

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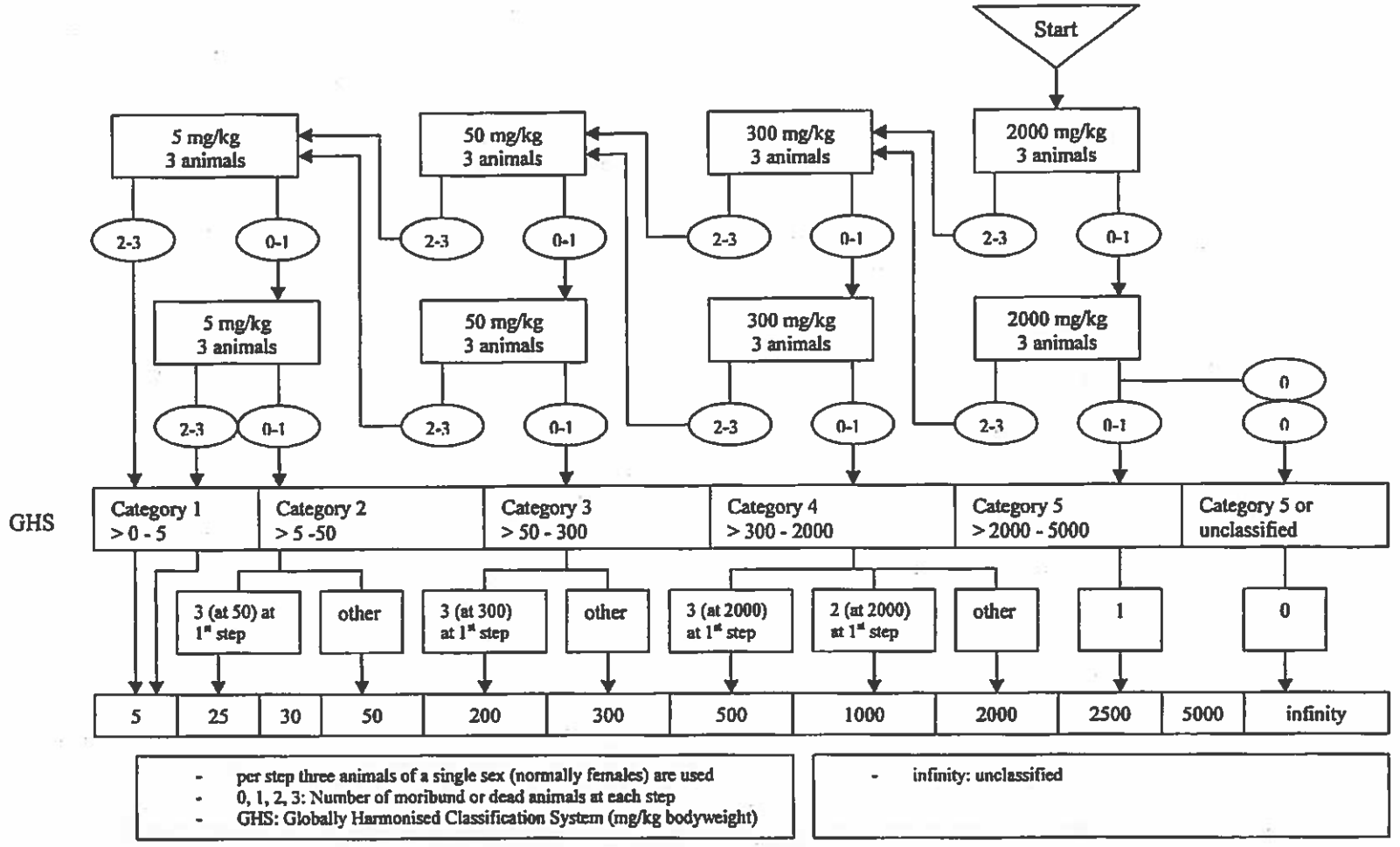
Appendix 1 Test Procedure with a Starting Dose of 2000 mg/kg Bodyweight



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Appendix 2 Statement of GLP Compliance in Accordance with Directive 2004/9/EC

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

Bryan J Wright 21/11/05.

Mr. Bryan J. Wright
Head, UK GLP Monitoring Authority

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SafePharm Laboratories

S-Face VL-212:
Glyceryl / Polyglyceryl-6 Isostearate / Behenate Esters
REVERSE MUTATION ASSAY "AMES TEST"
USING *SALMONELLA TYPHIMURIUM* AND
ESCHERICHIA COLI

SPL PROJECT NUMBER: 0432/0089

AUTHORS: P W Thompson
A J Bowles

STUDY SPONSOR:

Sakamoto Yakuhin Kogyo Co., Ltd.
1-20, Rinkaicho
Izumiotsu-city
OSAKA 595-01075
JAPAN

TEST FACILITY:

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

Telephone: +44 (0) 1332 792896

Facsimile: +44 (0) 1332 799018

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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:

	15 July 2004	Standard Test Method Compliance Audit
	25 September 2006	Test Material Preparation
	11 September 2006	Test System Preparation
	19 September 2006	Exposure
	25 September 2006	Assessment of Response
§	16 October 2006	Draft Report Audit
§	Date of QA Signature	Final Report Audit

§ Evaluation specific to this study

JVJohnson DATE: 01 NOV 2006

For Safepharm Quality Assurance Unit*

*Authorised QA Signatures:

Head of Department:	JR Pateman CBiol MIBiol DipRQA AIQA FRQA
Deputy Head of Department:	JM Crowther MIScT MRQA
Senior Audit Staff:	JV Johnson BSc MRQA; G Wren ONC MRQA

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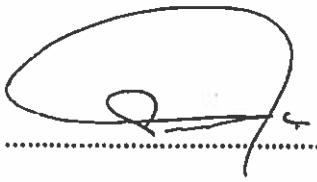
GLP COMPLIANCE STATEMENT

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: 31 OCT 2006

P W Thompson HNC
Study Director

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S-Face VL-212:

REVERSE MUTATION ASSAY "AMES TEST" USING *SALMONELLA TYPHIMURIUM* AND *ESCHERICHIA COLI*

SUMMARY

Introduction. The method conforms to the guidelines for bacterial mutagenicity testing published by the major Japanese Regulatory Authorities including METI, MHLW and MAFF. It also meets the requirements of the OECD Guidelines for Testing of Chemicals No. 471 "Bacterial Reverse Mutation Test", Method B13/14 of Commission Directive 2000/32/EC and the USA, EPA (TSCA) OPPTS harmonised guidelines.

Methods. *Salmonella typhimurium* strains TA1535, TA1537, TA98, TA100 and *Escherichia coli* strain WP2uvrA⁻ were treated with the test material using the Ames plate incorporation method at five dose levels, in triplicate, both with and without the addition of a rat liver homogenate metabolising system (10% liver S9 in standard co-factors). The dose range was determined in a preliminary toxicity assay and was 50 to 5000 µg/plate in the first experiment. The experiment was repeated on a separate day using the same dose range as Experiment 1, fresh cultures of the bacterial strains and fresh test material formulations.

Results. The vehicle (acetone) control plates gave counts of revertant colonies within the normal range. All of the positive control chemicals used in the test induced marked increases in the frequency of revertant colonies, both with or without metabolic activation. Thus, the sensitivity of the assay and the efficacy of the S9-mix were validated.

The test material caused no visible reduction in the growth of the bacterial background lawn at any dose level. The test material was, therefore, tested up to the maximum recommended dose level of 5000 µg/plate. A pale, particulate precipitate was observed at and above 1500 µg/plate, this did not prevent the scoring of revertant colonies.

No significant increases in the frequency of revertant colonies were recorded for any of the bacterial strains, with any dose of the test material, either with or without metabolic activation.

Conclusion. The test material was considered to be non-mutagenic under the conditions of this test.

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S-Face VL-212:

REVERSE MUTATION ASSAY "AMES TEST" USING *SALMONELLA TYPHIMURIUM* AND *ESCHERICHIA COLI*

1. INTRODUCTION

This study was designed to assess the mutagenic potential of the test material using a bacterial/ microsome test system. The study was based on the *in vitro* technique described by Ames and his co-workers (1, 2, 3) and Garner et al (4) in which mutagenic activity is assessed by exposing histidine auxotrophs of *Salmonella typhimurium* and tryptophan auxotrophs of *Escherichia coli* to various concentrations of the test material. The method conforms to the guidelines for bacterial mutagenicity testing published by the major Japanese Regulatory Authorities including METI, MHLW and MAFF. It also meets the requirements of the OECD Guidelines for Testing of Chemicals No. 471 "Bacterial Reverse Mutation Test", Method B13/14 of Commission Directive 2000/32/EC and the USA, EPA (TSCA) OPPTS harmonised guidelines. A copy of the Certificate of Compliance with GLP, issued by the UK Department of Health, is included.

These mutant strains of *Salmonella* are incapable of synthesising histidine and are, therefore, dependent on an external source of this particular amino acid for normal growth. When exposed to a mutagenic agent these bacteria may undergo a reverse mutation to histidine independent forms which are detected by their ability to grow on a histidine deficient medium. Using various strains of this organism, revertants produced after exposure to a chemical mutagen may arise as a result of base-pair substitution in the genetic material (miscoding) or frame-shift mutation in which genetic material is either added or deleted. In order to make the bacteria more sensitive to mutation by chemical and physical agents, several additional traits have been introduced. These include a deletion through the excision repair gene (uvrB$^-$ *Salmonella* strains) which renders the organism incapable of DNA excision repair and deep rough mutation (rfa) which increases the permeability of the cell wall. A mutant strain of *Escherichia coli* (WP2uvrA$^-$), which requires tryptophan and which can be reverse mutated by base substitution to tryptophan independence was used to complement the *Salmonella* strains. This strain also has a deletion in an excision repair gene (uvrA$^-$). Since many compounds do not exert a mutagenic effect until they have been metabolised by enzyme systems not available in the bacterial cell, the test material and the bacteria are also incubated in the presence of a liver microsomal preparation (S9-mix) prepared from rats pre-treated with a mixture known to induce an elevated level of these enzymes.

The experimental phase of this study was performed between 28 June 2006 and 14 September 2006.

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2. TEST MATERIAL

Sponsor's identification	:	S-Face VL-212
Description	:	White waxy solid
Chemical name	:	(Isostearate/Behenate)/(Glyceryl/polyglyceryl-6) esters
Purity	:	99%
Batch number	:	60209
Date received	:	05 June 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.

3. METHODS

3.1 Tester Strains

Salmonella typhimurium TA1535, TA1537, TA98 and TA100

Escherichia coli WP2uvrA$^-$

The *Salmonella* strains were obtained from the University of California at Berkeley on culture discs on 4 August 1995 whilst *Escherichia coli* strain WP2uvrA$^-$ was obtained from the British Industrial Biological Research Association on 17 August 1987. All of the strains were stored at -196°C in a Statebourne liquid nitrogen freezer, model SXR 34. Prior to the master strains being used, characterisation checks were carried out to confirm the amino-acid requirement, presence of rfa, R factors, uvrB or uvrA mutation and the spontaneous reversion rate.

In this assay, overnight sub-cultures of the appropriate coded stock cultures were prepared in nutrient broth (Oxoid Limited; lot number 434849 01/11) and incubated at 37°C for approximately 10 hours. Each culture was monitored spectrophotometrically for turbidity with titres determined by viable count analysis on nutrient agar plates.

3.2 Preparation of Test and Control Materials

According to information from the sponsor, the test material was insoluble in sterile distilled water. In solubility checks performed in-house, the test material was insoluble in dimethyl sulphoxide, dimethyl formamide and acetonitrile at 50 mg/ml but was fully soluble in acetone at

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50 mg/ml and tetrahydrofuran at 200 mg/ml. Although the test material was also soluble in tetrahydrofuran, acetone was selected as the vehicle because it is a more preferable solvent.

The test material was accurately weighed and approximate half-log dilutions prepared in acetone by mixing on a vortex mixer and sonication for 15 minutes at 40°C on the day of each experiment. The test material formulations at and above 5 mg/ml were retained at approximately 40°C to maintain solubility. Analysis for concentration, homogeneity and stability of the test material formulations is not a requirement of the test guidelines and was, therefore, not determined. Prior to use, the solvent was dried using molecular sieves (sodium alumino-silicate) ie 2 mm pellets with a nominal pore diameter of 4×10^{-4} microns.

Vehicle and positive controls were used in parallel with the test material. A solvent treatment group was used as the vehicle control and the positive control materials used in the series of plates without S9-mix were as follows:

N-ethyl-N'-nitro-N-nitrosoguanidine (ENNG):	3 µg/plate for TA100, 5 µg/plate for TA1535 and 2 µg/plate for WP2uvrA$^-$
9-Aminoacridine (9AA):	80 µg/plate for TA1537
4-Nitroquinoline-1-oxide (4NQO):	0.2 µg/plate for TA98

In addition, 2-Aminoanthracene (2AA) and Benzo(a)pyrene (BP), which are non-mutagenic in the absence of metabolising enzymes, were used in the series of plates with S9-mix at the following concentrations:

2AA at 1 µg/plate for TA100
2AA at 2 µg/plate for TA1535 and TA1537
BP at 5 µg/plate for TA98
2AA at 10 µg/plate for WP2uvrA$^-$

3.3 Microsomal Enzyme Fraction

S9 was prepared in-house on 01 April 2006 (Preliminary Toxicity Test), 08 July 2006 (Experiment 1) and 03 September 2006 (Experiment 2) from the livers of male Sprague-Dawley rats weighing ~ 250g. These had each orally received three consecutive daily doses of phenobarbitone/β-naphthoflavone (80/100 mg per kg per day) prior to S9 preparation on Day 4. Before use, each batch of S9 was assayed for its ability to metabolise appropriate indirect mutagens used in the Ames Test. The S9 was stored at -196°C.

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3.4 S9-Mix and Agar

The S9-mix was prepared immediately before use using sterilised co-factors and maintained on ice for the duration of the test.

S9	5.0 ml
1.65 M KCl/0.4 M $MgCl_2$	1.0 ml
0.1 M Glucose-6-phosphate	2.5 ml
0.1 M NADPH	2.0 ml
0.1 M NADH	2.0 ml
0.2 M Sodium phosphate buffer (pH 7.4)	25.0 ml
Sterile distilled water	12.5 ml

A 0.5 ml aliquot of S9-mix and 2 ml of molten, trace histidine or tryptophan supplemented, top agar were overlaid onto a sterile Vogel-Bonner Minimal agar plate in order to assess the sterility of the S9-mix. This procedure was repeated, in triplicate, on the day of each experiment.

Top agar was prepared using 0.6% Difco Bacto agar (lot numbers 5102667 01/10 and 5279941 08/10) and 0.5% sodium chloride with 5 ml of 1.0 mM histidine and 1.0 mM biotin or 1.0 mM tryptophan solution added to each 100 ml of top agar. Vogel-Bonner Minimal agar plates were purchased from ILS Limited (lot numbers 951578-02 04/11 and 954961-02 05/11).

3.5 Test Procedure

3.5.1 Preliminary Toxicity Test

In order to select appropriate dose levels for use in the main test, a preliminary test was carried out to determine the toxicity of the test material. The concentrations tested were 0, 0.15, 0.5, 1.5, 5, 15, 50, 150, 500, 1500 and 5000 µg/plate. The test was performed by mixing 0.1 ml of bacterial culture (TA100 or WP2uvrA$^-$), 2 ml of molten, trace histidine or tryptophan supplemented, top agar, 0.1 ml of test material formulation and 0.5 ml of S9-mix or phosphate buffer and overlaying onto sterile plates of Vogel-Bonner Minimal agar (30 ml/plate). Ten concentrations of the test material formulation and a vehicle control (acetone) were tested. In addition, 0.1 ml of the maximum concentration of the test material and 2 ml of molten, trace histidine or tryptophan supplemented, top agar were overlaid onto a sterile Nutrient agar plate in order to assess the sterility of the test material. After approximately 48 hours incubation at 37°C the plates were assessed for numbers of revertant colonies using a Domino colony counter and examined for effects on the growth of the bacterial background lawn.

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3.5.2 Mutation Test - Experiment 1

Five concentrations of the test material (50, 150, 500, 1500 and 5000 µg/plate) were assayed in triplicate against each tester strain, using the direct plate incorporation method.

Measured aliquots (0.1 ml) of one of the bacterial cultures were dispensed into sets of test tubes followed by 2.0 ml of molten, trace histidine or tryptophan supplemented, top agar, 0.1 ml of the test material formulation, vehicle or positive control and either 0.5 ml of S9-mix or phosphate buffer. The contents of each test tube were mixed and equally distributed onto the surface of Vogel-Bonner Minimal agar plates (one tube per plate). This procedure was repeated, in triplicate, for each bacterial strain and for each concentration of test material both with and without S9-mix.

All of the plates were incubated at 37°C for approximately 48 hours and the frequency of revertant colonies assessed using a Domino colony counter. Manual counts were performed at 5000 µg/plate because of excessive test material precipitation.

3.5.3 Mutation Test - Experiment 2

The second experiment was performed using methodology as described for Experiment 1, using fresh bacterial cultures, test material and control solutions. The test material dose range was the same as Experiment 1 (50 to 5000 µg/plate).

3.6 Acceptance Criteria

The reverse mutation assay may be considered valid if the following criteria are met:

All tester strain cultures exhibit a characteristic number of spontaneous revertants per plate in the vehicle and untreated controls. Acceptable ranges are presented in the standard test method section 3 with historical control ranges for 2004 and 2005 in Appendix 1.

The appropriate characteristics for each tester strain have been confirmed, eg rfa cell-wall mutation and pKM101 plasmid R-factor etc.

All tester strain cultures should be in the approximate range of 1 to 9.9 x 10^9 bacteria per ml.

Each mean positive control value should be at least twice the respective vehicle control value for each strain, thus demonstrating both the intrinsic sensitivity of the tester strains to mutagenic

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exposure and the integrity of the S9-mix. The historical control ranges for 2004 and 2005 are presented in Appendix 1.

There should be a minimum of four non-toxic test material dose levels.

There should be no evidence of excessive contamination.

3.7 Evaluation Criteria

There are several criteria for determining a positive result, such as a dose-related increase in revertant frequency over the dose range tested and/or a reproducible increase at one or more concentrations in at least one bacterial strain with or without metabolic activation. Biological relevance of the results will be considered first, statistical methods, as recommended by the UKEMS (5) can also be used as an aid to evaluation, however, statistical significance will not be the only determining factor for a positive response.

A test material will be considered non-mutagenic (negative) in the test system if the above criteria are not met.

Although most experiments will give clear positive or negative results, in some instances the data generated will prohibit a definitive judgement about the test material activity. Results of this type will be reported as equivocal.

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.

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5. RESULTS

5.1 Preliminary Toxicity Test

The test material was non-toxic to the strains of bacteria used (TA100 and WP2uvrA⁻). The test material formulation and S9-mix used in this experiment were both shown to be sterile.

The numbers of revertant colonies for the toxicity assay were:

With (+) or without (-) S9-mix	Strain	Dose (µg/plate)										
		0	0.15	0.5	1.5	5	15	50	150	500	1500	5000
-	TA100	97	99	103	84	91	92	112	88	92	118	99P
+	TA100	79	109	108	87	86	84	78	95	97	78	98P
-	WP2uvrA⁻	15	14	22	15	18	13	16	19	14	14	14P
+	WP2uvrA⁻	27	26	31	33	25	23	15	26	27	25	29P

P Precipitate

5.2 Mutation Test

Prior to use, the master strains were checked for characteristics, viability and spontaneous reversion rate (all were found to be satisfactory). These data are not given in the report. The amino acid supplemented top agar and the S9-mix used in both experiments was shown to be sterile.

Results for the negative controls (spontaneous mutation rates) are presented in Table 1 and were considered to be acceptable. These data are for concurrent untreated control plates performed on the same day as the Mutation Test.

The individual plate counts, the mean number of revertant colonies and the standard deviations, for the test material, positive and vehicle controls, both with and without metabolic activation, are presented in Table 2 and Table 3 for Experiment 1 and Table 4 and Table 5 for Experiment 2.

A history profile of vehicle and positive control values for 2004 and 2005 is presented in Appendix 1.

The test material caused no visible reduction in the growth of the bacterial background lawn at any dose level. The test material was, therefore, tested up to the maximum recommended dose

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level of 5000 µg/plate. A pale, particulate precipitate was observed at and above 1500 µg/plate, this did not prevent the scoring of revertant colonies.

No significant increases in the frequency of revertant colonies were recorded for any of the strains of bacteria, at any dose level either with or without metabolic activation.

All of the positive control chemicals used in the test induced marked increases in the frequency of revertant colonies thus confirming the activity of the S9-mix and the sensitivity of the bacterial strains.

6. CONCLUSION

The test material was considered to be non-mutagenic under the conditions of this test.

7. REFERENCES

1. Ames B N, Durston W E, Yamasaki E and Lee F D (1973b) *Proc. Natl. Acad. Sci. (USA)*, **70**, 2281-2285.

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USING *SALMONELLA TYPHIMURIUM* AND *ESCHERICHIA COLI*

Table 1 Spontaneous Mutation Rates (Concurrent Negative Controls)

EXPERIMENT 1

Number of revertants (mean number of colonies per plate)									
Base-pair substitution type						Frameshift type			
TA100		TA1535		WP2uvrA⁻		TA98		TA1537	
85		23		36		24		16	
103	(91)	10	(17)	45	(41)	24	(25)	13	(16)
86		19		42		26		20	

EXPERIMENT 2

Number of revertants (mean number of colonies per plate)									
Base-pair substitution type						Frameshift type			
TA100		TA1535		WP2uvrA⁻		TA98		TA1537	
123		22		26		40		9	
141	(125)	22	(23)	26	(27)	43	(38)	6	(7)
112		24		28		31		5	

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S-Face VL-212: REVERSE MUTATION ASSAY "AMES TEST"
USING *SALMONELLA TYPHIMURIUM* AND *ESCHERICHIA COLI*

Table 2 Test Results: Experiment 1 – Without Metabolic Activation

Test Period		From: 15 August 2006						To: 18 August 2006			
With or without S9-Mix	Test substance concentration (µg/plate)	Number of revertants (mean number of colonies per plate)									
		Base-pair substitution type						Frameshift type			
		TA100		TA1535		WP2uvrA-		TA98		TA1537	
-	0	93 89 89	(90) 2.3#	16 10 16	(14) 3.5	44 54 47	(48) 5.1	22 26 25	(24) 2.1	10 18 11	(13) 4.4
-	50	101 98 90	(96) 5.7	15 13 14	(14) 1.0	43 24 37	(35) 9.7	32 14 33	(26) 10.7	14 24 15	(18) 5.5
-	150	70 77 88	(78) 9.1	11 10 8	(10) 1.5	38 44 37	(40) 3.8	22 26 30	(26) 4.0	15 13 14	(14) 1.0
-	500	87 92 84	(88) 4.0	7 15 12	(11) 4.0	32 40 42	(38) 5.3	22 27 19	(23) 4.0	16 19 10	(15) 4.6
-	1500	84 P 90 P 102 P	(92) 9.2	22 P 18 P 16 P	(19) 3.1	46 P 36 P 43 P	(42) 5.1	25 P 27 P 31 P	(28) 3.1	18 P 13 P 19 P	(17) 3.2
-	5000	88 P 79 P 91 P	(86) 6.2	12 P 18 P 13 P	(14) 3.2	38 P 34 P 36 P	(36) 2.0	11 P 14 P 18 P	(14) 3.5	15 P 14 P 16 P	(15) 1.0
Positive controls	Name	ENNG		ENNG		ENNG		4NQO		9AA	
	Concentration (µg/plate)	3		5		2		0.2		80	
S9-Mix +	No. colonies per plate	395 443 404	(414) 25.5	142 111 102	(118) 21.0	792 757 721	(757) 35.5	164 170 148	(161) 11.4	715 720 690	(708) 16.1

ENNG N-ethyl-N'-nitro-N-nitrosoguanidine
4NQO 4-Nitroquinoline-1-oxide
9AA 9-Aminoacridine
P Precipitate
Standard deviation

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S-Face VL-212: REVERSE MUTATION ASSAY "AMES TEST"
USING *SALMONELLA TYPHIMURIUM* AND *ESCHERICHIA COLI*

Table 3 Test Results: Experiment 1 – With Metabolic Activation

Test Period		From: 15 August 2006						To: 18 August 2006			
With or without S9-Mix	Test substance concentration (µg/plate)	Number of revertants (mean number of colonies per plate)									
		Base-pair substitution type						Frameshift type			
		TA100		TA1535		WP2uvrA-		TA98		TA1537	
+	0	85 75 87	(82) 6.4#	8 12 12	(11) 2.3	52 45 47	(48) 3.6	8 16 21	(15) 6.6	12 11 10	(11) 1.0
+	50	80 88 62	(77) 13.3	12 11 12	(12) 0.6	51 38 40	(43) 7.0	18 20 16	(18) 2.0	9 12 7	(9) 2.5
+	150	73 81 66	(73) 7.5	7 12 13	(11) 3.2	54 49 48	(50) 3.2	13 14 15	(14) 1.0	7 8 10	(8) 1.5
+	500	76 76 88	(80) 6.9	5 12 14	(10) 4.7	40 49 42	(44) 4.7	27 18 13	(19) 7.1	16 7 10	(11) 4.6
+	1500	76 P 74 P 70 P	(73) 3.1	8 P 13 P 14 P	(12) 3.2	56 P 37 P 53 P	(49) 10.2	18 P 21 P 20 P	(20) 1.5	12 P 8 P 7 P	(9) 2.6
+	5000	78 P 83 P 92 P	(84) 7.1	10 P 13 P 13 P	(12) 1.7	42 P 45 P 29 P	(39) 8.5	22 P 19 P 16 P	(19) 3.0	8 P 9 P 7 P	(8) 1.0
Positive controls	Name	2AA		2AA		2AA		BP		2AA	
	Concentration (µg/plate)	1		2		10		5		2	
S9-Mix +	No. colonies per plate	597 608 637	(614) 20.7	208 222 238	(223) 15.0	405 379 414	(399) 18.2	180 166 165	(170) 8.4	443 477 526	(482) 41.7

2AA 2-Aminoanthracene
BP Benzo(a)pyrene
P Precipitate
Standard deviation

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S-Face VL-212: REVERSE MUTATION ASSAY "AMES TEST"
USING *SALMONELLA TYPHIMURIUM* AND *ESCHERICHIA COLI*

Table 4 Test Results: Experiment 2 – Without Metabolic Activation

Test Period		From: 11 September 2006						To: 14 September 2006					
With or without S9-Mix	Test substance concentration (µg/plate)	Number of revertants (mean number of colonies per plate)											
		Base-pair substitution type								Frameshift type			
		TA100		TA1535		WP2uvrA-				TA98		TA1537	
-	0	115 126 146	(129) 15.7#	31 24 10	(22) 10.7	25 23 31	(26) 4.2			23 16 19	(19) 3.5	25 6 9	(13) 10.2
-	50	114 93 104	(104) 10.5	20 25 27	(24) 3.6	30 29 33	(31) 2.1			33 18 27	(26) 7.5	13 7 14	(11) 3.8
-	150	103 114 129	(115) 13.1	22 21 18	(20) 2.1	22 21 43	(29) 12.4			20 23 23	(22) 1.7	5 9 18	(11) 6.7
-	500	105 137 103	(115) 19.1	22 20 14	(19) 4.2	18 19 24	(20) 3.2			23 21 18	(21) 2.5	10 9 6	(8) 2.1
-	1500	103 P 123 P 102 P	(109) 11.8	13 P 19 P 16 P	(16) 3.0	34 P 27 P 39 P	(33) 6.0			30 P 43 P 17 P	(30) 13.0	9 P 9 P 15 P	(11) 3.5
-	5000	90 P 99 P 89 P	(93) 5.5	18 P 20 P 11 P	(16) 4.7	29 P 25 P 24 P	(26) 2.6			19 P 18 P 18 P	(18) 0.6	9 P 6 P 8 P	(8) 1.5
Positive controls	Name	ENNG		ENNG		ENNG				4NQO		9AA	
	Concentration (µg/plate)	3		5		2				0.2		80	
S9-Mix -	No. colonies per plate	376 385 447	(403) 38.7	174 142 135	(150) 20.8	517 613 635	(588) 62.7			147 179 149	(158) 17.9	1209 962 1012	(1061) 130.6

ENNG N-ethyl-N'-nitro-N-nitrosoguanidine
4NQO 4-Nitroquinoline-1-oxide
9AA 9-Aminoacridine
P Precipitate
Standard deviation

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S-Face VL-212: REVERSE MUTATION ASSAY "AMES TEST"
USING *SALMONELLA TYPHIMURIUM* AND *ESCHERICHIA COLI*

Table 5 Test Results: Experiment 2 – With Metabolic Activation

Test Period		From: 11 September 2006						To: 14 September 2006			
With or without S9-Mix	Test substance concentration (µg/plate)	Number of revertants (mean number of colonies per plate)									
		Base-pair substitution type						Frameshift type			
		TA100		TA1535		WP2uvrA-		TA98		TA1537	
+	0	107 104 90	(100) 9.1#	19 14 6	(13) 6.6	36 38 34	(36) 2.0	26 21 17	(21) 4.5	11 15 9	(12) 3.1
+	50	87 110 91	(96) 12.3	15 7 10	(11) 4.0	43 37 42	(41) 3.2	23 15 29	(22) 7.0	13 13 15	(14) 1.2
+	150	96 88 78	(87) 9.0	16 14 10	(13) 3.1	33 32 41	(35) 4.9	24 28 30	(27) 3.1	9 6 9	(8) 1.7
+	500	86 82 89	(86) 3.5	15 13 9	(12) 3.1	29 28 32	(30) 2.1	21 19 29	(23) 5.3	9 6 6	(7) 1.7
+	1500	96 P 97 P 90 P	(94) 3.8	14 P 11 P 5 P	(10) 4.6	27 P 25 P 38 P	(30) 7.0	44 P 19 P 27 P	(30) 12.8	11 P 5 P 15 P	(10) 5.0
+	5000	86 P 90 P 73 P	(83) 8.9	13 P 13 P 13 P	(13) 0.0	28 P 22 P 30 P	(27) 4.2	27 P 25 P 17 P	(23) 5.3	5 P 4 P 8 P	(6) 2.1
Positive controls	Name	2AA		2AA		2AA		BP		2AA	
	Concentration (µg/plate)	1		2		10		5		2	
S9-Mix +	No. colonies per plate	2174 1883 2037	(2031) 145.6	186 168 167	(174) 10.7	374 418 317	(370) 50.6	154 183 194	(177) 20.7	571 530 499	(533) 36.1

2AA 2-Aminoanthracene
BP Benzo(a)pyrene
P Precipitate
Standard deviation

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S-Face VL-212: REVERSE MUTATION ASSAY "AMES TEST"
USING *SALMONELLA TYPHIMURIUM* AND *ESCHERICHIA COLI*

Appendix 1 History Profile of Vehicle and Positive Control Values

COMBINED VEHICLE AND UNTREATED CONTROL VALUES 2004

Strain	TA100		TA1535		WP2uvrA-		TA102		TA98		TA1537		WP2uvrA-pKM101	
S9-Mix	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Mean	94	98	24	14	23	27	333	367	21	31	12	18	147	183
SD	16.5	14.8	7.0	4.8	5.3	6.7	34.9	24.5	5.1	6.5	3.4	4.0	28.5	42.9
Min	61	60	9	7	9	11	192	263	10	11	5	6	92	131
Max	163	157	40	39	43	52	397	406	49	57	26	35	190	247
Values	993	798	911	713	735	579	291	183	917	740	889	708	15	12

POSITIVE CONTROL VALUES 2004

Strain	TA100		TA1535		WP2uvrA-		TA102		TA98		TA1537		WP2uvrA-pKM101	
S9-Mix	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Mean	557	1124	440	265	923	482	1250	893	175	219	999	366	2386	1954
SD	163.5	417.4	275.3	80.5	240.1	202.5	239.3	124.8	52.5	68.9	511.6	130.1	867.9	188.6
Min	189	348	93	162	371	153	728	660	95	114	130	137	1120	1737
Max	1215	2942	1634	1026	1714	1087	1960	1288	435	495	2964	832	3009	2075
Values	180	178	176	175	160	159	103	103	179	178	176	175	4	3

COMBINED VEHICLE AND UNTREATED CONTROL VALUES 2005

Strain	TA100		TA1535		WP2uvrA-		TA102		TA98		TA1537	
S9-Mix	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Mean	106	102	24	14	24	29	311	357	22	30	13	17
SD	19.5	19.5	6.6	4.2	5.3	5.6	43.9	27.7	5.1	6.2	3.5	4.9
Min	64	66	8	7	11	11	215	277	11	16	4	5
Max	196	177	40	38	49	57	391	393	49	51	25	29
Values	838	704	787	647	662	553	199	142	793	677	761	640

POSITIVE CONTROL VALUES 2005

Strain	TA100		TA1535		WP2uvrA-		TA102		TA98		TA1537	
S9-Mix	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Mean	576	1230	431	272	904	549	1483	957	210	289	854	413
SD	217.5	485.8	302.1	69.9	312.0	210.2	324.8	217.3	85.6	112.5	461.9	183.9
Min	261	334	77	126	251	188	646	646	91	109	252	123
Max	1649	2735	1966	577	2075	1339	2200	1787	579	679	2781	1061
Values	127	126	127	126	115	115	66	66	130	129	125	125

SD = Standard deviation
Min = Minimum value
Max = Maximum value

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Appendix 2 Statement of GLP Compliance in Accordance with Directive 2004/9/EC

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.

Bryan J Wright 21/11/05.

Mr. Bryan J. Wright
Head, UK GLP Monitoring Authority

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Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: Lillian Gill, D.P.A.
Director - COSMETIC INGREDIENT REVIEW (CIR)

FROM: Beth A. Lange, Ph.D.
Industry Liaison to the CIR Expert Panel

DATE: March 25, 2016

SUBJECT: Information on Macadamia Seed Oil Polyglyceryl-6 Esters Behenate (SLR posted January 28, 2016)

SafePharm Laboratories. 2006. Acute oral toxicity in the rat - acute toxic class method Macadamia Seed Oil Polyglyceryl-6 Esters Behenate.

SafePharm Laboratories. 2006. Reverse mutation assay "Ames test" using *Salmonella typhimurium* and *Escherichia coli* (Macadamia Seed Oil Polyglyceryl-6 Esters Behenate).

Japan Hair Science Association. 2010. Patch test on the cosmetic ingredient (Macadamia Seed Oil Polyglyceryl-6 Esters Behenate).

SafePharm Laboratories. 2006. Acute dermal irritation in the rabbit (Macadamia Seed Oil Polyglyceryl-6 Esters Behenate).

Phycher. 2006. Skin sensitization in the guinea pig- Magnusson and Kligman maximisation method (Macadamia Seed Oil Polyglyceryl-6 Esters Behenate).

SafePharm Laboratories. 2006. Acute eye irritation in the rabbit (Macadamia Seed Oil Polyglyceryl-6 Esters Behenate).

1620 L Street, N.W., Suite 1200 | Washington, D.C. 20036 | 202.331.1770 | 202.331.1969 (fax) | www.personalcarecouncil.org

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SafePharm Laboratories

S-Face VL-211:
Macadamia Seed Oil Polyglyceryl-6 Esters Behenate
ACUTE ORAL TOXICITY IN THE RAT
- ACUTE TOXIC CLASS METHOD

SPL PROJECT NUMBER: 0432/0080

AUTHOR: A Pooles

STUDY SPONSOR:

Sakamoto Yakuhin Kogyo Co., Ltd.
1-20, Rinkaicho
Izumiotsu-city
OSAKA 595-01075
JAPAN

TEST FACILITY:

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

Telephone: +44 (0) 1332 792896

Facsimile: +44 (0) 1332 799018

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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:

	29 October 2004	Standard Test Method Compliance Audit
	08 August 2006	Test Material Preparation
	17 August 2006	Animal Preparation
	08, 15 August 2006	Dosing
	08, 12 August 2006	Assessment of Response
	17 August 2006	Necropsy
§	10 September 2006	Draft Report Audit
§	Date of QA Signature	Final Report Audit

§ Evaluation specific to this study

JVJohnson ..... DATE: 01 NOV 2006

For Safepharm Quality Assurance Unit*

*Authorised QA Signatures:

Head of Department:	JR Pateman CBiol MIBiol DipRQA AIQA FRQA
Deputy Head of Department:	JM Crowther MIScT MRQA
Senior Audit Staff:	JV Johnson BSc MRQA; G Wren ONC MRQA

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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.

A. Pooles DATE: 31/10/06

A Pooles BA (Hons)
Study Director

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S-Face VL-211:

ACUTE ORAL TOXICITY IN THE RAT – ACUTE TOXIC CLASS METHOD

SUMMARY

Introduction. The study was performed to assess the acute oral toxicity of the test material following a single oral administration in the Sprague-Dawley CD strain rat. The method was designed to meet the requirements of the following:

- OECD Guidelines for the Testing of Chemicals No. 423 "Acute Oral Toxicity – Acute Toxic Class Method" (adopted 17 December 2001)
- Method B1 *tris* Acute Toxicity (Oral) of Commission Directive 2004/73/EC

Method. A group of three fasted females was treated with the test material at a dose level of 2000 mg/kg bodyweight. This was followed by a further group of three fasted females at the same dose level.

The test material was administered orally as a solution in arachis oil BP. Clinical signs and bodyweight development were monitored during the study. All animals were subjected to gross necropsy.

Mortality. There were no deaths.

Clinical Observations. There were no signs of systemic toxicity.

Bodyweight. All animals showed expected gains in bodyweight over the study period.

Necropsy. No abnormalities were noted at necropsy.

Conclusion. The acute oral median lethal dose (LD_{50}) of the test material in the female Sprague-Dawley CD strain rat was estimated to be greater than 2500 mg/kg bodyweight (Globally Harmonised Classification System – Unclassified).

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S-Face VL-211:

ACUTE ORAL TOXICITY IN THE RAT - ACUTE TOXIC CLASS METHOD

1. INTRODUCTION

The study was performed to assess the acute oral toxicity of the test material following a single oral administration in the Sprague-Dawley CD strain rat. The method was designed to meet the requirements of the following:

- OECD Guidelines for the Testing of Chemicals No. 423 "Acute Oral Toxicity – Acute Toxic Class Method" (adopted 17 December 2001)
- Method B1 *tris* Acute Toxicity (Oral) of Commission Directive 2004/73/EC

The rat was selected for this study as it is a readily available rodent species, historically used in safety evaluation studies, and is acceptable to appropriate regulatory authorities. The oral route was selected as the most appropriate route of exposure and the results are believed to be of value in predicting the likely toxicity of the test material to man.

The study was performed between 12 July 2006 and 02 August 2006.

2. TEST MATERIAL AND EXPERIMENTAL PREPARATION

2.1 Description, Identification and Storage Conditions

Sponsor's identification	:	S-Face VL-211
Description	:	white waxy solid
Batch number	:	60210
Date received	:	05 June 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.

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2.2 Preparation of Test Material

For the purpose of the study the test material was freshly prepared, as required, as a solution at the appropriate concentration in arachis oil BP. To aid preparation the test material formulation was heated at 70°C in a warming bath until dissolved. Arachis oil BP was used because the test material did not dissolve/suspend in distilled water.

Determination by analysis of the concentration, homogeneity and stability of the test material preparations was not appropriate because it was not specified in the Study Plan and is not a requirement of the Test Guideline.

3. METHODS

3.1 Animals and Animal Husbandry

Female Sprague-Dawley CD (Crl: CD® (SD) IGS BR) strain rats were supplied by Charles River (UK) Ltd, Margate, Kent, UK. On receipt the animals were randomly allocated to cages. The animals were nulliparous and non-pregnant. After an acclimatisation period of at least five days the animals were selected at random and given a number unique within the study by indelible ink-marking on the tail and a number written on a cage card. At the start of the study the animals were eight to twelve weeks of age. The bodyweights fell within an interval of ± 20% of the mean initial bodyweight of the first treated group.

The animals were housed in groups of three 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 (Certified Rat and Mouse Diet (Code 5LF2) supplied by BCM IPS Limited, London, UK) was allowed throughout the study. The diet, drinking water and bedding were routinely analysed 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. 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.

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3.2 Procedure

Using available information on the toxicity of the test material, 2000 mg/kg was chosen as the starting dose.

Groups of fasted animals were treated as follows:

Dose Level (mg/kg)	Concentration (mg/ml)	Dose Volume (ml/kg)	Number of Rats
			Female
2000	200	10	3
2000	200	10	3

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 group 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 fourteen 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.

3.3 Evaluation of Data

Data evaluations included the relationship, if any, between the exposure of the animal to the test material and the incidence and severity of all abnormalities including behavioural and clinical observations, gross lesions, bodyweight 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 material was made as shown in the schematic diagram in Appendix 1.

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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.

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5. RESULTS

5.1 Mortality Data

Individual mortality data are given in Table 1.

There were no deaths.

5.2 Clinical Observations

Individual clinical observations are given in Table 2.

There were no signs of systemic toxicity.

5.3 Bodyweight

Individual bodyweights and weekly bodyweight changes are given in Table 3.

All animals showed expected gains in bodyweight over the study period.

5.4 Necropsy

Individual necropsy findings are given in Table 4.

No abnormalities were noted at necropsy.

6. CONCLUSION

The acute oral median lethal dose (LD_{50}) of the test material in the female Sprague-Dawley CD strain rat was estimated to be greater than 2500 mg/kg bodyweight (Globally Harmonised Classification System – Unclassified).

Table 1 Mortality Data

Dose Level mg/kg	Sex	Number of Animals Treated	Deaths During Day of Dosing (Hours)				Deaths During Period After Dosing (Days)								Deaths
			½	1	2	4	1	2	3	4	5	6	7	8-14	
2000	Female	3	0	0	0	0	0	0	0	0	0	0	0	0	0/3
	Female	3	0	0	0	0	0	0	0	0	0	0	0	0	0/3

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Table 2 Individual Clinical Observations

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
2000	1-0 Female	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	1-1 Female	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	1-2 Female	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2-0 Female	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2-1 Female	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2-2 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

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Table 3 Individual Bodyweights and Weekly Bodyweight Changes

Dose Level mg/kg	Animal Number and Sex	Bodyweight (g) at Day			Bodyweight Gain (g) During Week	
		0	7	14	1	2
2000	1-0 Female	225	246	268	21	22
	1-1 Female	221	239	271	18	32
	1-2 Female	223	234	268	11	34
	2-0 Female	250	288	300	38	12
	2-1 Female	229	246	264	17	18
	2-2 Female	217	251	271	34	20

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Table 4 Individual Necropsy Findings

Dose Level mg/kg	Animal Number and Sex	Time of Death	Macroscopic Observations
2000	1-0 Female	Killed Day 14	No abnormalities detected
	1-1 Female	Killed Day 14	No abnormalities detected
	1-2 Female	Killed Day 14	No abnormalities detected
	2-0 Female	Killed Day 14	No abnormalities detected
	2-1 Female	Killed Day 14	No abnormalities detected
	2-2 Female	Killed Day 14	No abnormalities detected

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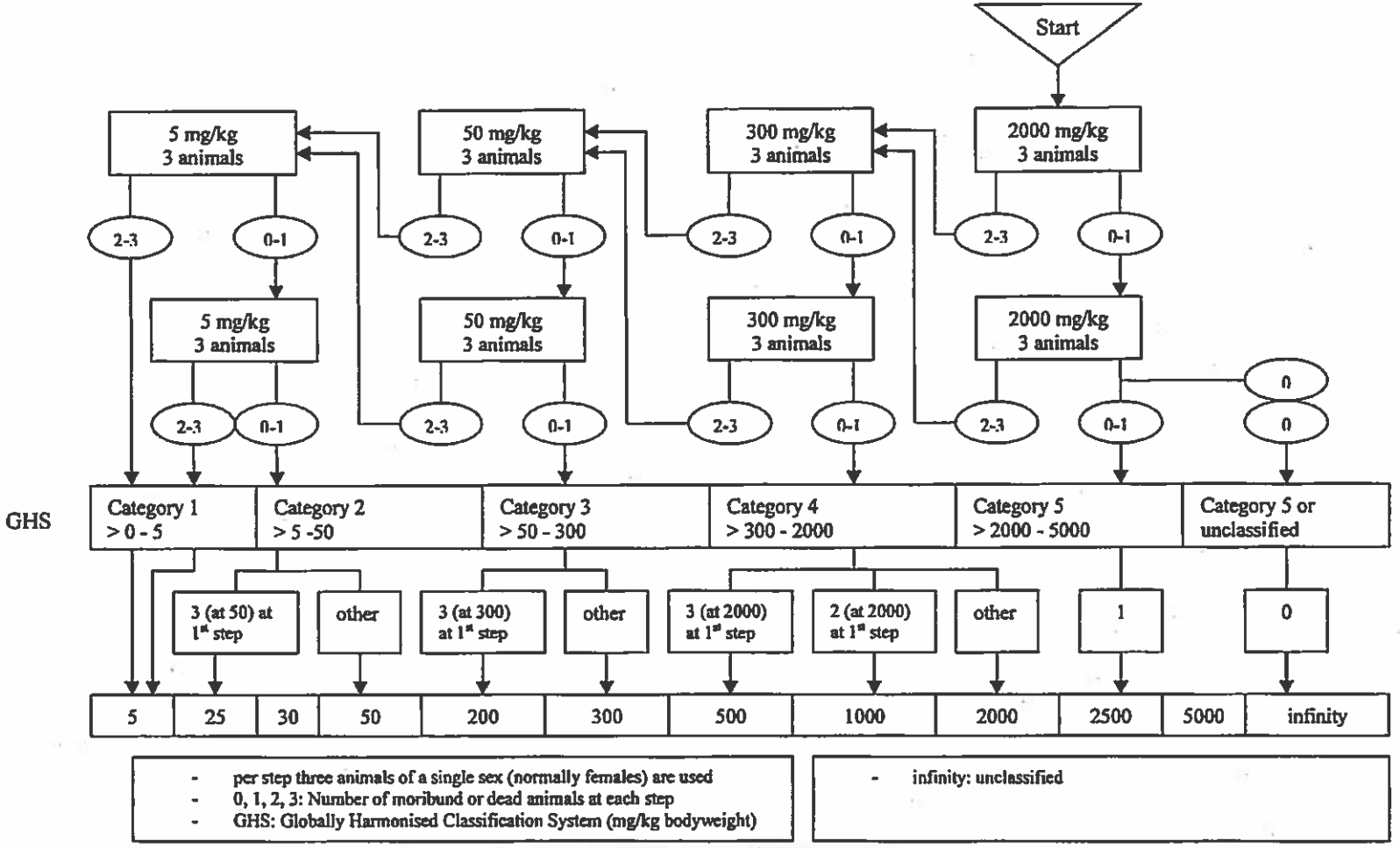
Appendix 1 Test Procedure with a Starting Dose of 2000 mg/kg Bodyweight



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Appendix 2 Statement of GLP Compliance in Accordance with Directive 2004/9/EC

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

Bryan J Wright 21/11/05.

Mr. Bryan J. Wright
Head, UK GLP Monitoring Authority

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SafePharm Laboratories

S-Face VL-211:
Macadamia Seed Oil Polyglyceryl-6 Esters Behenate
REVERSE MUTATION ASSAY "AMES TEST"
USING *SALMONELLA TYPHIMURIUM* AND
ESCHERICHIA COLI

SPL PROJECT NUMBER: 0432/0084

AUTHORS: P W Thompson
A J Bowles

STUDY SPONSOR:

Sakamoto Yakuhin Kogyo Co., Ltd.
1-20, Rinkaicho
Izumiotsu-city
OSAKA 595-01075
JAPAN

TEST FACILITY:

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

Telephone: +44 (0) 1332 792896

Facsimile: +44 (0) 1332 799018

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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:

	15 July 2004	Standard Test Method Compliance Audit
	25 September 2006	Test Material Preparation
	11 September 2006	Test System Preparation
	19 September 2006	Exposure
	25 September 2006	Assessment of Response
§	16 October 2006	Draft Report Audit
§	Date of QA Signature	Final Report Audit

§ Evaluation specific to this study

JVJohnson DATE: 01 NOV 2006

For Safepharm Quality Assurance Unit*

*Authorised QA Signatures:
Head of Department: JR Pateman CBiol MIBiol DipRQA AIQA FRQA
Deputy Head of Department: JM Crowther MIScT MRQA
Senior Audit Staff: JV Johnson BSc MRQA; G Wren ONC MRQA

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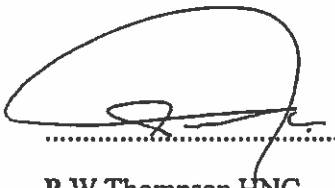
GLP COMPLIANCE STATEMENT

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: 31 OCT 2006

P W Thompson HNC
Study Director

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S-Face VL-211:

REVERSE MUTATION ASSAY "AMES TEST" USING *SALMONELLA TYPHIMURIUM* AND *ESCHERICHIA COLI*

SUMMARY

Introduction. The method conforms to the guidelines for bacterial mutagenicity testing published by the major Japanese Regulatory Authorities including METI, MHLW and MAFF. It also meets the requirements of the OECD Guidelines for Testing of Chemicals No. 471 "Bacterial Reverse Mutation Test", Method B13/14 of Commission Directive 2000/32/EC and the USA, EPA (TSCA) OPPTS harmonised guidelines.

Methods. *Salmonella typhimurium* strains TA1535, TA1537, TA98, TA100 and *Escherichia coli* strain WP2uvrA$^-$ were treated with the test material using the Ames plate incorporation method at five dose levels, in triplicate, both with and without the addition of a rat liver homogenate metabolising system (10% liver S9 in standard co-factors). The dose range was determined in a preliminary toxicity assay and was 50 to 5000 µg/plate in the first experiment. The experiment was repeated on a separate day using the same dose range as Experiment 1, fresh cultures of the bacterial strains and fresh test material formulations.

Results. The vehicle (acetone) control plates gave counts of revertant colonies within the normal range. All of the positive control chemicals used in the test induced marked increases in the frequency of revertant colonies, both with or without metabolic activation. Thus, the sensitivity of the assay and the efficacy of the S9-mix were validated.

The test material caused no visible reduction in the growth of the bacterial background lawn at any dose level. The test material was, therefore, tested up to the maximum recommended dose level of 5000 µg/plate. An oily precipitate was observed at and above 1500 µg/plate, this did not prevent the scoring of revertant colonies.

No significant increases in the frequency of revertant colonies were recorded for any of the bacterial strains, with any dose of the test material, either with or without metabolic activation.

Conclusion. The test material was considered to be non-mutagenic under the conditions of this test.

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S-Face VL-211:

REVERSE MUTATION ASSAY "AMES TEST" USING *SALMONELLA TYPHIMURIUM* AND *ESCHERICHIA COLI*

1. INTRODUCTION

This study was designed to assess the mutagenic potential of the test material using a bacterial/ microsome test system. The study was based on the *in vitro* technique described by Ames and his co-workers (1, 2, 3) and Garner et al (4) in which mutagenic activity is assessed by exposing histidine auxotrophs of *Salmonella typhimurium* and tryptophan auxotrophs of *Escherichia coli* to various concentrations of the test material. The method conforms to the guidelines for bacterial mutagenicity testing published by the major Japanese Regulatory Authorities including METI, MHLW and MAFF. It also meets the requirements of the OECD Guidelines for Testing of Chemicals No. 471 "Bacterial Reverse Mutation Test", Method B13/14 of Commission Directive 2000/32/EC and the USA, EPA (TSCA) OPPTS harmonised guidelines. A copy of the Certificate of Compliance with GLP, issued by the UK Department of Health, is included.

These mutant strains of *Salmonella* are incapable of synthesising histidine and are, therefore, dependent on an external source of this particular amino acid for normal growth. When exposed to a mutagenic agent these bacteria may undergo a reverse mutation to histidine independent forms which are detected by their ability to grow on a histidine deficient medium. Using various strains of this organism, revertants produced after exposure to a chemical mutagen may arise as a result of base-pair substitution in the genetic material (miscoding) or frame-shift mutation in which genetic material is either added or deleted. In order to make the bacteria more sensitive to mutation by chemical and physical agents, several additional traits have been introduced. These include a deletion through the excision repair gene (uvrB$^-$ *Salmonella* strains) which renders the organism incapable of DNA excision repair and deep rough mutation (rfa) which increases the permeability of the cell wall. A mutant strain of *Escherichia coli* (WP2uvrA$^-$), which requires tryptophan and which can be reverse mutated by base substitution to tryptophan independence was used to complement the *Salmonella* strains. This strain also has a deletion in an excision repair gene (uvrA$^-$). Since many compounds do not exert a mutagenic effect until they have been metabolised by enzyme systems not available in the bacterial cell, the test material and the bacteria are also incubated in the presence of a liver microsomal preparation (S9-mix) prepared from rats pre-treated with a mixture known to induce an elevated level of these enzymes.

The experimental phase of this study was performed between 28 June 2006 and 14 September 2006.

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2. TEST MATERIAL

Sponsor's identification	:	S-Face VL-211
Description	:	White waxy solid
Chemical name	:	Macadamia nut oil Glyceryl/Polyglyceryl-6 esters Behenate
Purity	:	>99%
Batch number	:	60210
Date received	:	05 June 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.

3. METHODS

3.1 Tester Strains

Salmonella typhimurium TA1535, TA1537, TA98 and TA100

Escherichia coli WP2uvrA$^-$

The *Salmonella* strains were obtained from the University of California at Berkeley on culture discs on 4 August 1995 whilst *Escherichia coli* strain WP2uvrA$^-$ was obtained from the British Industrial Biological Research Association on 17 August 1987. All of the strains were stored at -196°C in a Stateboume liquid nitrogen freezer, model SXR 34. Prior to the master strains being used, characterisation checks were carried out to confirm the amino-acid requirement, presence of rfa, R factors, uvrB or uvrA mutation and the spontaneous reversion rate.

In this assay, overnight sub-cultures of the appropriate coded stock cultures were prepared in nutrient broth (Oxoid Limited; lot number 434849 01/11) and incubated at 37°C for approximately 10 hours. Each culture was monitored spectrophotometrically for turbidity with titres determined by viable count analysis on nutrient agar plates.

3.2 Preparation of Test and Control Materials

According to information from the sponsor, the test material was insoluble in sterile distilled water. In solubility checks performed in-house, the test material was insoluble in dimethyl sulphoxide, dimethyl formamide and acetonitrile at 50 mg/ml but was fully soluble in acetone at

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50 mg/ml and tetrahydrofuran at 200 mg/ml. Although the test material was soluble in tetrahydrofuran, acetone, was selected as the vehicle because it is a more preferable solvent.

The test material was accurately weighed and approximate half-log dilutions prepared in acetone by mixing on a vortex mixer and sonication for 15 minutes at 40°C on the day of each experiment. The test material formulations at and above 1.5 mg/ml were retained at approximately 40°C to maintain solubility. Analysis for concentration, homogeneity and stability of the test material formulations is not a requirement of the test guidelines and was, therefore, not determined. Prior to use, the solvent was dried using molecular sieves (sodium alumino-silicate) ie 2 mm pellets with a nominal pore diameter of 4×10^{-4} microns.

Vehicle and positive controls were used in parallel with the test material. A solvent treatment group was used as the vehicle control and the positive control materials used in the series of plates without S9-mix were as follows:

N-ethyl-N'-nitro-N-nitrosoguanidine (ENNG):	3 µg/plate for TA100, 5 µg/plate for TA1535 and 2 µg/plate for WP2uvrA$^-$
9-Aminoacridine (9AA):	80 µg/plate for TA1537
4-Nitroquinoline-1-oxide (4NQO):	0.2 µg/plate for TA98

In addition, 2-Aminoanthracene (2AA) and Benzo(a)pyrene (BP), which are non-mutagenic in the absence of metabolising enzymes, were used in the series of plates with S9-mix at the following concentrations:

2AA at 1 µg/plate for TA100
2AA at 2 µg/plate for TA1535 and TA1537
BP at 5 µg/plate for TA98
2AA at 10 µg/plate for WP2uvrA$^-$

3.3 Microsomal Enzyme Fraction

S9 was prepared in-house on 01 April 2006 (Preliminary Toxicity Test), 08 July 2006 (Experiment 1) and 03 September 2006 (Experiment 2) from the livers of male Sprague-Dawley rats weighing ~ 250g. These had each orally received three consecutive daily doses of phenobarbitone/β-naphthoflavone (80/100 mg per kg per day) prior to S9 preparation on Day 4. Before use, each batch of S9 was assayed for its ability to metabolise appropriate indirect mutagens used in the Ames Test. The S9 was stored at -196°C.

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3.4 S9-Mix and Agar

The S9-mix was prepared immediately before use using sterilised co-factors and maintained on ice for the duration of the test.

S9	5.0 ml
1.65 M KCl/0.4 M $MgCl_2$	1.0 ml
0.1 M Glucose-6-phosphate	2.5 ml
0.1 M NADPH	2.0 ml
0.1 M NADH	2.0 ml
0.2 M Sodium phosphate buffer (pH 7.4)	25.0 ml
Sterile distilled water	12.5 ml

A 0.5 ml aliquot of S9-mix and 2 ml of molten, trace histidine or tryptophan supplemented, top agar were overlaid onto a sterile Vogel-Bonner Minimal agar plate in order to assess the sterility of the S9-mix. This procedure was repeated, in triplicate, on the day of each experiment.

Top agar was prepared using 0.6% Difco Bacto agar (lot numbers 5102667 01/10 and 5279941 08/10) and 0.5% sodium chloride with 5 ml of 1.0 mM histidine and 1.0 mM biotin or 1.0 mM tryptophan solution added to each 100 ml of top agar. Vogel-Bonner Minimal agar plates were purchased from ILS Limited (lot numbers 948188-02 03/11 and 951578-02 04/11).

3.5 Test Procedure

3.5.1 Preliminary Toxicity Test

In order to select appropriate dose levels for use in the main test, a preliminary test was carried out to determine the toxicity of the test material. The concentrations tested were 0, 0.15, 0.5, 1.5, 5, 15, 50, 150, 500, 1500 and 5000 µg/plate. The test was performed by mixing 0.1 ml of bacterial culture (TA100 or WP2uvrA$^-$), 2 ml of molten, trace histidine or tryptophan supplemented, top agar, 0.1 ml of test material formulation and 0.5 ml of S9-mix or phosphate buffer and overlaying onto sterile plates of Vogel-Bonner Minimal agar (30 ml/plate). Ten concentrations of the test material formulation and a vehicle control (acetone) were tested. In addition, 0.1 ml of the maximum concentration of the test material and 2 ml of molten, trace histidine or tryptophan supplemented, top agar were overlaid onto a sterile Nutrient agar plate in order to assess the sterility of the test material. After approximately 48 hours incubation at 37°C the plates were assessed for numbers of revertant colonies using a Domino colony counter and examined for effects on the growth of the bacterial background lawn.

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3.5.2 Mutation Test - Experiment 1

Five concentrations of the test material (50, 150, 500, 1500 and 5000 µg/plate) were assayed in triplicate against each tester strain, using the direct plate incorporation method.

Measured aliquots (0.1 ml) of one of the bacterial cultures were dispensed into sets of test tubes followed by 2.0 ml of molten, trace histidine or tryptophan supplemented, top agar, 0.1 ml of the test material formulation, vehicle or positive control and either 0.5 ml of S9-mix or phosphate buffer. The contents of each test tube were mixed and equally distributed onto the surface of Vogel-Bonner Minimal agar plates (one tube per plate). This procedure was repeated, in triplicate, for each bacterial strain and for each concentration of test material both with and without S9-mix.

All of the plates were incubated at 37°C for approximately 48 hours and the frequency of revertant colonies assessed using a Domino colony counter. Manual counts were performed at 5000 µg/plate because of excessive test material precipitation.

3.5.3 Mutation Test - Experiment 2

The second experiment was performed using methodology as described for Experiment 1, using fresh bacterial cultures, test material and control solutions. The test material dose range was the same as Experiment 1 (50 to 5000 µg/plate).

3.6 Acceptance Criteria

The reverse mutation assay may be considered valid if the following criteria are met:

All tester strain cultures exhibit a characteristic number of spontaneous revertants per plate in the vehicle and untreated controls. Acceptable ranges are presented in the standard test method section 3 with historical control ranges for 2004 and 2005 in Appendix 1.

The appropriate characteristics for each tester strain have been confirmed, eg rfa cell-wall mutation and pKM101 plasmid R-factor etc.

All tester strain cultures should be in the approximate range of 1 to 9.9 x 10^9 bacteria per ml.

Each mean positive control value should be at least twice the respective vehicle control value for each strain, thus demonstrating both the intrinsic sensitivity of the tester strains to mutagenic

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exposure and the integrity of the S9-mix. The historical control ranges for 2004 and 2005 are presented in Appendix 1.

There should be a minimum of four non-toxic test material dose levels.

There should be no evidence of excessive contamination.

3.7 Evaluation Criteria

There are several criteria for determining a positive result, such as a dose-related increase in revertant frequency over the dose range tested and/or a reproducible increase at one or more concentrations in at least one bacterial strain with or without metabolic activation. Biological relevance of the results will be considered first, statistical methods, as recommended by the UKEMS (5) can also be used as an aid to evaluation, however, statistical significance will not be the only determining factor for a positive response.

A test material will be considered non-mutagenic (negative) in the test system if the above criteria are not met.

Although most experiments will give clear positive or negative results, in some instances the data generated will prohibit a definitive judgement about the test material activity. Results of this type will be reported as equivocal.

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.

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5. RESULTS

5.1 Preliminary Toxicity Test

The test material was non-toxic to the strains of bacteria used (TA100 and WP2uvrA$^-$). The test material formulation and S9-mix used in this experiment were both shown to be sterile.

The numbers of revertant colonies for the toxicity assay were:

With (+) or without (-) S9-mix	Strain	Dose (µg/plate)										
		0	0.15	0.5	1.5	5	15	50	150	500	1500	5000
-	TA100	108	100	97	88	100	91	115	77	97	118	121P
+	TA100	79	87	81	81	95	75	71	73	85	124	75P
-	WP2uvrA$^-$	22	19	13	22	22	10	25	27	27	18	30P
+	WP2uvrA$^-$	20	23	25	18	20	25	22	33	18	32	32P

P Precipitate

5.2 Mutation Test

Prior to use, the master strains were checked for characteristics, viability and spontaneous reversion rate (all were found to be satisfactory). These data are not given in the report. The amino acid supplemented top agar and the S9-mix used in both experiments was shown to be sterile.

Results for the negative controls (spontaneous mutation rates) are presented in Table 1 and were considered to be acceptable. These data are for concurrent untreated control plates performed on the same day as the Mutation Test.

The individual plate counts, the mean number of revertant colonies and the standard deviations, for the test material, positive and vehicle controls, both with and without metabolic activation, are presented in Table 2 and Table 3 for Experiment 1 and Table 4 and Table 5 for Experiment 2.

A history profile of vehicle and positive control values for 2004 and 2005 is presented in Appendix 1.

The test material caused no visible reduction in the growth of the bacterial background lawn at any dose level. The test material was, therefore, tested up to the maximum recommended dose

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level of 5000 µg/plate. An oily precipitate was observed at and above 1500 µg/plate, this did not prevent the scoring of revertant colonies.

No significant increases in the frequency of revertant colonies were recorded for any of the strains of bacteria, at any dose level either with or without metabolic activation.

All of the positive control chemicals used in the test induced marked increases in the frequency of revertant colonies thus confirming the activity of the S9-mix and the sensitivity of the bacterial strains.

6. CONCLUSION

The test material was considered to be non-mutagenic under the conditions of this test.

7. REFERENCES

1. Ames B N, Durston W E, Yamasaki E and Lee F D (1973b) *Proc. Natl. Acad. Sci. (USA)*, 70, 2281-2285.

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5. Kirkland D J (Ed) (1989) Statistical Evaluation of Mutagenicity Test Data. *UKEMS Sub-committee on Guidelines for Mutagenicity Testing*, **Report - Part III**, Cambridge University Press.

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USING *SALMONELLA TYPHIMURIUM* AND *ESCHERICHIA COLI*

Table 1 Spontaneous Mutation Rates (Concurrent Negative Controls)

EXPERIMENT 1

Number of revertants (mean number of colonies per plate)									
Base-pair substitution type						Frameshift type			
TA100		TA1535		WP2uvrA⁻		TA98		TA1537	
85		23		36		24		16	
103	(91)	10	(17)	45	(41)	24	(25)	13	(16)
86		19		42		26		20	

EXPERIMENT 2

Number of revertants (mean number of colonies per plate)									
Base-pair substitution type						Frameshift type			
TA100		TA1535		WP2uvrA⁻		TA98		TA1537	
123		22		26		40		9	
141	(125)	22	(23)	26	(27)	43	(38)	6	(7)
112		24		28		31		5	

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USING *SALMONELLA TYPHIMURIUM* AND *ESCHERICHIA COLI*

Table 2 Test Results: Experiment 1 – Without Metabolic Activation

Test Period		From: 15 August 2006						To: 18 August 2006			
With or without S9-Mix	Test substance concentration (µg/plate)	Number of revertants (mean number of colonies per plate)									
		Base-pair substitution type						Frameshift type			
		TA100		TA1535		WP2uvrA-		TA98		TA1537	
-	0	95 115 80	(97) 17.6#	21 12 15	(16) 4.6	43 37 44	(41) 3.8	12 15 18	(15) 3.0	19 9 18	(15) 5.5
-	50	84 76 99	(86) 11.7	19 9 18	(15) 5.5	54 46 35	(45) 9.5	21 20 22	(21) 1.0	15 15 12	(14) 1.7
-	150	92 82 114	(96) 16.4	14 11 19	(15) 4.0	36 34 38	(36) 2.0	18 18 22	(19) 2.3	16 9 13	(13) 3.5
-	500	92 114 91	(99) 13.0	12 16 12	(13) 2.3	47 47 46	(47) 0.6	13 22 15	(17) 4.7	12 13 19	(15) 3.8
-	1500	92 P 114 P 115 P	(107) 13.0	13 P 19 P 15 P	(16) 3.1	32 P 46 P 37 P	(38) 7.1	23 P 16 P 15 P	(18) 4.4	19 P 12 P 15 P	(15) 3.5
-	5000	99 P 81 P 102 P	(94) 11.4	12 P 10 P 10 P	(11) 1.2	43 P 37 P 40 P	(40) 3.0	18 P 17 P 10 P	(15) 4.4	11 P 15 P 14 P	(13) 2.1
Positive controls	Name	ENNG		ENNG		ENNG		4NQO		9AA	
	Concentration (µg/plate)	3		5		2		0.2		80	
S9-Mix -	No. colonies per plate	395 443 404	(414) 25.5	142 111 102	(118) 21.0	792 757 721	(757) 35.5	164 170 148	(161) 11.4	715 720 690	(708) 16.1

ENNG N-ethyl-N'-nitro-N-nitrosoguanidine
4NQO 4-Nitroquinoline-1-oxide
9AA 9-Aminoacridine
P Precipitate
Standard deviation

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USING *SALMONELLA TYPHIMURIUM* AND *ESCHERICHIA COLI*

Table 3 Test Results: Experiment 1 – With Metabolic Activation

Test Period		From: 15 August 2006						To: 18 August 2006			
With or without S9-Mix	Test substance concentration (µg/plate)	Number of revertants (mean number of colonies per plate)									
		Base-pair substitution type						Frameshift type			
		TA100		TA1535		WP2uvrA-		TA98		TA1537	
+	0	80 80 75	(78) 2.9#	11 15 16	(14) 2.6	43 42 54	(46) 6.7	20 21 19	(20) 1.0	12 13 12	(12) 0.6
+	50	88 101 88	(92) 7.5	4 10 19	(11) 7.5	34 30 44	(36) 7.2	22 20 18	(20) 2.0	16 16 16	(16) 0.0
+	150	97 81 67	(82) 15.0	16 5 8	(10) 5.7	41 51 45	(46) 5.0	15 30 21	(22) 7.5	16 13 11	(13) 2.5
+	500	91 82 78	(84) 6.7	15 11 10	(12) 2.6	51 45 56	(51) 5.5	20 15 16	(17) 2.6	13 11 10	(11) 1.5
+	1500	89 P 73 P 77 P	(80) 8.3	18 P 15 P 24 P	(19) 4.6	57 P 38 P 43 P	(46) 9.8	24 P 19 P 23 P	(22) 2.6	10 P 16 P 12 P	(13) 3.1
+	5000	74 P 75 P 80 P	(76) 3.2	13 P 17 P 14 P	(15) 2.1	42 P 42 P 46 P	(43) 2.3	13 P 22 P 25 P	(20) 6.2	15 P 9 P 12 P	(12) 3.0
Positive controls	Name	2AA		2AA		2AA		BP		2AA	
	Concentration (µg/plate)	1		2		10		5		2	
S9-Mix +	No. colonies per plate	597 608 637	(614) 20.7	208 222 238	(223) 15.0	405 379 414	(399) 18.2	180 166 165	(170) 8.4	443 477 526	(482) 41.7

2AA 2-Aminoanthracene
BP Benzo(a)pyrene
P Precipitate
Standard deviation

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S-Face VL-211: REVERSE MUTATION ASSAY "AMES TEST"
USING *SALMONELLA TYPHIMURIUM* AND *ESCHERICHIA COLI*

Table 4 Test Results: Experiment 2 -- Without Metabolic Activation

Test Period		From: 11 September 2006						To: 14 September 2006			
With or without S9-Mix	Test substance concentration (µg/plate)	Number of revertants (mean number of colonies per plate)									
		Base-pair substitution type						Frameshift type			
		TA100		TA1535		WP2uvrA-		TA98		TA1537	
-	0	82 106 106	(98) 13.9#	21 10 26	(19) 8.2	33 37 22	(31) 7.8	19 29 26	(25) 5.1	13 14 19	(15) 3.2
-	50	85 74 106	(88) 16.3	13 21 18	(17) 4.0	30 33 32	(32) 1.5	23 16 32	(24) 8.0	11 15 21	(16) 5.0
-	150	104 89 102	(98) 8.1	19 12 14	(15) 3.6	27 35 30	(31) 4.0	21 15 18	(18) 3.0	15 5 13	(11) 5.3
-	500	98 118 98	(105) 11.5	15 31 30	(25) 9.0	36 23 24	(28) 7.2	31 33 24	(29) 4.7	14 15 5	(11) 5.5
-	1500	90 P 88 P 85 P	(88) 2.5	33 P 34 P 25 P	(31) 4.9	29 P 29 P 29 P	(29) 0.0	14 P 16 P 16 P	(15) 1.2	4 P 10 P 12 P	(9) 4.2
-	5000	72 P 98 P 99 P	(90) 15.3	25 P 20 P 17 P	(21) 4.0	25 P 24 P 27 P	(25) 1.5	20 P 22 P 19 P	(20) 1.5	12 P 13 P 10 P	(12) 1.5
Positive controls	Name	ENNG		ENNG		ENNG		4NQO		9AA	
	Concentration (µg/plate)	3		5		2		0.2		80	
S9-Mix -	No. colonies per plate	376 385 447	(403) 38.7	174 142 135	(150) 20.8	517 613 635	(588) 62.7	147 179 149	(158) 17.9	1209 962 1012	(1061) 130.6

ENNG N-ethyl-N'-nitro-N-nitrosoguanidine
4NQO 4-Nitroquinoline-1-oxide
9AA 9-Aminoacridine
P Precipitate
Standard deviation

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S-Face VL-211: REVERSE MUTATION ASSAY "AMES TEST"
USING *SALMONELLA TYPHIMURIUM* AND *ESCHERICHIA COLI*

Table 5 Test Results: Experiment 2 – With Metabolic Activation

Test Period		From: 11 September 2006						To: 14 September 2006			
With or without S9-Mix	Test substance concentration (µg/plate)	Number of revertants (mean number of colonies per plate)									
		Base-pair substitution type						Frameshift type			
		TA100		TA1535		WP2uvrA-		TA98		TA1537	
+	0	84 89 78	(84) 5.5#	26 22 10	(19) 8.3	43 40 46	(43) 3.0	22 33 32	(29) 6.1	25 14 21	(20) 5.6
+	50	90 97 85	(91) 6.0	10 8 15	(11) 3.6	31 41 41	(38) 5.8	24 29 25	(26) 2.6	9 22 16	(16) 6.5
+	150	82 85 76	(81) 4.6	20 12 19	(17) 4.4	26 42 32	(33) 8.1	23 22 25	(23) 1.5	23 15 20	(19) 4.0
+	500	100 100 76	(92) 13.9	9 8 9	(9) 0.6	26 33 34	(31) 4.4	25 27 31	(28) 3.1	15 21 23	(20) 4.2
+	1500	85 P 112 P 70 P	(89) 21.3	4 P 8 P 13 P	(8) 4.5	45 P 41 P 33 P	(40) 6.1	29 P 31 P 26 P	(29) 2.5	13 P 19 P 10 P	(14) 4.6
+	5000	75 P 92 P 83 P	(83) 8.5	12 P 11 P 16 P	(13) 2.6	30 P 27 P 20 P	(26) 5.1	25 P 20 P 22 P	(22) 2.5	5 P 17 P 11 P	(11) 6.0
Positive controls	Name	2AA		2AA		2AA		BP		2AA	
	Concentration (µg/plate)	1		2		10		5		2	
S9-Mix +	No. colonies per plate	2174 1883 2037	(2031) 145.6	186 168 167	(174) 10.7	374 418 317	(370) 50.6	154 183 194	(177) 20.7	571 530 499	(533) 36.1

2AA 2-Aminoanthracene
BP Benzo(a)pyrene
P Precipitate
Standard deviation

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S-Face VL-211: REVERSE MUTATION ASSAY "AMES TEST"
USING *SALMONELLA TYPHIMURIUM* AND *ESCHERICHIA COLI*

Appendix 1 History Profile of Vehicle and Positive Control Values

COMBINED VEHICLE AND UNTREATED CONTROL VALUES 2004

Strain	TA100		TA1535		WP2uvrA-		TA102		TA98		TA1537		WP2uvrA-pKM101	
S9-Mix	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Mean	94	98	24	14	23	27	333	367	21	31	12	18	147	183
SD	16.5	14.8	7.0	4.8	5.3	6.7	34.9	24.5	5.1	6.5	3.4	4.0	28.5	42.9
Min	61	60	9	7	9	11	192	263	10	11	5	6	92	131
Max	163	157	40	39	43	52	397	406	49	57	26	35	190	247
Values	993	798	911	713	735	579	291	183	917	740	889	708	15	12

POSITIVE CONTROL VALUES 2004

Strain	TA100		TA1535		WP2uvrA-		TA102		TA98		TA1537		WP2uvrA-pKM101	
S9-Mix	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Mean	557	1124	440	265	923	482	1250	893	175	219	999	366	2386	1954
SD	163.5	417.4	275.3	80.5	240.1	202.5	239.3	124.8	52.5	68.9	511.6	130.1	867.9	188.6
Min	189	348	93	162	371	153	728	660	95	114	130	137	1120	1737
Max	1215	2942	1634	1026	1714	1087	1960	1288	435	495	2964	832	3009	2075
Values	180	178	176	175	160	159	103	103	179	178	176	175	4	3

COMBINED VEHICLE AND UNTREATED CONTROL VALUES 2005

Strain	TA100		TA1535		WP2uvrA-		TA102		TA98		TA1537	
S9-Mix	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Mean	106	102	24	14	24	29	311	357	22	30	13	17
SD	19.5	19.5	6.6	4.2	5.3	5.6	43.9	27.7	5.1	6.2	3.5	4.9
Min	64	66	8	7	11	11	215	277	11	16	4	5
Max	196	177	40	38	49	57	391	393	49	51	25	29
Values	838	704	787	647	662	553	199	142	793	677	761	640

POSITIVE CONTROL VALUES 2005

Strain	TA100		TA1535		WP2uvrA-		TA102		TA98		TA1537	
S9-Mix	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Mean	576	1230	431	272	904	549	1483	957	210	289	854	413
SD	217.5	485.8	302.1	69.9	312.0	210.2	324.8	217.3	85.6	112.5	461.9	183.9
Min	261	334	77	126	251	188	646	646	91	109	252	123
Max	1649	2735	1966	577	2075	1339	2200	1787	579	679	2781	1061
Values	127	126	127	126	115	115	66	66	130	129	125	125

SDMinMax

SD = Standard deviation
Min = Minimum value
Max = Maximum value

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Appendix 2 Statement of GLP Compliance in Accordance with Directive 2004/9/EC

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.

Bryan J Wright 21/11/05.

Mr. Bryan J. Wright
Head, UK GLP Monitoring Authority

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STUDY REPORT

Patch test on the cosmetic ingredient

Japan Hair Science Association
(Incorporated Association)

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To SAKAMOTO YAKUHIN KOGYO CO.,LTD.
I hereby certify that this document is a duplicate copy of the original issued as Nichimokyo №18018-(1) on 5th August, 2006.

1th July, 2010

Prof. Dr. Mitsuyoshi Honda

Prof. Dr. med. Mitsuyoshi Honda
Chairman of Directors
Japan Hair Science Association
(Incorporated Association)

Contract Study Report

This is the report sponsored study on the patch test of the cosmetic ingredient as you requested on 2th June, 2006. See Attachment for study results.

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Nichimokyo №18018-(1)
5th August, 2006

To SAKAMOTO YAKUHIN KOGYO CO.,LTD.

This report presents the results of patch test on a cosmetic ingredient performed by Japan Hair Science Association. It was sponsored by SAKAMOTO YAKUHIN KOGYO CO.,LTD. on 2th June, 2006. This report is an English version of the original, which was written in Japanese. The undersigned hereby declare that this version faithfully reflects the original report to the best of my knowledge.

TEST FOR IRRITATION EFFECTS OF COSMETIC INGREDIENT ON HUMAN SKIN

1.Purpose of the test

This patch test was performed to assess possible irritation potential of the cosmetic ingredient on healthy human skin, sponsored by SAKAMOTO YAKUHIN KOGYO CO.,LTD. on 2th June, 2006.

2.Test sample

The supplied sample is the following cosmetic ingredient.

Sample : S-Face VL-211 Lot.№60210 Macadamia Seed Oil Polyglyceryl-Esters Behenate

Control①:Petrolatum(SUNWHITE P-1)

Control②:Soap(0.5%)

3.Test procedure

(1)Volunteers

16 males and 29 females (45 people in total), age 20 to 65, were selected as volunteers.

(2)Applied amount

0.01g of test sample was applied to each volunteer.

(3)Method of patch test

0.01g of test sample was placed on FINN CHAMBER(EPITEST Ltd. Oy)and applied to the crooked side of upper arm of each volunteer for 24 hours in occlusive condition to for evaluation.

At the end of 24 hours exposure period, the test sample was removed. Skin reactions were evaluated 1 hour and 24 hours after removing the test sample.

(4)Criteria for evaluation

The reaction was evaluated using the following criteria.

− :	No reaction
± :	Very slight erythema
+ :	Well-defined erythema
++ :	Erythema with swelling or with papule
+++ :	Blisters in addition to the above symptom
++++ :	Large blister

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Takashi Nagashima, M.D., a Vice-chairmam of Directors of Japan Hair Science Association(Dermatologist) was in overall charge of the tests and observations reported herein.

4.Results

The results of this test on 45 volunteers were as shown in the Table. In all volunteers, no reaction was observed in 1 hour after removing the test sample (shown in Table as 24-hours Evaluation time). Similarly, no reaction was observed in 24 hours after removing the test sample (shown in Table as 48-hours Evaluation time) in all volunteers.

5.Conclusion

The result of patch test carried out based on the request from your company is as above-mentioned.

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Table Results of patch test(1)

No.	Volunteer	Sex	Age	Evaluationtime(hrs.)	Sample	Control①	Control②	Note(anamnesis)
1	S. A.	F	48	24	—	—	—	Healthy
				48	—	—	—	
2	A. I.	F	46	24	—	—	—	Healthy
				48	—	—	—	
3	S. I.	F	20	24	—	—	—	Healthy
				48	—	—	—	
4	M. O.	F	21	24	—	—	—	Healthy
				48	—	—	—	
5	M. O.	F	20	24	—	—	—	Healthy
				48	—	—	—	
6	R. O.	F	33	24	—	—	—	Healthy
				48	—	—	—	
7	Y. K.	F	30	24	—	—	—	Healthy
				48	—	—	—	
8	S. K.	F	25	24	—	—	—	Healthy
				48	—	—	—	
9	A. K.	F	26	24	—	—	—	Healthy
				48	—	—	—	
10	K. K.	M	55	24	—	—	—	Healthy
				48	—	—	—	
11	J. K.	F	35	24	—	—	—	Healthy
				48	—	—	—	
12	A. S.	F	48	24	—	—	—	Healthy
				48	—	—	—	
13	I. S.	F	20	24	—	—	—	Healthy
				48	—	—	—	
14	M. S.	F	32	24	—	—	—	Healthy
				48	—	—	—	
15	I. S.	M	43	24	—	—	—	Healthy
				48	—	—	—	
16	Y. S.	M	20	24	—	—	—	Healthy
				48	—	—	—	
17	Y. S.	M	35	24	—	—	—	Healthy
				48	—	—	—	
18	T. S.	F	60	24	—	—	—	Healthy
				48	—	—	—	
19	M. T.	F	35	24	—	—	—	Healthy
				48	±	—	—	
20	K. T.	F	24	24	—	—	—	Healthy
				48	—	—	—	
21	S. T.	F	57	24	—	—	—	Healthy
				48	—	—	—	
22	J. T.	M	54	24	—	—	—	Healthy
				48	—	—	—	
23	K. T.	M	54	24	—	—	—	Healthy
				48	—	—	—	
24	T. T.	F	21	24	—	—	—	Healthy
				48	—	—	—	
25	K. T.	F	46	24	—	—	—	Healthy
				48	—	—	—	

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Table Results of patch test(2)

No.	Volunteer	Sex	Age	Evaluationtime(hrs.)	Sample	Control①	Control②	Note(anamnesis)
26	J. T.	F	43	24	−	−	−	Healthy
				48	−	−	−	
27	H. N.	M	23	24	−	−	−	Healthy
				48	−	−	−	
28	Y. N.	M	55	24	−	−	−	Healthy
				48	−	−	−	
29	E. N.	F	27	24	−	−	−	Healthy
				48	−	−	−	
30	T. H.	F	34	24	−	−	−	Healthy
				48	−	−	−	
31	N. B.	F	49	24	−	−	−	Healthy
				48	−	−	−	
32	M. H.	F	31	24	−	−	−	Healthy
				48	−	−	−	
33	R. F.	M	20	24	−	−	−	Healthy
				48	−	−	−	
34	S. F.	F	36	24	−	−	−	Healthy
				48	−	−	−	
35	K. H.	M	65	24	−	−	−	Healthy
				48	−	−	−	
36	S. M.	M	57	24	−	−	−	Healthy
				48	−	−	−	
37	N. M.	F	25	24	−	−	−	Healthy
				48	−	−	−	
38	T. M.	M	35	24	−	−	−	Healthy
				48	−	−	−	
39	M. Y.	F	38	24	−	−	−	Healthy
				48	−	−	−	
40	K. Y.	M	28	24	−	−	−	Healthy
				48	−	−	−	
41	K. Y.	F	30	24	−	−	−	Healthy
				48	−	−	−	
42	T. Y.	M	40	24	−	−	−	Healthy
				48	−	−	−	
43	S. Y.	M	60	24	+	−	−	Healthy
				48	−	−	−	
44	S. Y.	M	51	24	−	−	−	Healthy
				48	−	−	−	
45	A. Y.	F	43	24	−	−	−	Healthy
				48	−	−	−	

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SafePharm Laboratories

S-Face VL-211:
Macadamid Seed Oil Polyglyceryl-6 Esters Behenate
ACUTE DERMAL IRRITATION
IN THE RABBIT

SPL PROJECT NUMBER: 0432/0081

AUTHOR: A Pooles

STUDY SPONSOR:

Sakamoto Yakuhin Kogyo Co., Ltd.
1-20, Rinkaicho
Izumiotsu-city
OSAKA 595-01075
JAPAN

TEST FACILITY:

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

Telephone: +44 (0) 1332 792896

Facsimile: +44 (0) 1332 799018

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QUALITY ASSURANCE REPORT

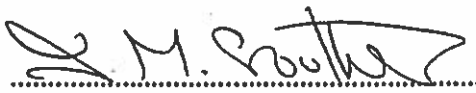
This study type is classed as short-term. The standard test method for this study type ("General Study Plan" in OECD terminology) was reviewed for compliance once only on initial production. Inspection of the routine and repetitive procedures that constitute the study is carried out as a continuous process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

	15 June 2004	Standard Test Method Compliance Audit
	03 October 2006	Test Material Preparation
	03 October 2006	Animal Preparation
	03 October 2006	Dosing
	25 October 2006	Assessment of Response
§	22 November 2006	Draft Report Audit
§	Date of QA Signature	Final Report Audit

§ Evaluation specific to this study



DATE: 11 DEC 2006

For Safepharm Quality Assurance Unit*

*Authorised QA Signatures:
Head of Department: JR Pateman CBiol MIBiol DipRQA AIQA FRQA
Deputy Head of Department: JM Crowther MIScT MRQA
Senior Audit Staff: JV Johnson BSc MRQA; G Wren ONC MRQA

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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.

A. Pools DATE: 8/12/06

A Pooles BA (Hons)
Study Director

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S-Face VL-211:

ACUTE DERMAL IRRITATION IN THE RABBIT

SUMMARY

Introduction. The study was performed to assess the irritancy potential of the test material to the skin 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. 404 "Acute Dermal Irritation/Corrosion" (adopted 24 April 2002)
- Method B4 Acute Toxicity (Skin Irritation) of Commission Directive 2004/73/EC

Results. A single 4-hour, semi-occluded application of the test material to the intact skin of three rabbits produced very slight erythema. One treated skin site appeared normal throughout the study and the remaining two treated skin sites appeared normal at the 24-hour observation.

3-minute and 1-hour semi-occluded applications of the test material to the intact skin of one rabbit produced no evidence of skin irritation.

Conclusion. The test material produced a primary irritation index of 0.0 and was classified as non-irritant to rabbit skin according to the Draize classification scheme.

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S-Face VL-211:

ACUTE DERMAL IRRITATION IN THE RABBIT

1. INTRODUCTION

The study was performed to assess the irritancy potential of the test material following single, 3-minute, 1 and 4-hour, semi-occluded applications to the intact rabbit skin. The method was designed to meet the requirements of the following:

- OECD Guidelines for the Testing of Chemicals No. 404 "Acute Dermal Irritation/Corrosion" (adopted 24 April 2002)
- Method B4 Acute Toxicity (Skin 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 skin irritancy potential of the test material to man.

The study was performed between 19 September 2006 and 10 November 2006.

2. TEST MATERIAL

2.1 Description, Identification and Storage Conditions

Sponsor's identification	:	S-Face VL-211
Description	:	white waxy solid
Batch number	:	60210
Date received	:	05 June 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.

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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 .
10% w/w aqueous preparation of the test material	approximately 7.1

3. METHODS

3.1 Animals and Animal Husbandry

Three New Zealand White rabbits were supplied by Charles River (France), Charlaronne, France and Harlan UK Limited, Bicester, Oxon, UK. This deviation from the Standard Test Method was considered not to affect the purpose or integrity of the study. 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 (Code 5322) supplied by BCM IPS Limited, London, UK) 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

On the day before the test each rabbit was clipped free of fur from the dorsal/flank area using veterinary clippers. Only animals with a healthy intact epidermis by gross observation were selected for the study.

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One rabbit was initially treated. Three suitable sites were selected on the back of the rabbit. At each test site a quantity of 0.5ml of the test material was introduced under a 2.5 cm x 2.5 cm cotton gauze patch and placed in position on the shorn skin. Each patch was secured in position with a strip of surgical adhesive tape. To prevent the animal interfering with the patches, the trunk of the rabbit was wrapped in an elasticated corset and the animal was returned to its cage for the duration of the 1 and 4 hour exposure periods.

One patch was removed at each of three time points: 3 minutes, 1 hour and 4 hours after application. Any residual test material was removed by gentle swabbing with cotton wool soaked in distilled water.

After consideration of the skin reactions produced in the first animal, two additional animals were treated with 0.5 ml. One patch was applied to the back of each rabbit, and was allowed to remain in contact with the skin for a period of four hours.

Approximately one hour following the removal of the patches, and 24, 48 and 72 hours later, the test sites were examined for evidence of primary irritation and scored according to the following scale:

EVALUATION OF SKIN REACTIONS

Erythema and Eschar Formation	**Value**
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beef redness) to eschar formation 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

Any other skin reactions, if present, were also recorded.

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3.3 Interpretation of Results

Calculation of Primary Irritation Index and Grading of Irritancy Potential Using the Draize Scheme

The scores for erythema and oedema at the 24 and 72-hour readings were totalled for the three test rabbits (12 values) and this total was divided by six to give the primary irritation index of the test material. The test material was classified according to the following scheme devised by Draize J H (1959) "Dermal Toxicity" In: Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics. Association of Food and Drug Officials of the United States, Austin, Texas, p.47:

Primary Irritation Index	Classification of Irritancy
0	Non-irritant
> 0 to 2	Mild irritant
> 2 to 5	Moderate irritant
> 5 to 8	Severe irritant

If irreversible alteration of the dermal tissue is noted in any rabbit, as judged by the Study Director, which include ulceration and clear necrosis or signs of scar tissue, the test material is classified as corrosive to rabbit skin. Classification according to Draize may, therefore, not be applicable.

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.

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5. RESULTS

5.1 4-Hour Exposure Period

The individual scores for erythema/eschar and oedema are given in Table 1.

Very slight erythema was noted at two treated skin sites one hour after patch removal.

One treated skin site appeared normal throughout the study and the remaining two treated skin sites appeared normal at the 24-hour observation.

5.2 1-Hour Exposure Period

The individual scores for erythema/eschar and oedema are given in Appendix 1.

No evidence of skin irritation was noted during the study.

5.3 3-Minute Exposure Period

The individual scores for erythema/eschar and oedema are given in Appendix 1.

No evidence of skin irritation was noted during the study.

6. CONCLUSION

The test material produced a primary irritation index of 0.0 and was classified as NON-IRRITANT to rabbit skin according to the Draize classification scheme. No corrosive effects were noted.

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S-Face VL-211 : ACUTE DERMAL IRRITATION IN THE RABBIT

Table 1 Individual Skin Reactions Following 4-Hour Exposure

Skin Reaction	Observation Time	Individual Scores – Rabbit Number and Sex			Total
		64940 Male	65084 Male	65085 Male	
Erythema/Eschar Formation	1 Hour	0	1	1	(2)
	24 Hours	0	0	0	0
	48 Hours	0	0	0	(0)
	72 Hours	0	0	0	0
Oedema Formation	1 Hour	0	0	0	(0)
	24 Hours	0	0	0	0
	48 Hours	0	0	0	(0)
	72 Hours	0	0	0	0
Sum of 24 and 72-hour Readings (S)	:	0			
Primary Irritation Index (S/6)	:	0/6 = 0.0			
Classification	:	NON-IRRITANT			

( ) = Total values not used for calculation of primary irritation index

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S-Face VL-211 : ACUTE DERMAL IRRITATION IN THE RABBIT

Appendix 1 Individual Skin Reactions Following 1-Hour and 3-Minute Exposures

Skin Reaction	Observation Time	Individual Scores - Rabbit Number and Sex	
		64940 Male	
		1-Hour Exposure	3-Minute Exposure
Erythema/Eschar Formation	1 Hour	0	0
	24 Hours	0	0
	48 Hours	0	0
	72 Hours	0	0
Oedema Formation	1 Hour	0	0
	24 Hours	0	0
	48 Hours	0	0
	72 Hours	0	0

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

Bryan J Wright 21/11/05.

Mr. Bryan J. Wright
Head, UK GLP Monitoring Authority

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Phycher
Bio développement

S-FACE VL-211
Macadamia Seed Oil Polyglyceryl-6 Esters Behenate
SKIN SENSITISATION IN THE GUINEA PIG - MAGNUSSON AND KLIGMAN MAXIMISATION METHOD

SPL PROJECT NUMBER: 0432/0083
Study n°: SMK-PH-06/0231

AUTHOR: F Richeux

STUDY SPONSOR:

Sakamoto Yakuhin Kogyo Co., Ltd.
1-20, Rinkai-cho
Izumiotsu-city
OSAKA 595-0075
JAPAN

STUDY MONITOR:

A. Pooles
Safepharm Laboratories Limited
Shardlow Business Park
Shardlow
Derbyshire
DE72 2GD
UK

TEST FACILITY:

Phycher Bio Développement
ZI de Toctoucau
18 Chemin de Lou Tribail
33611 CESTAS Cedex
FRANCE

Telephone: (33) 5 57 97 16 80

Facsimile: (33) 5 57 97 16 81

Siège social : 18, chemin de Lou Tribail ZI de Toctoucau 33611 CESTAS Cedex - ✆ 05 57 97 16 80 - Fax 05 57 97 16 81
info@phycher.com - www.phycher.com
S.A.R.L. AU CAPITAL DE 60 979,61 Euros - APE 7312 - SIRET 402 924 088 000 30

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QUALITY ASSURANCE REPORT

The routine inspections of the toxicity tests performed at Phycher Bio Développement are carried out as a continuous process designed to encompass the major phases of at least one similar test at or about the time this study was in progress.

This report has been audited by Phycher Bio Développement 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 within twenty-four hours of evaluation. Audits of study documentation, and process inspections appropriate to the type and schedule of this study were as follows:

§	12 June 2006	Protocol Compliance Audit
	01 June 2006	Main study: skin reactions at the reading 48-hours after topical challenge
§	23 August 2006	Draft Report Audit
§	30 August 2006	Final Report Audit

§ Evaluation specific to this study

..... DATE: 30 August 2006

Bernard BENECH
Quality Assurance Manager

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GLP COMPLIANCE STATEMENT

I, the undersigned, François Richeux, Study director, certify that the study SMK-PH-06/0231 was performed on the premises of the Laboratory PHYCHER Bio Développement.

I, certify that the objectives laid down in the protocol were achieved and no undesirable event occurred to affect the quality or the integrity of the study. I consider the data generated to be valid. This report fully and accurately reflects the operating procedures used and data generated.

The entire work was devised and performed in compliance with the principles of the Good Laboratory Practice (G.L.P.), as defined in *the directive 2004/9/EC, the O.E.C.D. ruling relative to the mutual acceptance of data in the evaluation of chemical substances (C(81) 30 (final) Appendix 2 – May 12th , 1981; C (97) 186, November 26th, 1987),* and in the French decree n° 98-1312 dated December 31st, 1998 of the Journal Officiel de la République Française (see certificate of G.L.P. in appendix 7).

..... DATE: 30 August 2006

François Richeux
Study Director
Expert Toxicologist (PhD, ERT)

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S-FACE VL-211 :

SKIN SENSITISATION IN THE GUINEA PIG - MAGNUSSON AND KLIGMAN MAXIMISATION METHOD

SUMMARY

Introduction. The study was performed to assess the contact sensitisation potential of the test material in the albino guinea pig. The method was designed to meet the requirements of the following:

- OECD Guidelines for the Testing of Chemicals No. 406 "Skin Sensitisation" (adopted 17 July 1992)
- Method B6 Acute Toxicity (Skin Sensitisation) of Commission Directive 96/54/EC
- Japanese Ministry of Agriculture, Forestry and Fisheries (JMAFF), Testing Guidelines for Toxicology Studies, 12 NohSan No. 8147, Guideline No. 2-1-6, revised on 24 November 2004 and 13 SeiSan No. 3986 of October 10, 2001

Method. Ten test and five control animals were used for the main study. Two phases were involved in the main test; an induction of a response by intradermal injection and topical application and a topical challenge of that response.

Based on the results of sighting tests, the concentrations of test material for the topical induction and topical challenge phases were selected as:

Intradermal Induction	:	25% in olive oil
Topical Induction	:	100%
Topical Challenge	:	100% and 50% in liquid paraffin

Conclusion. Under the conditions of the test, the test material produced a 0% (0/10) sensitisation rate and was classified as a non-sensitiser to guinea pig skin. The test material did not meet the criteria for classification as a sensitiser according to EU labelling regulations Commission Directive 2001/59/EC. No symbol and risk phrase are required.

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S-FACE VL-211 :

SKIN SENSITISATION IN THE GUINEA PIG - MAGNUSSON AND KLIGMAN MAXIMISATION METHOD

1. INTRODUCTION

The study was performed to assess the contact sensitisation potential of the test material in the albino guinea pig. The method was designed to meet the requirements of the following:

- OECD Guidelines for the Testing of Chemicals No. 406 "Skin Sensitisation" (adopted 17 July 1992)
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- Japanese Ministry of Agriculture, Forestry and Fisheries (JMAFF), Testing Guidelines for Toxicology Studies, 12 NohSan No. 8147, Guideline No. 2-1-6, revised on 24 November 2004 and 13 SeiSan No. 3986 of October 10, 2001

The albino guinea pig has been shown to be a suitable species for this type of study and is recommended in the test method. The strain used in these laboratories has been shown to produce satisfactory sensitisation responses using known positive sensitisers (see Appendix 6). The results of the study are believed to be of value in predicting the likely contact sensitisation potential of the test material to man.

The study was performed between 27 June 2006 and 03 August 2006.

2. TEST MATERIAL

2.1 Description, Identification and Storage Conditions

Sponsor's identification	:	S-FACE VL-211
Code number	:	PH-06/0231
Description	:	White solid
CAS number	:	-
Batch number	:	60210
Date received	:	16 June 2006
Storage conditions	:	room temperature

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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 this study the test material was freshly prepared in olive oil and paraffin oil. The concentrations used are stated in the procedure section.

To collect and liquefy the test material, it has been heated at 50°C.

The absorption of the test material was not determined.

Determination by analysis of the concentration, homogeneity and stability of the test material preparations was not appropriate because it was not specified in the Study Plan and is not a requirement of the Test Guideline.

3. METHODS

3.1 Animals and Animal Husbandry

Female albino Dunkin Hartley guinea pigs were supplied by Charles River (F-69592 L'Arbresle). Females were nulliparous and non-pregnant. After an acclimatisation period of at least five days, each animal was selected at random and identified individually by marking with picric acid and an ear tattoo. At the start of the main study the animals were in the weight range of 328 to 367 g.

The animals were housed in groups of two or three in makrolon cages furnished with woodflakes. Free access to mains tap water and food (Safe 91360 Villemoisson s/Orge, France) was allowed throughout the study. The diet, drinking water and bedding were routinely analysed and were considered not to contain any contaminant that could 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 58 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 ten changes per hour and the lighting was controlled by a time switch to give twelve hours continuous light (07:00 to 19:00) and twelve hours darkness.

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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

The method used for assessing the sensitising properties of the test material was based on the Guinea Pig Maximisation Test of Magnusson B & Kligman A M, (1969) J. Invest. Dermatol. 52: 268 - 276.

3.2.1 Selection of Concentrations for Main Study (Sighting Tests)

The concentrations of test material to be used at each stage of the main study were determined by 'sighting tests' in which groups of guinea pigs were treated with various concentrations of test material. The procedures were as follows:

3.2.1.1 Selection of Concentration for Intradermal Induction

Intradermal injections (0.1 ml/injection site) were made on the clipped shoulder of two guinea pigs, at the concentrations of 100%, 50%, 25%, 12.5%, 6.25% and 3.125% by successive dilution (v/v) in olive oil. A macroscopic evaluation of the injection sites was conducted approximately 24 hours after injection to determine whether these concentrations caused necrosis. Any evidence of systemic toxicity was also recorded. The highest concentration which did not cause necrosis was selected for the intradermal induction stage of the main study. The results are given in Appendix 1.

3.2.1.2 Selection of Concentration for Topical Induction

Two guinea pigs were treated with preparations of the test material at 100% and diluted at 50% 25% and 12.5% by successive dilution (v/v) in liquid paraffin. Applications were made to the clipped flanks under occlusive dressings for an exposure period of 24 hours. The degree of erythema and oedema was evaluated approximately 24 hours after dressing removal. The highest concentration producing only moderate dermal irritation was selected for the topical induction stage of the main study. The results are given in Appendix 2.

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3.2.1.3 Selection of Concentration for Topical Challenge

Four preparations of the test material at 100% and diluted at 50%, 25% and 12.5% by successive dilution (v/v) in liquid paraffin were applied to the clipped flanks of three guinea pigs under occlusive dressings for an exposure period of 24 hours. These guinea pigs did not form part of the main study but had been treated identically to the control animals of the main study, up to Day 14. The degree of erythema and oedema was evaluated approximately 24 hours after dressing removal. The highest non-irritant concentration of the test material and one lower concentration were selected for the topical challenge stage of the main study. The results are given in Appendix 3.

3.2.2 Main Study

A group of fifteen guinea pigs was used for the main study, ten test and five control. The bodyweight of each animal was recorded at the start and the end of the study and are presented in Appendix 4.

Two phases were involved in the main study; (a) an induction of a response and (b) a challenge of that response.

3.2.2.1 Induction

Induction of the Test Animals: Shortly before treatment on Day 0 the hair was removed from an area approximately 40 mm x 60 mm on the shoulder region of each animal with veterinary clippers. A row of three injections (0.1 ml each) was made on each side of the spine. The injections were:

a) Freund's Complete Adjuvant plus isotonic sodium chloride in the ratio 1:1

b) a 25% formulation of the test material in olive oil

c) a 25% formulation of the test material in a 1:1 preparation of Freund's Complete Adjuvant plus isotonic sodium chloride

On Day 6, the scapular region of all test and control animals was shaved.

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On Day 7 the same area on the shoulder region used previously for intradermal injections was clipped again and treated with a topical application of the test material formulation. A topical application of the test material at 100% under occlusive dressing was made. The occlusive dressing was kept in place for 48 hours.

Induction of the Control Animals: On Day 0, the intradermal induction was performed using an identical procedure to that used for the test animals except that the test material was omitted from the intradermal injections. Injection b) was therefore the vehicle alone, injection c) was a 50% formulation of the vehicle in a 1:1 preparation of Freund's Complete Adjuvant plus isotonic sodium chloride. Similarly, on Day 7, the topical induction procedure was identical to that used for the test animals except that the test material was omitted.

3.2.2.2 Challenge

The flanks of each animal were previously clipped free of hair with veterinary clippers.

The test material formulation at the maximum non-irritant concentration (100%) was applied to one side of the shorn flank of each animal under an occlusive dressing. To ensure that the maximum non-irritant concentration was used at challenge, the test material at a concentration of 50% (v/v) in liquid paraffin was similarly applied under an occlusive dressing to the opposite skin site on the shorn flank.

After 24 hours, the dressing was carefully removed and discarded. The topical challenge sites were cleaned if required.

Prior to the 24-hour observation the flanks were clipped to remove regrown hair.

Approximately 24 and 48 hours after challenge dressing removal, the degree of erythema and oedema was quantified using the scale shown in Appendix 5.

Any other reactions were also recorded.

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3.3 Interpretation of Results

The percentage of test animals that showed a more severe reaction at the test material challenge site than the most severe reaction seen in the control animals, was compared with the following scale:

PERCENTAGE OF ANIMALS SENSITISED	CLASSIFICATION OF SENSITISATION POTENTIAL
0	Non sensitiser
> 0 - 8	Weak sensitiser
> 8 - 28	Mild sensitiser
>28 – 64	Moderate sensitiser
>64 - 80	Strong sensitiser
>80 - 100	Extreme sensitiser

The results were evaluated according to Commission Directive 2001/59/EC for classification and labelling of dangerous substances.

4. ARCHIVES

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

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5. RESULTS

5.1 Preliminary Sighting Tests

5.1.1 Skin Reactions Observed After Intradermal Induction

Individual skin reactions at the intradermal induction sites of the preliminary sighting test animals are presented in Appendix 1.

Necrosis was noted at the intradermal induction sites of both preliminary sighting test animals following treatment with the test material at concentrations of 100% and 50%. A moderate erythema was noted at the intradermal induction sites of both preliminary sighting test animals following treatment with the test material at concentration of 25%. No skin reactions were noted at the intradermal induction sites of the preliminary sighting test animals following treatment with the test material at the concentrations of 12.5%, 6.25% and 3.125%.

The concentration chosen for use in the Intradermal Induction phase of the main test was 25% in olive oil (maximal non necrotising concentration (MNNC)).

5.1.2 Skin Reactions Observed After Topical Induction

Individual skin reactions at the topical induction sites of the preliminary sighting test animals are presented in Appendix 2.

No skin reactions were noted at the topical induction sites of the preliminary sighting test animals following treatment with the test material at concentrations of 100%, 50%, 25% and 12.5%.

The concentration chosen for use in the 2nd topical Induction of the main test was 100% and the 1st concentration chosen for the MNIC (maximal non irritant concentration) determination was 100%.

5.1.3 Skin Reactions Observed After Topical Challenge

Individual skin reactions at the topical challenge sites of the preliminary sighting test animals are presented in Appendix 3.

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No skin reactions were noted at the topical challenge sites of the preliminary sighting test animals following treatment with the test material at concentrations of 100%, 50%, 25% and 12.5%.

The concentrations chosen for use in the Topical Challenge phase of the main test were 100% (maximal non irritant concentration (MNIC)) and 50% (1/2 MNIC) in liquid paraffin.

5.2 Skin Reactions Observed After Topical Challenge

Individual skin reactions at the topical challenge sites of the test and control group animals are given in Table 1 and overall results are presented in Table 2.

No mortality occurred during this study.

100%

No skin reactions were noted at the topical challenge site of the test or control group animals at the 24 and 48-hour observations.

50% in liquid paraffin

No skin reactions were noted at the topical challenge site of the test or control group animals at the 24 and 48-hour observations.

6. CONCLUSION

Under the conditions of the test, the test material produced a 0% (0/10) sensitisation rate and was classified as a non-sensitiser to guinea pig skin

The test material did not meet the criteria for classification as a sensitiser according to EU labelling regulations Commission Directive 2001/59/EC. No symbol and risk phrase are required.

Table 1 Individual Skin Reactions at Topical Challenge

CHALLENGE CONCENTRATIONS: **100% and 50%**

VEHICLE: **liquid paraffin**

Group	Animal Number	Skin Reactions (Hours after Removal of Dressings)											
		100%						50%					
		24 Hours			48 Hours			24 Hours			48 Hours		
		Er	Oe	Other	Er	Oe	Other	Er	Oe	Other	Er	Oe	Other
TEST	C5656	0	0	-	0	0	-	0	0	-	0	0	-
	C5657	0	0	-	0	0	-	0	0	-	0	0	-
	C5658	0	0	-	0	0	-	0	0	-	0	0	-
	C5659	0	0	-	0	0	-	0	0	-	0	0	-
	C5660	0	0	-	0	0	-	0	0	-	0	0	-
	C5661	0	0	-	0	0	-	0	0	-	0	0	-
	C5662	0	0	-	0	0	-	0	0	-	0	0	-
	C5663	0	0	-	0	0	-	0	0	-	0	0	-
	C5664	0	0	-	0	0	-	0	0	-	0	0	-
	C5665	0	0	-	0	0	-	0	0	-	0	0	-

Er = Erythema Oe = Oedema - = No other reactions noted

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Table 1 (continued) Individual Skin Reactions at Topical Challenge

CHALLENGE CONCENTRATIONS: **100% and 50%**

VEHICLE: **liquid paraffin**

Group	Animal Number	Skin Reactions (Hours after Removal of Dressings)											
		100%						50%					
		24 Hours			48 Hours			24 Hours			48 Hours		
		Er	Oe	Other	Er	Oe	Other	Er	Oe	Other	Er	Oe	Other
CONTROL	C5651	0	0	-	0	0	-	0	0	-	0	0	-
	C5652	0	0	-	0	0	-	0	0	-	0	0	-
	C5653	0	0	-	0	0	-	0	0	-	0	0	-
	C5654	0	0	-	0	0	-	0	0	-	0	0	-
	C5655	0	0	-	0	0	-	0	0	-	0	0	-

Er = Erythema Oe = Oedema - = No other reactions noted

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Table 2 Assessment of the Sensitising Potential following Topical Challenge

Group	Observation (Hours after Removal of Dressings)	Concentration	Number of Animals Showing Sensitisation Reactions				Positive Responses	Animals Sensitised
			0	1	2	3 or >		
Test	24	100%	10	0	0	0	0%	0%
	48	100%	10	0	0	0	0%	0%
	24	50%	10	0	0	0	0%	0%
	48	50%	10	0	0	0	0%	0%
Control	24	100%	5	0	0	0	0%	
	48	100%	5	0	0	0	0%	
	24	50%	5	0	0	0	0%	
	48	50%	5	0	0	0	0%	

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Appendix 1 Intradermal Sighting Test – Summary of Results

VEHICLE: **olive oil**

Animal Identification	Concentration of Test Material (%)	Time of Observation	Grade of Erythema at Injection Sites
C5556	100	24 Hours	Necrosis
	50	24 Hours	Necrosis
	25	48 Hours	2 (Er)
	12.5	24 Hours	0
	6.25	24 Hours	0
	3.125	24 Hours	0
C5557	100	24 Hours	Necrosis
	50	24 Hours	Necrosis
	25	48 Hours	2 (Er)
	12.5	24 Hours	0
	6.25	24 Hours	0
	3.125	24 Hours	0

The concentration of the test material selected for the intradermal induction test of the main test was 25% in olive oil

Er = Erythema

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Appendix 2 Topical Sighting Test for Topical Induction Application (24-Hour Exposure) – Individual Skin Reactions

VEHICLE: liquid paraffin

Animal Identification	Concentration of Test Material (%)	Skin Reactions (24 Hours After Removal of Patches)		
		Er	Oe	Other
C5556	100	0	0	-
	50	0	0	-
	25	0	0	-
	12.5	0	0	-
C5557	100	0	0	-
	50	0	0	-
	25	0	0	-
	12.5	0	0	-

The concentration of the test material selected for the main test topical induction was 100%

Er = Erythema Oe = Oedema - = No other reactions noted

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Appendix 3 Topical Sighting Test for Topical Challenge Application (24-Hour Exposure) – Individual Skin Reactions

VEHICLE: liquid paraffin

Animal Identification	Concentration of Test Material (%)	Skin Reactions (24 Hours After Removal of Patches)		
		Er	Oe	Other
C5560	100	0	0	-
	50	0	0	-
	25	0	0	-
	12.5	0	0	-
C5561	100	0	0	-
	50	0	0	-
	25	0	0	-
	12.5	0	0	-
C5562	100	0	0	-
	50	0	0	-
	25	0	0	-
	12.5	0	0	-

The concentrations of the test material selected for the main test topical challenge were 100% and 50% in liquid paraffin

Er = Erythema Oe = Oedema - = No other reactions noted

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Appendix 4 Individual Bodyweights and Bodyweight Gains

Group	Animal Number	Bodyweight (g)		Bodyweight (g) Increase
		Day 0	Day 30	
TEST	C5656	352	508	156
	C5657	367	520	153
	C5658	337	492	155
	C5659	332	471	139
	C5660	344	477	133
	C5661	333	539	206
	C5662	347	563	216
	C5663	358	540	182
	C5664	332	508	176
	C5665	337	476	139

Bodyweight increases of the guinea pigs in the test group between Day 0 and Day 30 were comparable to those noted in the control group over the same period.

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Appendix 4 (continued) Individual Bodyweights and Bodyweight Gains

Group	Animal Number	Bodyweight (g)		Bodyweight (g) Increase
		Day 0	Day 30	
CONTROL	C5651	340	544	204
	C5652	347	521	174
	C5653	350	517	167
	C5654	334	507	173
	C5655	328	494	166

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S-FACE VL-211: SKIN SENSITISATION IN THE GUINEA PIG - MAGNUSSON AND KLIGMAN MAXIMISATION METHOD

Appendix 5 Scales for Evaluation of Skin Reactions

EVALUATION OF ERYTHEMA #	VALUE
No visible modification	0
Slight or patches of erythema	1
Moderate confluent erythema	2
Intense erythema and swelling	3

EVALUATION OF OEDEMA †	VALUE
No visible modification	0
Slight oedema	1
Moderate oedema	2
Severe oedema	3

From: Modified OECD Test Guideline 406, 1992 and Method B6 Skin Sensitisation of Commission Directive 96/54/EC.

† 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, p31.

Appendix 6 Summary of Positive Control Data for the Magnusson and Kligman Maximisation Study

Test Number	Date Start	Number of Animals Test	Positive Control Material	Challenge Concentration	Observation (Hours after Removal of Dressings)	Incidence of Sensitisation
10	06/09/2005	10	α-Hexylcinnamaldehyde CAS [101-86-0]	50%	24	100%
					48	70%
				25%	24	100%
					48	60%
11	31/01/2006	10	α-Hexylcinnamaldehyde CAS [101-86-0]	50%	24	70%
					48	70%
				25%	24	50%
					48	30%
12	07/02/2006	10	α-Hexylcinnamaldehyde CAS [101-86-0]	50%	24	100%
					48	90%
				25%	24	90%
					48	90%

Conclusion: Based on these results, under the conditions of the test, the Positive control material, α-Hexylcinnamaldehyde, was classified sensitisers according to EU labelling regulations Commission Directive 2001/59/EC. The symbol "Xi", the indication of danger 'irritant' and risk phrase R 43 "May Cause Sensitisation by Skin Contact" are therefore required.

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Appendix 7 Statement of GLP Compliance in Accordance with Directive 88/320/EEC

FRENCH REPUBLIC
INTERMINISTERIAL GROUP OF CHEMICALS (GIPC)

Paris, 13 December 2005

(Translated from the original version in French)

Subject: Evaluation of conformity to GLP according to the directive 88/320/EEC.

In the application of the 81-278 decree dated 25th March 1981 modified by the 90-206 decree dated 7th March 1990 and the 98-1312 decree dated 31 December 1998 concerning the Good Laboratory Practice, I confirm that, following the inspection visit made by the Comité Français d'Accréditation (COFRAC – Section Essais) in accordance with its usual procedures and upon your request, the GIPC was able to establish that the assays perform in your test facility comply with GLP.

Compliance with the GLP principles.
Areas of expertise
- **2 Toxicity studies**
- **3 Mutagenicity studies**

Inspection date: 03 October 2005

- **Periodical inspection (i.p.)**
- **Extension inspection (i.e.)**

Date of GIPC decision: 09 December 2005
Date of validity: 03 October 2005
Year of 1st validity: 1998
Period of validity: 18 months

PHYCHER BIO Developpement
ZI de Toctoucau
18, chemin de Lou Tribail
33611 CESTAS Cedex

President of the Interministerial
Group of Chemicals
Mr Pierre CREYSSEL

General Secretary of GIPC – DiGITIP – Simap – 12, rue Viliot – 75572 Paris Cedex 12
Tel: 01 53 44 96 10 Telecopy: 01 53 44 91 72

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SafePharm Laboratories

S-Face VL-211:
Macadamia Seed Oil Polyglyceryl-6 Esters Behenate
ACUTE EYE IRRITATION IN THE RABBIT

SPL PROJECT NUMBER: 0432/0082

AUTHOR: A Pooles

STUDY SPONSOR:

Sakamoto Yakuhin Kogyo Co., Ltd.
1-20, Rinkaicho
Izumiotsu-city
OSAKA 595-01075
JAPAN

TEST FACILITY:

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

Telephone: +44 (0) 1332 792896

Facsimile: +44 (0) 1332 799018

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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
	03 October 2006	Test Material Preparation
	18, 21 November 2006	Animal Preparation
	21 November 2006	Dosing
	18, 20, 21 November 2006	Assessment of Response
§	05 December 2006	Draft Report Audit
§	Date of QA Signature	Final Report Audit

§ Evaluation specific to this study

..... DATE: 12 DEC 2006

For Safepharm Quality Assurance Unit*

*Authorised QA Signatures:

Head of Department:	JR Pateman CBiol MIBiol DipRQA AIQA FRQA
Deputy Head of Department:	JM Crowther MIScT MRQA
Senior Audit Staff:	JV Johnson BSc MRQA; G Wren ONC MRQA

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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.

A. Pooles DATE: 11/12/06

A Pooles BA (Hons)
Study Director

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S-Face VL-211:

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. All treated eyes appeared normal at the 24-hour observation.

Conclusion. The test material produced a maximum group mean score of 3.3 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.

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S-Face VL-211:

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 13 November 2006 and 23 November 2006.

2. TEST MATERIAL

2.1 Description, Identification and Storage Conditions

Sponsor's identification	:	S-Face VL-211
Description	:	white waxy solid
Batch number	:	60210
Date received	:	05 June 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.

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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
10% w/w aqueous preparation of the test material	approximately 7.1

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.

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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	=	$(A + B + C) \times 2$
Score for iris	=	$D \times 5$
Score for cornea	=	$(E \times F) \times 5$

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.

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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.

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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 all treated eyes one hour after treatment.

All treated eyes appeared normal at the 24-hour observation.

6. CONCLUSION

The test material produced a maximum group mean score of 3.3 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	65133 Male				65167 Male				65168 Male			
	IPR = 2				IPR = 1				IPR = 1			
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	1	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	1	0	0	0	0	0	0	0
Score (A + B + C) x 2	4	0	0	0	4	0	0	0	2	0	0	0
Total Score	4	0	0	0	4	0	0	0	2	0	0	0

IPR = Initial pain reaction

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S-Face VL-211 : 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
65133 Male	4	0	0	0
65167 Male	4	0	0	0
65168 Male	2	0	0	0
Group Total	10	0	0	0
Group Mean Score	3.3	0.0	0.0	0.0

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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.

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S-Face VL-211 : 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

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S-Face VL-211 : 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)

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

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

Bryan J Wright 21/11/05.

Mr. Bryan J. Wright
Head, UK GLP Monitoring Authority

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Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: Lillian Gill, D.P.A.
Director - COSMETIC INGREDIENT REVIEW (CIR)

FROM: Beth A. Lange, Ph.D.
Industry Liaison to the CIR Expert Panel

DATE: March 25, 2016

SUBJECT: Information on Polyglyceryl-8 Decabehenate/Caprate (SLR posted January 28, 2016)

SafePharm Laboratories. 2008. Reverse mutation assy "Ames test" using *Salmonella typhimurium* and *Escherichia coli* (Polyglyceryl-8 Decabehenate/Caprate).

SafePharm Laboratories. 2008. Acute dermal irritation in the rabbit Polyglyceryl-8 Decabehenate/Caprate).

Japan Hair Science Association. 2007. Test for irritation effects of cosmetic ingredient on human skin (Polyglyceryl-8 Decabehenate/Caprate).

SafePharm Laboratories. 2008. Acute eye irritation in the rabbit Polyglyceryl-8 Decabehenate/Caprate).

SafePharm Laboratories. 2008. Skin senistization in the guinea pig - Magnusson and Kligman maximisation method (Polyglyceryl-8 Decabehenate/Caprate).

SafePharm Laboratories. 2008. Acute oral toxicity in the rat - acute toxic class method (Polyglyceryl-8 Decabehenate/Caprate).

1620 L Street, N.W., Suite 1200 | Washington, D.C. 20036 | 202.331.1770 | 202.331.1969 (fax) | www.personalcarecouncil.org

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SafePharm Laboratories

S-Face BC-810:

Polyglyceryl-8 Decabehenate / Caprate

REVERSE MUTATION ASSAY "AMES TEST"

USING *SALMONELLA TYPHIMURIUM*

AND *ESCHERICHIA COLI*

SPL PROJECT NUMBER: 0432/0105

AUTHOR: P W Thompson

STUDY SPONSOR:

Sakamoto Yakuhin Kogyo Co., Ltd.
1-20, Rinkai-cho
Izumiotsu-shi
Osaka 595-0075
JAPAN

TEST FACILITY:

Safepharm Laboratories Limited
Shardlow Business Park
Shardlow
Derbyshire
DE72 2GD

Telephone: +44 (0) 1332 792896

Facsimile: +44 (0) 1332 799018

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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. In addition, inspection of general facilities not specifically related to this study are done monthly or annually in accordance with QA Standard Procedure.

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:

	14 May 2007	Standard Test Method Compliance Audit
	14 April 2008	Test Material Preparation
	15 April 2008	Test System Preparation
	14 April 2008	Exposure
	07 April 2008	Assessment of Response
§	14 May 2008	Draft Report Audit
§	Date of QA Signature	Final Report Audit

§ Evaluation specific to this study

..... DATE: 09 JUL 2008

For Safepharm Quality Assurance Unit*

*Authorised QA Signatures:

Head of Department:	JR Pateman CBiol MIBiol DipRQA ACQI FRQA
Deputy Head of Department:	JM Crowther MIScT MRQA
Senior Audit Staff:	G Wren ONC MRQA

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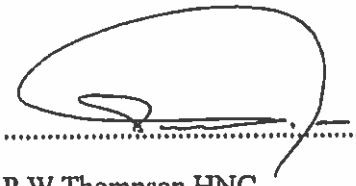
GLP COMPLIANCE STATEMENT

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.

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



..... DATE: 07 JUL 2008

P W Thompson HNC
Study Director

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S-Face BC-810:

REVERSE MUTATION ASSAY "AMES TEST" USING *SALMONELLA TYPHIMURIUM* AND *ESCHERICHIA COLI*

SUMMARY

Introduction. The method was designed to conform to the guidelines for bacterial mutagenicity testing published by the major Japanese Regulatory Authorities including METI, MHLW and MAFF. It also meets the requirements of the OECD Guidelines for Testing of Chemicals No. 471 "Bacterial Reverse Mutation Test", Method B13/14 of Commission Directive 2000/32/EC and the USA, EPA (TSCA) OPPTS harmonised guidelines.

Methods. *Salmonella typhimurium* strains TA1535, TA1537, TA98 and TA100 and *Escherichia coli* strain WP2uvrA$^-$ were treated with the test material using the Ames plate incorporation method at five dose levels, in triplicate, both with and without the addition of a rat liver homogenate metabolising system (10% liver S9 in standard co-factors). The dose range for the range-finding test was determined in a preliminary toxicity assay and was 50 to 5000 µg/plate. The experiment was repeated on a separate day using the same dose range as the range-finding test, fresh cultures of the bacterial strains and fresh test material formulations.

Results. The vehicle (tetrahydrofuran) control plates gave counts of revertant colonies within the normal range. All of the positive control chemicals used in the test induced marked increases in the frequency of revertant colonies, both with or without metabolic activation. Thus, the sensitivity of the assay and the efficacy of the S9-mix were validated.

The test material caused no visible reduction in the growth of the bacterial background lawn at any dose level. The test material was, therefore, tested up to the maximum recommended dose level of 5000 µg/plate. A pale, particulate precipitate was observed at and above 500 µg/plate, this did not prevent the scoring of revertant colonies.

No significant increases in the frequency of revertant colonies were recorded for any of the bacterial strains, with any dose of the test material, either with or without metabolic activation.

Conclusion. The test material was considered to be non-mutagenic under the conditions of this test.

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S-Face BC-810:

REVERSE MUTATION ASSAY "AMES TEST" USING *SALMONELLA TYPHIMURIUM* AND *ESCHERICHIA COLI*

1. INTRODUCTION

This study was designed to assess the mutagenic potential of the test material using a bacterial/microsome test system. The study was based on the *in vitro* technique described by Ames and his co-workers (1, 2, 3) and Garner *et al* (4) in which mutagenic activity is assessed by exposing histidine auxotrophs of *Salmonella typhimurium* and tryptophan auxotrophs of *Escherichia coli* to various concentrations of the test material. The method conforms to the guidelines for bacterial mutagenicity testing published by the major Japanese Regulatory Authorities including METI, MHLW and MAFF. This method also conforms with the OECD Guidelines for the Testing of Chemicals, Protocol No. 471, Method B13/14 in EC Commission Directive 2000/32/EC and the USA, EPA (TSCA) OPPTS harmonised guidelines. A copy of the Certificate of Compliance with GLP, issued by the UK Department of Health, is included.

The mutant strains of *Salmonella* are incapable of synthesising histidine and are, therefore, dependent on an external source of this particular amino acid for normal growth. When exposed to a mutagenic agent these bacteria may undergo a reverse mutation to histidine independent forms which are detected by their ability to grow on a histidine deficient medium. Using various strains of this organism, revertants produced after exposure to a chemical mutagen may arise as a result of base-pair substitution in the genetic material (miscoding) or frame-shift mutation in which genetic material is either added or deleted. In order to make the bacteria more sensitive to mutation by chemical and physical agents, several additional traits have been introduced. These include a deletion through the excision repair gene (uvrB$^-$ *Salmonella* strains) which renders the organism incapable of DNA excision repair and deep rough mutation (rfa) which increases the permeability of the cell wall. A mutant strain of *Escherichia coli* (WP2uvrA$^-$), which requires tryptophan and which can be reverse mutated by base substitution to tryptophan independence, was used to complement the *Salmonella* strains. This strain also has a deletion in an excision repair gene (uvrA$^-$).

Since many compounds do not exert a mutagenic effect until they have been metabolised by enzyme systems not available in the bacterial cell, the test material and the bacteria are also

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incubated in the presence of a liver microsomal preparation (S9-mix) prepared from rats pre-treated with a mixture known to induce an elevated level of these enzymes.

The experimental phase of this study was performed between 20 March 2008 and 14 April 2008.

2. TEST MATERIAL

Sponsor's identification	:	S-Face BC-810
Description	:	Extremely pale yellow solid
Batch number	:	80121
Date received	:	25 February 2008
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. A copy of the Test Material Certificate of Analysis is included as Appendix 2.

3. METHODS

3.1 Tester Strains

Salmonella typhimurium	TA1535, TA1537, TA98 and TA100
Escherichia coli	WP2uvrA$^-$

The *Salmonella typhimurium* strains were obtained from the University of California at Berkeley on culture discs on 4 August 1995 whilst *Escherichia coli* strain WP2uvrA$^-$ was obtained from the British Industrial Biological Research Association on 17 August 1987. All of the strains were stored at -196°C in a Statebourne Liquid Nitrogen Freezer, Model SXR 34. Prior to the master strains being used, characterisation checks were carried out to confirm the amino-acid requirement, presence of rfa, R factors, uvrB or uvrA mutation and the spontaneous reversion rate.

In this assay, overnight sub-cultures of the appropriate coded stock cultures were prepared in nutrient broth and incubated at 37°C for approximately 10 hours. Each culture was monitored spectrophotometrically for turbidity with titres determined by viable count analysis on nutrient agar plates.

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3.2 Preparation of Test and Control Materials

The test material was insoluble in sterile distilled water, dimethyl sulphoxide, acetone, dimethyl formamide and acetonitrile at 50 mg/ml in solubility checks performed in–house. The test material was fully soluble in tetrahydrofuran at 200 mg/ml, therefore, this solvent was selected as the vehicle.

The test material was accurately weighed and approximate half-log dilutions prepared in tetrahydrofuran by mixing on a vortex mixer and sonication for 15 minutes at 40°C on the day of each experiment. Tetrahydrofuran is toxic to the bacterial cells at and above 50 µl, therefore all of the formulations were prepared at concentrations four times greater than required on Vogel-Bonner agar plates. To compensate, each formulation was dosed using 25 µl aliquots. Tetrahydrofuran is an acceptable vehicle for use in this test system (6). Analysis for concentration, homogeneity and stability of the test material formulations is not a requirement of the test guidelines and was, therefore, not determined. Prior to use, the solvent was dried using molecular sieves (sodium alumino-silicate) *ie* 2 mm pellets with a nominal pore diameter of 4×10^{-4} microns.

Vehicle and positive controls were used in parallel with the test material. A solvent treatment group was used as the vehicle control and the positive control materials used in the series of plates without S9-mix, were as follows:

N-ethyl-N'-nitro-N-nitrosoguanidine (ENNG):	2 µg/plate for WP2uvrA$^-$, 3 µg/plate for TA100 and 5 µg/plate for TA1535
9-Aminoacridine (9AA):	80 µg/plate for TA1537
4-Nitroquinoline-1-oxide (4NQO):	0.2 µg/plate for TA98

In addition, 2-Aminoanthracene (2AA) and Benzo(a)pyrene (BP), which are non-mutagenic in the absence of metabolising enzymes, were used in the series of plates with S9-mix at the following concentrations:

2AA at 1 µg/plate for TA100
2AA at 2 µg/plate for TA1535 and TA1537
2AA at 10 µg/plate for WP2uvrA$^-$
BP at 5 µg/plate for TA98

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3.3 Microsomal Enzyme Fraction

S9 was prepared in-house on 20 January 2008 from the livers of male Sprague-Dawley rats weighing ~ 250g. These had each orally received three consecutive daily doses of phenobarbitone/β-naphthoflavone (80/100 mg per kg per day) prior to S9 preparation on Day 4. Before use, each batch of S9 was assayed for its ability to metabolise appropriate indirect mutagens used in the Ames Test. The S9 was stored at -196°C.

3.4 S9-Mix and Agar

The S9-mix was prepared immediately before use using sterilised co-factors and maintained on ice for the duration of the test.

S9	5.0 ml
1.65 M KCl/0.4 M $MgCl_2$	1.0 ml
0.1 M Glucose-6-phosphate	2.5 ml
0.1 M NADP	2.0 ml
0.2 M Sodium phosphate buffer (pH 7.4)	25.0 ml
Sterile distilled water	14.5 ml

A 0.5 ml aliquot of S9-mix and 2 ml of molten, trace histidine or tryptophan supplemented, top agar were overlaid onto a sterile Vogel-Bonner Minimal agar plate in order to assess the sterility of the S9-mix. This procedure was repeated, in triplicate, on the day of each experiment.

Top agar was prepared using 0.6% Difco Bacto agar and 0.5% sodium chloride with 5 ml of 1.0 mM histidine and 1.0 mM biotin or 1.0 mM tryptophan solution added to each 100 ml of top agar. Vogel-Bonner Minimal agar plates were purchased from ILS Ltd.

3.5 Test Procedure

3.5.1 Preliminary Toxicity Test

In order to select appropriate dose levels for use in the main test, a preliminary assay was carried out to determine the toxicity of the test material. The concentrations tested were 0, 0.15, 0.5, 1.5, 5, 15, 50, 150, 500, 1500 and 5000 µg/plate. The assay was performed by mixing 0.1 ml of bacterial culture (TA100 or WP2uvrA$^-$), 0.025 ml of test material formulation, 0.5 ml of S9-mix or phosphate buffer and 2 ml of molten, trace histidine or tryptophan supplemented, top agar and

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overlaying onto sterile plates of Vogel-Bonner Minimal agar (30 ml/plate). Ten concentrations of the test material and a vehicle control (tetrahydrofuran) were tested. In addition, 0.025 ml of the maximum concentration of the test material and 2 ml of molten, trace histidine or tryptophan supplemented, top agar were overlaid onto a sterile Nutrient agar plate in order to assess the sterility of the test material. After approximately 48 hours incubation at 37°C the plates were assessed for numbers of revertant colonies using a Domino colony counter and examined for effects on the growth of the bacterial background lawn. Manual counts were performed at and above 1500 µg/plate because of excessive precipitation.

3.5.2 Mutation Test – Experiment 1 (Range-finding Test)

Five concentrations of the test material (50, 150, 500, 1500 and 5000 µg/plate) were assayed in triplicate against each tester strain, using the direct plate incorporation method.

Measured aliquots (0.1 ml) of one of the bacterial cultures were dispensed into sets of test tubes followed by 2.0 ml of molten, trace histidine or tryptophan supplemented, top agar, 0.025 ml of the vehicle or test material formulation and 0.1ml of positive control and either 0.5 ml of S9-mix or phosphate buffer. The contents of each test tube were mixed and equally distributed onto the surface of Vogel-Bonner Minimal agar plates (one tube per plate). This procedure was repeated, in triplicate, for each bacterial strain and for each concentration of test material both with and without S9-mix.

All of the plates were incubated at 37°C for approximately 48 hours and the frequency of revertant colonies assessed using a Domino colony counter. Manual counts were performed at and above 1500 µg/plate because of excessive precipitation.

3.5.3 Mutation Test – Experiment 2 (Main Test)

The second experiment was performed using methodology as described for the range-finding test, using fresh bacterial cultures, test material and control solutions. The test material dose range was the same as the range-finding test (50 to 5000 µg/plate).

3.6 Acceptance Criteria

The reverse mutation assay may be considered valid if the following criteria are met:

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All tester strain cultures exhibit a characteristic number of spontaneous revertants per plate in the vehicle and untreated controls. Acceptable ranges are presented in the standard test method section 3 with historical control ranges for 2006 and 2007 presented in Appendix 3.

The appropriate characteristics for each tester strain have been confirmed, eg rfa cell-wall mutation and pKM101 plasmid R-factor etc.

All tester strain cultures should be in the approximate range of 1 to 9.9 x 10^9 bacteria per ml.

Each mean positive control value should be at least twice the respective vehicle control value for each strain, thus demonstrating both the intrinsic sensitivity of the tester strains to mutagenic exposure and the integrity of the S9-mix. The positive control historical ranges for 2006 and 2007 are presented in Appendix 3.

There should be a minimum of four non-toxic test material dose levels.

There should not be an excessive loss of plates due to contamination.

3.7 Evaluation Criteria

There are several criteria for determining a positive result, such as a dose-related increase in revertant frequency over the dose range tested and/or a reproducible increase at one or more concentrations in at least one bacterial strain with or without metabolic activation. Biological relevance of the results will be considered first, statistical methods, as recommended by the UKEMS (5) can also be used as an aid to evaluation, however, statistical significance will not be the only determining factor for a positive response.

A test material will be considered non-mutagenic (negative) in the test system if the above criteria are not met.

Although most experiments will give clear positive or negative results, in some instances the data generated will prohibit a definitive judgement about the test material activity. Results of this type will be reported as equivocal.

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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.

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5. RESULTS

5.1 Preliminary Toxicity Test

The test material was non-toxic to the strains of bacteria used (TA100 and WP2uvrA$^-$). The test material formulation and S9-mix used in this experiment were both shown to be sterile.

The numbers of revertant colonies for the toxicity assay were:

With (+) or without (-) S9-mix	Strain	Dose (µg/plate)										
		0	0.15	0.5	1.5	5	15	50	150	500	1500	5000
-	TA100	112	90	106	94	102	100	99	95	96	98P	92P
+	TA100	89	116	118	96	83	84	82	99	104	83P	102P
-	WP2uvrA$^-$	31	32	31	28	23	29	20	28	28	15P	24P
+	WP2uvrA$^-$	29	28	32	23	21	23	32	29	19	25P	25P

P Precipitate

5.2 Mutation Test

Prior to use, the master strains were checked for characteristics, viability and spontaneous reversion rate (all were found to be satisfactory). These data are not given in the report. The amino acid supplemented top agar and the S9-mix used in both experiments was shown to be sterile.

Results for the negative controls (spontaneous mutation rates) are presented in Table 1 and were considered to be acceptable. These data are for concurrent untreated control plates performed on the same day as the Mutation Test.

The individual plate counts, the mean number of revertant colonies and the standard deviations for the test material, vehicle and positive controls both with and without metabolic activation, are presented in Table 2 to Table 5 with the results also expressed graphically in Figure 1 to Figure 4.

Information regarding the equipment and methods used in these experiments as required by the Japanese Ministry of Economy, Trade and Industry and Japanese Ministry of Health, Labour and Welfare are presented in Appendix 1.

A history profile of vehicle and positive control values is presented in Appendix 3.

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The test material caused no visible reduction in the growth of the bacterial background lawn at any dose level. The test material was, therefore, tested up to the maximum recommended dose level of 5000 µg/plate. A pale, particulate precipitate was observed at and above 500 µg/plate, this did not prevent the scoring of revertant colonies.

No significant increases in the frequency of revertant colonies were recorded for any of the bacterial strains, at any dose level either with or without metabolic activation.

All of the positive control chemicals used in the test induced marked increases in the frequency of revertant colonies thus confirming the activity of the S9-mix and the sensitivity of the bacterial strains.

6. CONCLUSION

The test material was considered to be non-mutagenic under the conditions of this test.

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USING *SALMONELLA TYPHIMURIUM* AND *ESCHERICHIA COLI*

Table 1 Spontaneous Mutation Rates (Concurrent Negative Controls)

Range-finding Test

Number of revertants (mean number of colonies per plate)									
Base-pair substitution type						Frameshift type			
TA100		TA1535		WP2uvrA⁻		TA98		TA1537	
95		24		37		18		13	
89	(97)	18	(22)	29	(30)	15	(15)	10	(11)
106		23		24		13		10	

Main Test

Number of revertants (mean number of colonies per plate)									
Base-pair substitution type						Frameshift type			
TA100		TA1535		WP2uvrA⁻		TA98		TA1537	
96		29		23		24		12	
100	(104)	26	(29)	20	(19)	18	(21)	8	(11)
117		32		15		21		14	

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USING *SALMONELLA TYPHIMURIUM* AND *ESCHERICHIA COLI*

Table 2 Test Results: Range-Finding Test– Without Metabolic Activation

Test Period		From: 31 March 2008						To: 03 April 2008					
With or without S9-Mix	Test substance concentration (µg/plate)	Number of revertants (mean number of colonies per plate)											
		Base-pair substitution type								Frameshift type			
		TA100		TA1535		WP2uvrA-				TA98		TA1537	
-	0	119 100 88	(102) 15.6#	23 22 18	(21) 2.6	37 27 41	(35) 7.2			13 22 19	(18) 4.6	16 18 16	(17) 1.2
-	50	89 103 119	(104) 15.0	21 21 19	(20) 1.2	34 37 33	(35) 2.1			14 18 23	(18) 4.5	8 15 10	(11) 3.6
-	150	118 130 110	(119) 10.1	20 23 26	(23) 3.0	46 35 40	(40) 5.5			9 29 20	(19) 10.0	8 13 16	(12) 4.0
-	500	115 P 133 P 97 P	(115) 18.0	22 P 20 P 20 P	(21) 1.2	49 P 29 P 20 P	(33) 14.8			20 P 45 P 29 P	(31) 12.7	14 P 10 P 9 P	(11) 2.6
-	1500	84 P 89 P 86 P	(86) 2.5	23 P 21 P 20 P	(21) 1.5	19 P 23 P 29 P	(24) 5.0			19 P 22 P 18 P	(20) 2.1	10 P 12 P 23 P	(15) 7.0
-	5000	105 P 96 P 76 P	(92) 14.8	22 P 12 P 19 P	(18) 5.1	43 P 38 P 22 P	(34) 11.0			28 P CP 21 P	(25) 4.9	12 P 13 P 15 P	(13) 1.5
Positive controls	Name	ENNG		ENNG		ENNG				4NQO		9AA	
	Concentration (µg/plate)	3		5		2				0.2		80	
S9-Mix -	No. colonies per plate	398 401 383	(394) 9.6	147 175 141	(154) 18.1	142 136 136	(138) 3.5			134 123 139	(132) 8.2	1173 947 605	(908) 286.0

ENNG N-ethyl-N'-nitro-N-nitrosoguanidine
4NQO 4-Nitroquinoline-1-oxide
9AA 9-Aminoacridine
C Contaminated
P Precipitate
Standard deviation

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USING *SALMONELLA TYPHIMURIUM* AND *ESCHERICHIA COLI*

Table 3 Test Results: Range-Finding Test– With Metabolic Activation

Test Period		From: 31 March 2008						To: 03 April 2008			
With or without S9-Mix	Test substance concentration (µg/plate)	Number of revertants (mean number of colonies per plate)									
		Base-pair substitution type						Frameshift type			
		TA100		TA1535		WP2uvrA-		TA98		TA1537	
+	0	85 99 77	(87) 11.1#	10 14 19	(14) 4.5	35 40 36	(37) 2.6	43 30 24	(32) 9.7	13 12 14	(13) 1.0
+	50	93 109 99	(100) 8.1	10 16 14	(13) 3.1	33 48 27	(36) 10.8	27 25 48	(33) 12.7	9 9 8	(9) 0.6
+	150	87 92 113	(97) 13.8	18 10 16	(15) 4.2	44 31 29	(35) 8.1	31 32 29	(31) 1.5	9 16 19	(15) 5.1
+	500	109 P 102 P 118 P	(110) 8.0	16 P 16 P 9 P	(14) 4.0	42 P 27 P 31 P	(33) 7.8	24 P 20 P 21 P	(22) 2.1	10 P 10 P 13 P	(11) 1.7
+	1500	115 P 86 P 93 P	(98) 15.1	10 P 9 P 12 P	(10) 1.5	29 P 23 P 22 P	(25) 3.8	27 P 20 P 33 P	(27) 6.5	14 P 13 P 16 P	(14) 1.5
+	5000	121 P 98 P 94 P	(104) 14.6	16 P 12 P 15 P	(14) 2.1	24 P 32 P 26 P	(27) 4.2	25 P 23 P 23 P	(24) 1.2	14 P 14 P 18 P	(15) 2.3
Positive controls	Name	2AA		2AA		2AA		BP		2AA	
	Concentration (µg/plate)	1		2		10		5		2	
S9-Mix +	No. colonies per plate	1768 1011 1067	(1282) 421.8	152 291 279	(241) 77.0	417 570 560	(516) 85.6	233 225 234	(231) 4.9	399 383 443	(408) 31.1

BP Benzo(a)pyrene
2AA 2-Aminoanthracene
P Precipitate
Standard deviation

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USING *SALMONELLA TYPHIMURIUM* AND *ESCHERICHIA COLI*

Table 4 Test Results: Main Test– Without Metabolic Activation

Test Period		From: 11 April 2008						To: 14 April 2008					
With or without S9-Mix	Test substance concentration (µg/plate)	Number of revertants (mean number of colonies per plate)											
		Base-pair substitution type						Frameshift type					
		TA100		TA1535		WP2uvrA-		TA98		TA1537			
-	0	109 128 129	(122) 11.3#	23 29 36	(29) 6.5	26 27 21	(25) 3.2	37 31 32	(33) 3.2	15 19 15	(16) 2.3		
-	50	102 118 98	(106) 10.6	25 23 23	(24) 1.2	23 14 23	(20) 5.2	23 40 31	(31) 8.5	20 14 15	(16) 3.2		
-	150	98 109 124	(110) 13.1	27 32 29	(29) 2.5	25 15 22	(21) 5.1	33 32 26	(30) 3.8	10 12 16	(13) 3.1		
-	500	112 P 103 P 104 P	(106) 4.9	38 P 32 P 26 P	(32) 6.0	25 P 19 P 18 P	(21) 3.8	27 P 23 P 26 P	(25) 2.1	21 P 12 P 18 P	(17) 4.6		
-	1500	121 P 123 P 108 P	(117) 8.1	43 P 22 P 37 P	(34) 10.8	24 P 24 P 15 P	(21) 5.2	24 P 37 P 32 P	(31) 6.6	13 P 12 P 14 P	(13) 1.0		
-	5000	112 P 106 P 118 P	(112) 6.0	21 P 33 P 23 P	(26) 6.4	20 P 25 P 23 P	(23) 2.5	27 P 32 P 30 P	(30) 2.5	19 P 19 P 14 P	(17) 2.9		
Positive controls	Name	ENNG		ENNG		ENNG		4NQO		9AA			
	Concentration (µg/plate)	3		5		2		0.2		80			
S9-Mix	No. colonies per plate	261 572 1043	(625) 393.7	342 338 277	(319) 36.4	392 354 274	(340) 60.2	163 170 189	(174) 13.5	1059 1078 1044	(1060) 17.0		
-													

ENNG N-ethyl-N'-nitro-N-nitrosoguanidine
4NQO 4-Nitroquinoline-1-oxide
9AA 9-Aminoacridine
P Precipitate
Standard deviation

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USING *SALMONELLA TYPHIMURIUM* AND *ESCHERICHIA COLI*

Table 5 Test Results: Main Test– With Metabolic Activation

Test Period		From: 11 April 2008			To: 14 April 2008								
With or without S9-Mix	Test substance concentration (µg/plate)	Number of revertants (mean number of colonies per plate)											
		Base-pair substitution type								Frameshift type			
		TA100			TA1535			WP2uvrA-			TA98			TA1537		
+	0	103 118 115	(112) 7.9#		16 15 16	(16) 0.6		34 26 24	(28) 5.3		36 30 18	(28) 9.2		12 16 15	(14) 2.1	
+	50	111 93 93	(99) 10.4		14 10 14	(13) 2.3		26 19 15	(20) 5.6		24 29 31	(28) 3.6		15 12 20	(16) 4.0	
+	150	96 93 115	(101) 11.9		13 11 14	(13) 1.5		15 22 29	(22) 7.0		33 29 23	(28) 5.0		8 16 13	(12) 4.0	
+	500	110 P 107 P 106 P	(108) 2.1		13 P 11 P 13 P	(12) 1.2		31 P 16 P 26 P	(24) 7.6		24 P 20 P 19 P	(21) 2.6		11 P 8 P 13 P	(11) 2.5	
+	1500	115 P 100 P 113 P	(109) 8.1		18 P 15 P 12 P	(15) 3.0		18 P 23 P 31 P	(24) 6.6		34 P 26 P 31 P	(30) 4.0		16 P 10 P 11 P	(12) 3.2	
+	5000	109 P 102 P 100 P	(104) 4.7		14 P 14 P 16 P	(15) 1.2		31 P 24 P 25 P	(27) 3.8		25 P 29 P 33 P	(29) 4.0		15 P 12 P 14 P	(14) 1.5	
Positive controls	Name	2AA			2AA			2AA			BP			2AA		
	Concentration (µg/plate)	1			2			10			5			2		
S9-Mix +	No. colonies per plate	1758 1724 1895	(1792) 90.5		293 275 272	(280) 11.4		521 498 538	(519) 20.1		244 290 285	(273) 25.2		566 451 231	(416) 170.2	

BP Benzo(a)pyrene
2AA 2-Aminoanthracene
P Precipitate
Standard deviation

Figure 1 **Dose Response Curve: Range-Finding Test– Without Metabolic Activation**

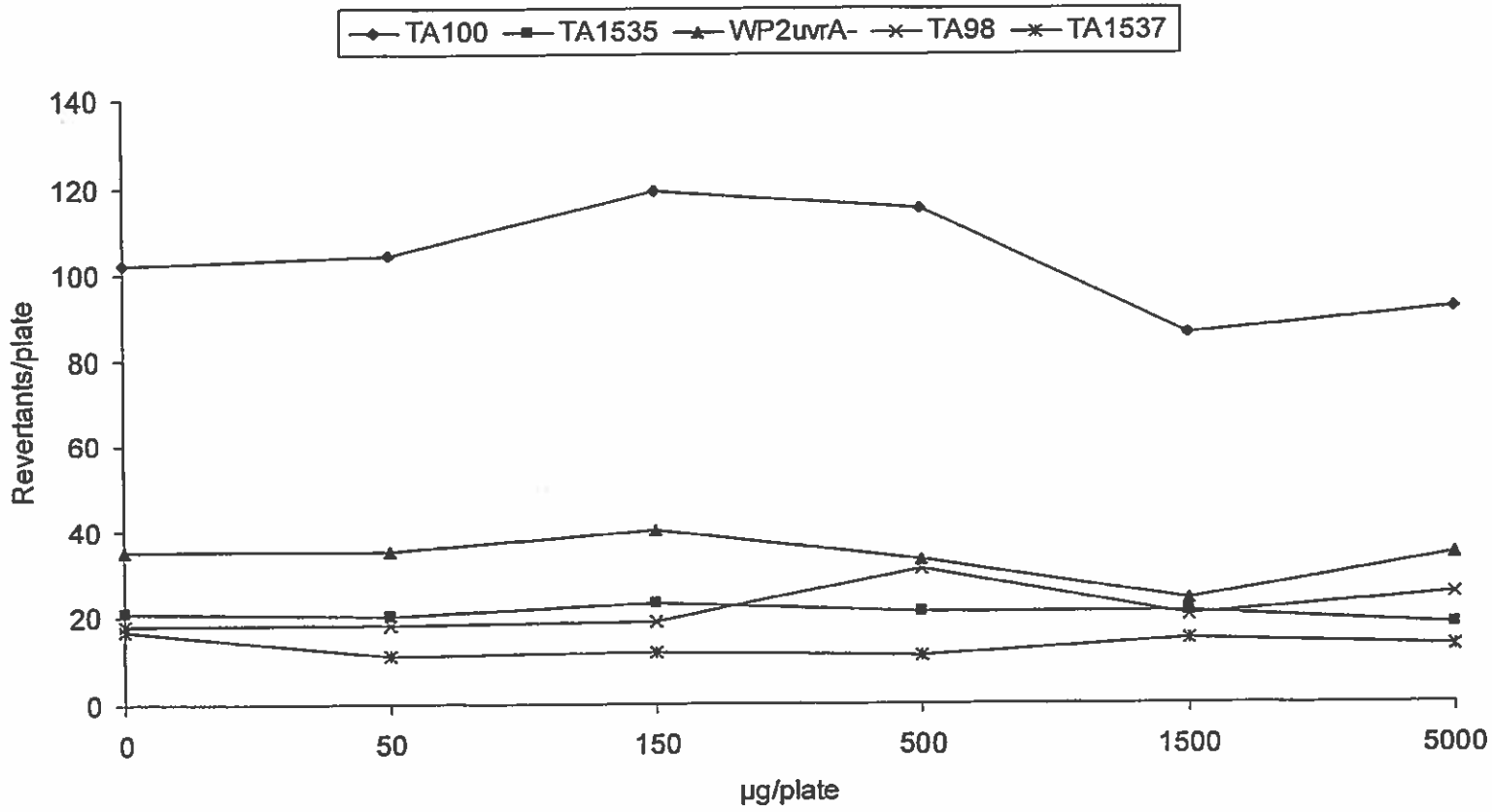

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Figure 2 **Dose Response Curve: Range-Finding Test– With Metabolic Activation**

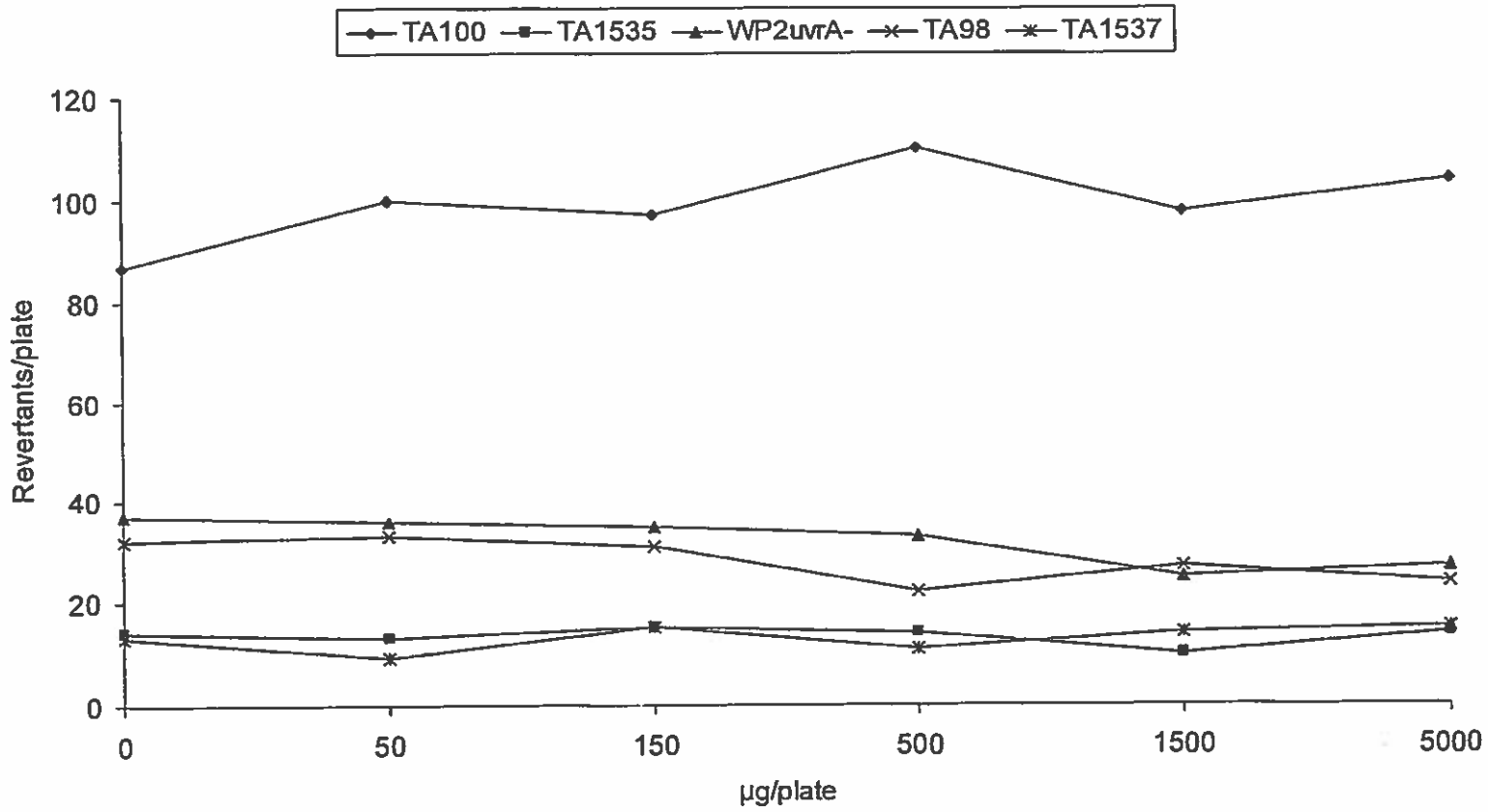

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Figure 3 **Dose Response Curve: Main Test– Without Metabolic Activation**

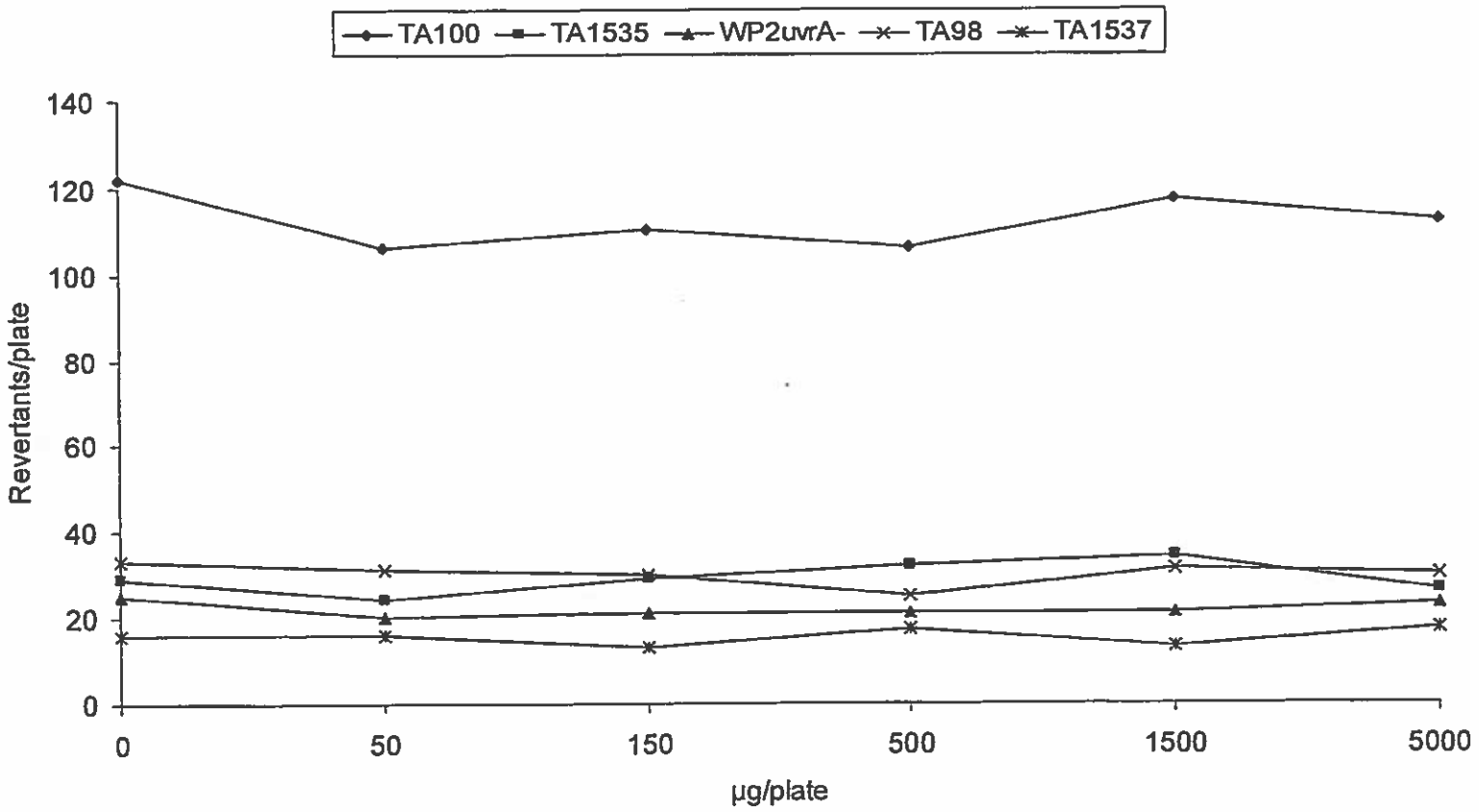

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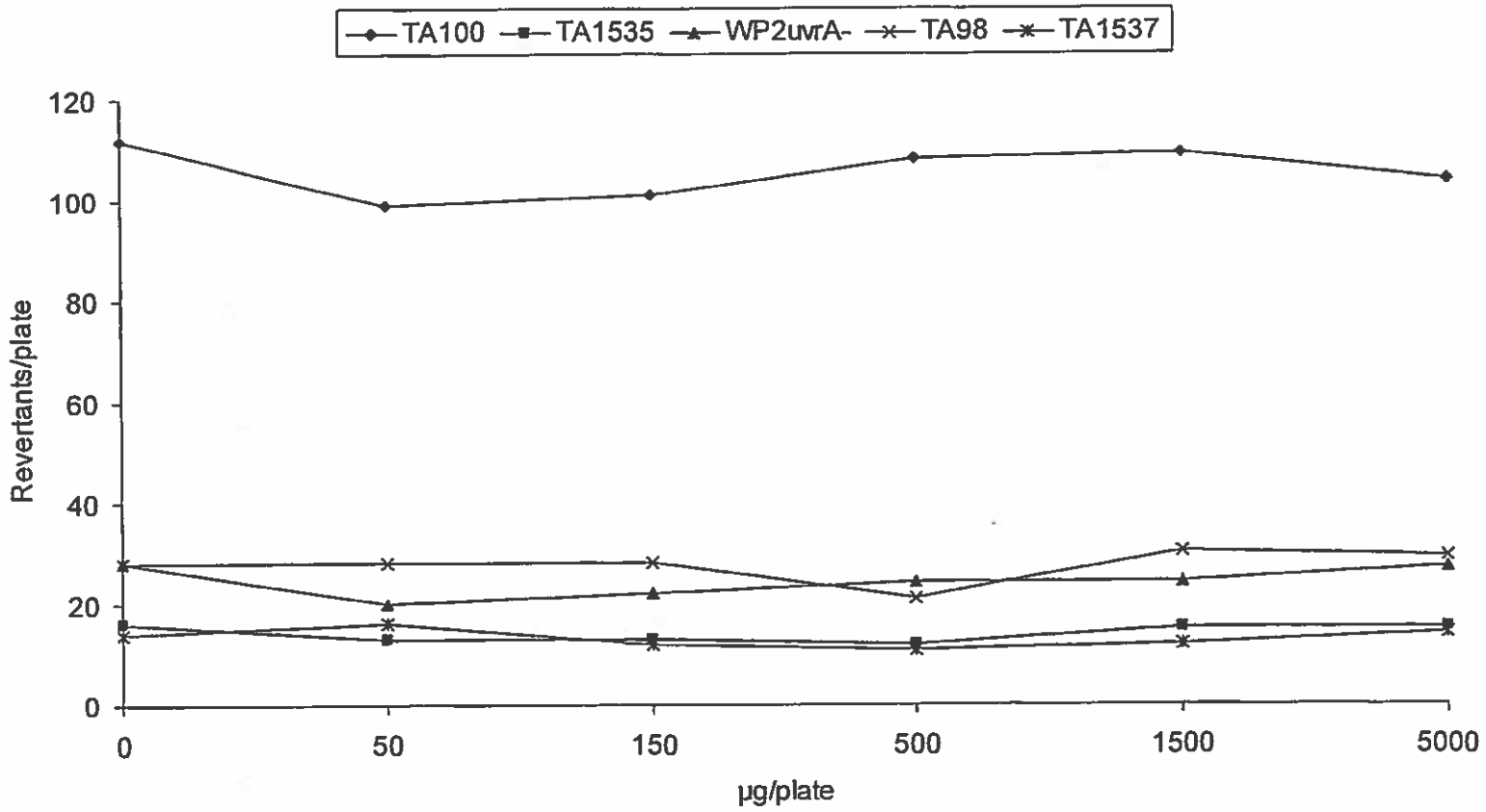
Figure 4 **Dose Response Curve: Main Test– With Metabolic Activation**



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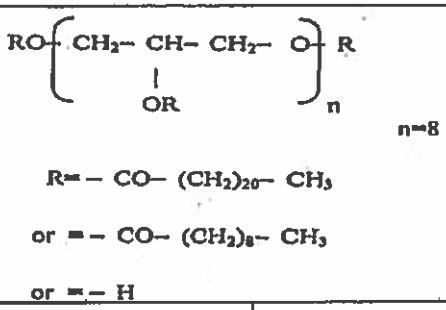
S-Face BC-810: REVERSE MUTATION ASSAY "AMES TEST"
USING *SALMONELLA TYPHIMURIUM* AND *ESCHERICHIA COLI*

Appendix 1 Report of Results in Mutagenicity Test using Micro-organisms

1. GENERAL ITEMS (to be completed by the sponsor)

Name of the new chemical substance (IUPAC nomenclature)	Poiyglyceryl-8 Dacabehenate / Decacaprate					
Other name	S-Face BC-810					
Structural formula or rational formula (or outline of manufacturing method, in case both are unknown)	 n=8 R= – CO– $(CH_2)_{20}$– CH_3 or = – CO– $(CH_2)_8$– CH_3 or = – H					
Purity of the new chemical substance tested	>99%		Lot No. of the new chemical substance tested	80121		
Name of impurities and concentration	-					
CAS No.	1001428-78-9		Vapour pressure	-		
Molecular weight	3593.3		Partition coefficient	-		
Melting point (°C)	68		Appearance at ordinary temperature	Extremely pale, yellow solid		
Boiling point (°C)	>350					
Stability	Stable under normal temperatures and pressure					
Degree of solubility in solvent	Solvent	Degree of solubility	Stability in solvent	Solvent	Degree of solubility	Stability in solvent
	Water	Insolubie at 50 mg/ml	-	DMSO	Insoluble at 50 mg/ml	-
	Acetone	Insolubie at 50 mg/ml	-	DMF	Insoluble at 50 mg/ml	-
	Acetonitrile	Insoluble at 50 mg/ml	-	THF	Soiuble at 200 mg/ml	*

Solubility data generated by the testing facility
* No visible change in test material formulations throughout each experiment, therefore, assumed stable

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S-Face BC-810: REVERSE MUTATION ASSAY "AMES TEST"
USING *SALMONELLA TYPHIMURIUM* AND *ESCHERICHIA COLI*

Appendix 1 (continued)

2. TESTER STRAINS

Tester strain	Obtained from	Date obtained
TA100	UCB*	4 August 1995
TA1535	UCB*	4 August 1995
WP2uvrA⁻	BIBRA**	17 August 1987
TA98	UCB*	4 August 1995
TA1537	UCB*	4 August 1995

3. S-9 MIX

(1) Source of S9

(encircle the applicable number and fill in the relevant entries)

Made in-house or purchase	(1.) Made in-house	2. Purchase (Supplier)
Prepared on	20 January 2008	
Lot No. (in case of purchase)	-	
Storage temperature	-196°C	

(2) Preparation of S9

Animal used		Inducing substance	
Species strain	Rat Sprague-Dawley	Name	Phenobarbitone/β-naphthoflavone
Sex	Male	Administration method	Orai
Age (in weeks)	6 - 8 weeks	Administration period and amount (mg/kg bodyweight/day)	3 days 80/100 mg per kg per day
Weight	~ 250g		

(3) Composition of S9-Mix

Constituents	Amount in 1 ml S9-Mix	Constituents	Amount in 1 ml S9-Mix
S9	0.1 ml	NADP	4.0 µmol
$MgCl_2$	8.0 µmol	Na-Phosphate buffer	100.0 µmol
KCl	33.0 µmol		
Glucose-6-phosphate	5.0 µmol		
Glucose-6-phosphate dehydrogenase	-		

* UCB: University of California at Berkeley
** BIBRA: British Industrial Biological Research Association

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S-Facc BC-810: REVERSE MUTATION ASSAY "AMES TEST"
USING *SALMONELLA TYPHIMURIUM* AND *ESCHERICHIA COLI*

Appendix 1 (continued)

4. PREPARATION OF THE TEST MATERIAL IN SOLUTION

(encircle the applicable response)

	Name	Supplier	Lot No.	Grade	Purity (%)
Solvent used	Tetrahydrofuran	Sigma Aidrich	72000	-	99.9
Reason for selection of solvent	Fully soluble at 200 mg/ml				
Appearance of the test material preparation	(Solution)	Suspension	Others ( )		
Suspension and other methods when test substance difficult to dissolve	Solution achieved by action on an autovortex mixer and sonication for 15 minutes at 40°C				
Storage time and temperature of solution from preparation until use	55 minutes at room temperature (range-finding test) 20 minutes at room temperature (main test)				
Conversion of purity	Yes	(No)			

5. CONDITIONS OF PRE-CULTURE ETC.

(1) Conditions

	Name	Manufacturer	Lot No.
Nutrient Broth	Oxoid NB No.2	Oxoid Ltd.	583667 09/2012
Period of pre-culture	10 hr		
Culture flask (form, size)	Costar 75 cm^2 tissue culture flasks		
Amount of culture medium	5 ml	Amount of strain inoculated	20µl

(2) Cell viability at the end of pre-culture

		Base-pair substitution type			Frameshift type		
		TA100	TA1535	WP2uvrA'	TA98	TA1537	
Cell number (x 10^9/ml)	Range-finding test	1.1	1.8	5.1	1.1	1.8	
	Main test	1.6	1.3	2.0	1.1	1.1	
Count method (encircle the applicable number)		1. Conversion from O.D. value (2.) Stepwise dilution method 3. Others ( )					

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S-Face BC-810: REVERSE MUTATION ASSAY "AMES TEST"
USING *SALMONELLA TYPHIMURIUM* AND *ESCHERICHIA COLI*

Appendix 1 (continued)

6. MINIMUM GLUCOSE AGAR PLATE MEDIUM

(encircle the applicable number and fill in the relevant entries)

Made in-house or purchase	1. Made in-house (2.) Purchase (ILS Ltd)
Prepared on	20 March 2008 (range-finding test) 02 April 2008 (main test)
Lot No. in case of purchase	1030205-02 12/2012

7. TEST METHOD (encircle the applicable number and fill in the relevant entries)

(1) Test Method and the reason why it was selected

Test method used	1. Pre-incubation method (2.) Plate incorporation method 3. Others ( )
Reason in case of others	-

(2) Test Conditions

		Pre-incubation	Plate Method
Composition	Bacterial suspension	ml	0.1 ml
	Test substance solution	ml	0.025 ml
	Na-phosphate buffer	ml	0.5 ml
	S9-mix (in case of metabolic activation method)	ml	0.5 ml
	Top agar solution	ml	2.0 ml
	Others ( )	ml	-
Pre-incubation	Temperature	°C	-
	Time	min	-
Incubation	Temperature	°C	37°C
	Time	hours	48 hours

8. COUNT METHOD OF COLONIES (Encircle 1 and 2 if both methods used)

Count method	(1.) By hand†	(2.) Colony counter
Conduction of correction	1. No	(2.) Yes (Correction method: Area and miscount correction)

† Manual counts performed at and above 1500 µg/plate because of test material precipitation

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S-Face BC-810: REVERSE MUTATION ASSAY "AMES TEST"
USING *SALMONELLA TYPHIMURIUM* AND *ESCHERICHIA COLI*

Appendix 1 (continued)

9. TEST RESULTS

(1) The results should be reported on the attached form

(2) Judgement of the results

Judgement (encircle one)	Positive	(Negative)
Reason for judgement: No significant increase in the frequency of revertant colonies was recorded for any bacterial strain used with any dose of the test material in two separate experiments either with or without metabolic activation.		

(when judged as positive, attach a table for relative activity)

(3) Referential matters

A precipitate was observed at and above 500 µg/plate, this did not prevent the scoring of revertant colonies. The test material was insoluble in sterile distilled water, dimethyl sulphoxide, acetone, dimethyl formamide and acetonitrile at 50 mg/ml in solubility checks performed in-house. The test material was fully soluble in tetrahydrofuran at 200 mg/ml, therefore, this solvent was selected as the vehicle. Tetrahydrofuran is an acceptable vehicle for use in this test system (6). Prior to use, the solvent was dried using molecular sieves (sodium alumino-silicate) ie 2 mm pellets with a nominal pore diameter of 4×10^{-4} microns.
There are several criteria for determining a positive result, such as a dose-related increase in revertant frequency over the dose range tested and/or a reproducible increase at one or more concentrations in at least one bacterial strain with or without metabolic activation. Biological relevance of the results will be considered first, statistical methods, as recommended by the UKEMS (5) can also be used as an aid to evaluation, however, statistical significance will not be the only determining factor for a positive response.
A test material will be considered non-mutagenic (negative) in the test system if the above criteria are not met.
Although most experiments will give clear positive or negative results, in some instances the data generated will prohibit a definitive judgement about the test material activity. Results of this type will be reported as equivocal.
Phenobarbitone/β-naphthoflavone was used to induce higher enzyme levels in the rat liver S9. The S9-mix used in each experiment of this study was shown to be sterile. The test material formulations showed no evidence of contamination when tested for sterility during the preliminary toxicity test. The use of approximate half-log dilutions instead of twofold dilutions was agreed with the JMOL in January 1995.

[REMARKS] "Referential matters" – (fill in the view etc of the Study Director on the test results)

10. OTHERS

	Name	Safepharm Laboratories Ltd.		
	Address	Shardlow Business Park, Shardlow, Derbyshire, DE72 2GD	Tel No: 0044 1332 792896 Fax No: 0044 1332 799018	
Study Director	Title	Senior Genetic Toxicologist	Name	P W Thompson HNC
	Years of experience: 22			
Study number	0432/0105			
Test dates	1. Each experiment date:	Experiment 1: From: 31 March 2008 To: 03 April 2008 Experiment 2: From: 11 April 2008 To: 14 April 2008		
	2. Test Period:	From: 11 March 2008 To: 07 JUL 2008		

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S-Face BC-810: REVERSE MUTATION ASSAY "AMES TEST"
USING *SALMONELLA TYPHIMURIUM* AND *ESCHERICHIA COLI*

Appendix 2 Certificate of Analysis

SAKAMOTO YAKUHIN KOGYO CO.,LTD.

HEAD OFFICE : 1-2-6 AWAJIMACHI,CHUO-KU,OSAKA CITY 541-0047,JAPAN
TEL (81)6-6231-1851 FAX (81)6-6222-0631
TOKYO BRANCH : 1-8-2 MARUNOUCHI,CHIYODA-KU,TOKYO 100-0005,JAPAN
TEL (81)3-3211-3818 FAX (81)3-3216-1769

date : Feb. 20, 2008

Product : S-Face BC-810
Lot No. : 80121
Quantity : 100g × 1

Certificate of analysis / Quality

	Result
Description	A pale yellow solid with a faint characteristic odor
Color (Gardner)	3 ~ 4
Acid value (mgKOH/g)	3.9
Saponification value (mgKOH/g)	150.6
Heavy metal (ppm)	less than 20
Arsenic (ppm)	less than 2
Residue on ignition (%)	less than 0.5

Y. Noguchi
Yasunori Noguchi
Quality Control Manager

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S-Face BC-810: REVERSE MUTATION ASSAY "AMES TEST"
USING *SALMONELLA TYPHIMURIUM* AND *ESCHERICHIA COLI*

Appendix 3 History Profile of Vehicle and Positive Control Values

COMBINED VEHICLE AND UNTREATED CONTROL VALUES 2006

Strain	TA100		TA1535		WP2uvrA⁻		TA102		TA98		TA1537		WP2uvrA⁻ pKM101	
S9-Mix	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Mean	99	90	20	13	25	30	286	337	22	26	12	14	133	157
SD	15.6	13.7	5.6	3.2	4.5	5.4	34.7	31.2	5.7	5.7	3.2	4.6	31.3	28.4
Min	66	62	8	6	12	14	197	245	11	13	4	3	77	121
Max	160	147	39	31	48	50	381	393	77	48	28	27	234	216
Values	897	733	880	716	787	632	151	88	906	732	868	703	81	22

POSITIVE CONTROL VALUES 2006

Strain	TA100		TA1535		WP2uvrA⁻		TA102		TA98		TA1537		WP2uvrA⁻ pKM101	
S9-Mix	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Mean	578	1585	352	245	855	541	1458	917	218	222	1023	498	1962	1850
SD	218.2	566.9	229.5	82.2	325.1	222.1	267.7	166.1	64.3	67.0	502.1	138.8	839.1	889.8
Min	228	363	111	108	276	199	919	621	101	102	175	186	553	110
Max	1955	2969	1448	621	1942	1991	2074	1430	480	417	2718	917	3438	3053
Values	162	162	164	164	159	158	64	64	164	164	164	164	19	20

COMBINED VEHICLE AND UNTREATED CONTROL VALUES 2007

Strain	TA100		TA1535		WP2uvrA⁻		TA102		TA98		TA1537		WP2uvrA⁻ pKM101		WP2pKM101	
S9-Mix	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Mean	94	87	21	13	26	28	273	309	21	26	11	13	132	162	100	128
SD	16.0	14.8	5.2	3.6	5.8	6.4	40.4	43.2	5.9	6.0	3.8	4.1	48.5	47.8	21.8	21.4
Min	63	50	9	6	12	11	194	208	7	11	2	4	65	78	67	98
Max	156	152	38	28	46	49	390	395	99	57	28	27	278	263	136	165
Values	807	629	767	601	541	384	159	107	1282	609	1245	569	197	128	27	15

POSITIVE CONTROL VALUES 2007

Strain	TA100		TA1535		WP2uvrA⁻		TA102		TA98		TA1537		WP2uvrA⁻ pKM101		WP2pKM101	
S9-Mix	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Mean	558	1231	343	231	652	505	1281	960	256	308	1697	339	960	1103	2134	997
SD	313.5	651.1	864.2	476.5	707.9	535.2	458.9	303.6	289.7	280.5	1193.6	254.4	1679.6	1010.6	1047.0	586.4
Min	258	320	79	73	138	139	567	401	78	106	117	107	332	355	1091	285
Max	1634	3777	2087	661	2023	1185	2422	1441	1483	2055	5618	677	2522	3023	3934	2024
Values	196	197	197	203	159	159	60	61	419	202	414	198	64	64	9	11

SD = Standard deviation
Min = Minimum value
Max = Maximum value

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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

TEST FACILITY	TEST TYPE
SafePharm Laboratories Ltd. Shardlow Business Park Shardlow Derbyshire DE72 2GD	Analytical Chemistry Environmental Fate Environmental Toxicity Mutagenicity Phys/Chem testing Toxicology

DATE OF INSPECTION

21st August 2007

A general inspection for compliance with the Principles of Good Laboratory Practice was carried out at the above test facility 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.

15/10/07

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

MHRA

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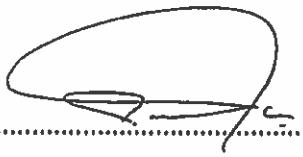
SAFEPHARM LABORATORIES LIMITED

S-Face BC-810

SPL PROJECT NUMBER: 0432/0105

I verify that this is an exact copy of the original report which is located in the Archives of SafePharm Laboratories Limited, Derby, UK.

P W Thompson HNC
Study Director ..... 

DATE ..... 14 JUL 2008

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SafePharm Laboratories

S-Face BC-810:

Polyglyceryl-8 Decabehenate / Caprate

ACUTE DERMAL IRRITATION

IN THE RABBIT

SPL PROJECT NUMBER: 0432/0102

AUTHOR: A Pooles

STUDY SPONSOR:

Sakamoto Yakuhin Kogyo Co., Ltd.
1-20, Rinkai-cho
Izumiotsu-shi
Osaka 595-0075
JAPAN

TEST FACILITY:

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

Telephone: +44 (0) 1332 792896

Facsimile: +44 (0) 1332 799018

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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. In addition, inspection of general facilities not specifically related to this study are done monthly or annually in accordance with QA Standard Procedure.

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:

	15 June 2004	Standard Test Method Compliance Audit
	11 March 2008	Test Material Preparation
	11 March 2008	Animal Preparation
	11 March 2008	Dosing
	11 March 2008	Assessment of Response
§	23 April 2008	Draft Report Audit
§	Date of QA Signature	Final Report Audit

§ Evaluation specific to this study

J. M. Crowther ..... DATE: 28 MAY 2008

For Safepharm Quality Assurance Unit*

***Authorised QA Signatures:**

Head of Department:	JR Pateman CBiol MIBiol DipRQA ACQI FRQA
Deputy Head of Department:	JM Crowther MIScT MRQA
Senior Audit Staff:	G Wren ONC MRQA

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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.

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

A. Poob ..... DATE: 28|5|08

A Pooles BA (Hons)
Study Director

This report may be presented in final form as a digital (pdf) document. Such documents produced by Safepharm are prepared by scanning the paper original, and are considered of equivalent integrity and authenticity to versions produced by optical photocopy. However, in all cases the hand-signed paper original, held in secure archives, is the definitive document.

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S-Face BC-810:

ACUTE DERMAL IRRITATION IN THE RABBIT

SUMMARY

Introduction. The study was performed to assess the irritancy potential of the test material to the skin 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. 404 "Acute Dermal Irritation/Corrosion" (adopted 24 April 2002)
- Method B4 Acute Toxicity (Skin Irritation) of Commission Directive 2004/73/EC

Results. 3-minute and 1-hour semi-occluded applications of the test material to the intact skin of one rabbit produced no evidence of skin irritation.

A single 4-hour, semi-occluded application of the test material to the intact skin of three rabbits produced no evidence of skin irritation. No corrosive effects were noted.

Conclusion. The test material produced a primary irritation index of 0.0 and was classified as non-irritant to rabbit skin according to the Draize classification scheme.

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S-Face BC-810:

ACUTE DERMAL IRRITATION IN THE RABBIT

1. INTRODUCTION

The study was performed to assess the irritancy potential of the test material following single, 3-minute, 1 and 4-hour, semi-occluded applications to the intact rabbit skin. The method was designed to meet the requirements of the following:

- OECD Guidelines for the Testing of Chemicals No. 404 "Acute Dermal Irritation/Corrosion" (adopted 24 April 2002)
- Method B4 Acute Toxicity (Skin 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 skin irritancy potential of the test material to man.

The study was performed between 13 March 2008 and 22 March 2008.

2. TEST MATERIAL

2.1 Description, Identification and Storage Conditions

Sponsor's identification	:	S-Face BC-810
Description	:	extremely pale yellow solid
Batch number	:	80121
Date received	:	25 February 2008
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.

A Certificate of Analysis supplied by the Sponsor is given in Appendix 1.

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2.2 Preparation of Test Material

For the purpose of the study the test material was ground to a powder prior to application.

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
10% w/w aqueous preparation of the test material	6.6	6.7

3. METHODS

3.1 Animals and Animal Husbandry

Three New Zealand White rabbits were supplied by Harlan France SARL, 03800 GANNAT, France. 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.

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3.2 Procedure

On the day before the test each rabbit was clipped free of fur from the dorsal/flank area using veterinary clippers. Only animals with a healthy intact epidermis by gross observation were selected for the study.

One rabbit was initially treated. Three suitable sites were selected on the back of the rabbit. At each test site a quantity of 0.5 g of test material moistened with 0.5 ml of distilled water, was introduced under a 2.5 cm x 2.5 cm cotton gauze patch and placed in position on the shorn skin. Each patch was secured in position with a strip of surgical adhesive tape. To prevent the animal interfering with the patches, the trunk of the rabbit was wrapped in an elasticated corset for the duration of the exposure period.

One patch was removed at each of three time points: 3 minutes, 1 hour and 4 hours after application. Any residual test material was removed by gentle swabbing with cotton wool soaked in distilled water.

After consideration of the skin reactions produced in the first animal, two additional animals were treated with 0.5 g of test material moistened with 0.5 ml of distilled water. One patch was applied to the back of each rabbit, and was allowed to remain in contact with the skin for a period of four hours.

Approximately one hour following the removal of the patches, and 24, 48 and 72 hours later, the test sites were examined for evidence of primary irritation and scored according to the following scale:

EVALUATION OF SKIN REACTIONS

Erythema and Eschar Formation	**Value**
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beef redness) to eschar formation preventing grading of erythema	4

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Oedema Formation	Value
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

Any other skin reactions, if present, were also recorded.

3.3 Interpretation of Results

Calculation of Primary Irritation Index and Grading of Irritancy Potential Using the Draize Scheme

The scores for erythema and oedema at the 24 and 72-hour readings were totalled for the three test rabbits (12 values) and this total was divided by six to give the primary irritation index of the test material. The test material was classified according to the following scheme devised by Draize J H (1959) "Dermal Toxicity" In: Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics. Association of Food and Drug Officials of the United States, Austin, Texas, p.46-59:

Primary Irritation Index	Classification of Irritancy
0	Non-irritant
> 0 to 2	Mild irritant
> 2 to 5	Moderate irritant
> 5 to 8	Severe irritant

If irreversible alteration of the dermal tissue is noted in any rabbit, as judged by the Study Director, which include ulceration and clear necrosis or signs of scar tissue, the test material is classified as corrosive to rabbit skin. Classification according to Draize may, therefore, not be applicable.

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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.

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5. RESULTS

5.1 3-Minute Exposure Period

The individual scores for erythema/eschar and oedema are given in Table 1.

No evidence of skin irritation was noted during the study.

5.2 1-Hour Exposure Period

The individual scores for erythema/eschar and oedema are given in Table 1.

No evidence of skin irritation was noted during the study.

5.3 4-Hour Exposure Period

The individual scores for erythema/eschar and oedema are given in Table 2.

No evidence of skin irritation was noted during the study.

6. CONCLUSION

The test material produced a primary irritation index of 0.0 and was classified as NON-IRRITANT to rabbit skin according to the Draize classification scheme. No corrosive effects were noted.

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S-Face BC-810 : ACUTE DERMAL IRRITATION IN THE RABBIT

Table 1 Individual Skin Reactions Following 3-Minute and 1-Hour Exposures

Skin Reaction	Observation Time	Individual Scores - Rabbit Number and Sex	
		67283 Male	
		3-Minute Exposure	1-Hour Exposure
Erythema/Eschar Formation	1 Hour	0	0
	24 Hours	0	0
	48 Hours	0	0
	72 Hours	0	0
Oedema Formation	1 Hour	0	0
	24 Hours	0	0
	48 Hours	0	0
	72 Hours	0	0

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S-Face BC-810 : ACUTE DERMAL IRRITATION IN THE RABBIT

Table 2 Individual Skin Reactions Following 4-Hour Exposure

Skin Reaction	Observation Time	Individual Scores – Rabbit Number and Sex			Total
		67283 Male	67313 Male	67314 Male	
Erythema/Eschar Formation	1 Hour	0	0	0	(0)
	24 Hours	0	0	0	0
	48 Hours	0	0	0	(0)
	72 Hours	0	0	0	0
Oedema Formation	1 Hour	0	0	0	(0)
	24 Hours	0	0	0	0
	48 Hours	0	0	0	(0)
	72 Hours	0	0	0	0
Sum of 24 and 72-hour Readings (S)	:	0			
Primary Irritation Index (S/6)	:	0/6 = 0.0			
Classification	:	NON-IRRITANT			

( ) = Total values not used for calculation of primary irritation index

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Appendix 1 Certificate of Analysis

SAKAMOTO YAKUHIN KOGYO CO.,LTD.

HEAD OFFICE : 1-2-6 AWAJIMACHI,CHUO-KU,OSAKA CITY 541-0047,JAPAN
TEL (81)6-6231-1861 FAX (81)6-6222-0631
TOKYO BRANCH : 1-8-2 MARUNOUCHI,CHIYODA-KU,TOKYO 100-0005,JAPAN
TEL (81)3-3211-3813 FAX (81)3-3215-1789

date : Feb. 20, 2008

Product : S-Face BC-810
Lot. No. : 80121
Quantity : 100g× 1

Certificate of analysis / Quality

	Result
Description	A pale yellow solid with a faint characteristic odor
Color (Gardner)	3 ~ 4
Acid value (mgKOH/g)	3.9
Saponification value (mgKOH/g)	150.6
Heavy metal (ppm)	less than 20
Arsenic (ppm)	less than 2
Residue on ignition (%)	less than 0.5

Y. Noguchi
Yasunori Noguchi
Quality Control Manager

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Appendix 2 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

TEST FACILITY	TEST TYPE
SafePharm Laboratories Ltd. Shardlow Business Park Shardlow Derbyshire DE72 2GD	Analytical Chemistry Environmental Fate Environmental Toxicity Mutagenicity Phys/Chem testing Toxicology

DATE OF INSPECTION

21st August 2007

A general inspection for compliance with the Principles of Good Laboratory Practice was carried out at the above test facility 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.

15/10/07

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

MHRA

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To SAKAMOTO YAKUHIN KOGYO CO.,LTD.
I hereby certify that this document is a duplicate copy of the original issued as Nichimokyo No.19012-(3) on 11th July, 2007.

8th March, 2010

Prof. Dr. med. Mitsuyoshi Honda
Chairman of Directors
Japan Hair Science Association
(Incorporated Association)

Contract Study Report

This is the report sponsored study on the patch test of the cosmetic ingredient as you requested on 6th June, 2007. See Attachment for study results.

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Nichimokyo №19012-(3)
11th July, 2007

To SAKAMOTO YAKUHIN KOGYO CO.,LTD.

This report presents the results of patch test on a cosmetic ingredient performed by Japan Hair Science Association. It was sponsored by SAKAMOTO YAKUHIN KOGYO CO.,LTD. on 6th June, 2007. This report is an English version of the original, which was written in Japanese. The undersigned hereby declare that this version faithfully reflects the original report to the best of my knowledge.

TEST FOR IRRITATION EFFECTS OF COSMETIC INGREDIENT ON HUMAN SKIN

1.Purpose of the test

This patch test was performed to assess possible irritation potential of a cosmetic ingredient on healthy human skin, sponsored by SAKAMOTO YAKUHIN KOGYO CO.,LTD. on 6th June, 2007.

2.Test sample

The supplied sample is the following cosmetic ingredient.

Sample : 50%w/w S Face BC-810 (Lot.№70510) and liquid paraffin solution Polyglyceryl-8 Decabehenate / Caprate

Control①:Petrolatum(SUNWHITE P-1)

Control②:Soap(0.5%)

3.Test procedure

(1)Volunteers

16 males and 27 females (43 people in total), age 21 to 43, were selected as volunteers.

(2)Applied amount

0.01g of test sample was applied to each volunteer.

(3)Method of patch test

0.01g of test sample was placed on FINN CHAMBER(Epitest Ltd.·Oy)and applied to the crooked side of upper arm of each volunteer for 24 hours in occlusive condition to for evaluation.

At the end of 24 hours exposure period, the test sample was removed. Skin reactions were evaluated 1 hour and 24 hours after removing the test sample.

(4)Criteria for evaluation

The reaction was evaluated using the following criteria.

− :	No reaction
± :	Very slight erythema
+ :	Well-defined erythema
++ :	Erythema with swelling or with papule
+++ :	Blisters in addition to the above symptom
++++ :	Large blister

Takashi Nagashima, M.D., a Vice-chairmam of Directors of Japan Hair Science Association(Dermatologist) was in overall charge of the tests and observations reported herein.

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4.Results

The results of this test on 43 volunteers were as shown in the Table. In all volunteers, no reaction was observed in 1 hour after removing the test sample (shown in Table as 24-hours Evaluation time). Similarly, no reaction was observed in 24 hours after removing the test sample (shown in Table as 48-hours Evaluation time) in all volunteers.

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Table Results of patch test(1)

No.	Volunteer	Sex	Age	Evaluationtime(hrs.)	Sample	Control①	Control②	Note(anamnesis)
1	K. A.	F	42	24	–	–	–	Healthy
				48	–	–	–	
2	H. A.	M	46	24	–	–	–	Healthy
				48	–	–	–	
3	T. A.	M	29	24	–	–	–	Healthy
				48	–	–	–	
4	N. A.	M	37	24	–	–	–	Healthy
				48	–	–	–	
5	Y. I.	F	30	24	–	–	–	Healthy
				48	–	–	–	
6	M. I.	F	34	24	–	–	–	Healthy
				48	–	–	–	
7	Y. I.	M	45	24	–	–	–	Healthy
				48	–	–	–	
8	A. I.	F	22	24	–	–	–	Healthy
				48	–	–	–	
9	K. I.	F	36	24	–	–	–	Healthy
				48	–	–	–	
10	H. U.	F	34	24	–	–	–	Healthy
				48	–	–	–	
11	Y. O.	F	26	24	–	–	–	Healthy
				48	–	–	–	
12	S. O.	M	34	24	–	–	–	Healthy
				48	–	–	–	
13	M. O.	F	34	24	–	–	–	Healthy
				48	–	–	–	
14	E. O.	M	46	24	–	–	–	Healthy
				48	–	–	–	
15	A. K.	M	41	24	–	–	–	Healthy
				48	–	–	–	
16	N. K.	M	25	24	–	–	–	Healthy
				48	–	–	–	
17	Y. K.	F	31	24	–	–	–	Healthy
				48	–	–	–	
18	M. K.	M	50	24	–	–	–	Healthy
				48	–	–	–	
19	K. G.	F	37	24	–	–	–	Healthy
				48	–	–	–	
20	N. N.	M	33	24	–	–	–	Healthy
				48	–	–	–	
21	Y. K.	F	24	24	–	–	–	Healthy
				48	–	–	–	
22	I. S.	M	48	24	–	–	–	Healthy
				48	–	–	–	
23	M. S.	M	37	24	–	–	–	Healthy
				48	–	–	–	
24	K. T.	F	36	24	–	–	–	Healthy
				48	–	–	–	
25	S. T.	F	27	24	–	–	–	Healthy
				48	–	–	–	

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Table Results of patch test(2)

No.	Volunteer	Sex	Age	Evaluationtime(hrs.)	Sample	Control①	Control②	Note(anamnesis)
26	M. T.	M	48	24	–	–	–	Healthy
				48	–	–	–	
27	M. C.	F	26	24	–	–	–	Healthy
				48	–	–	–	
28	M. T.	F	28	24	–	–	–	Healthy
				48	–	–	–	
29	A. T.	F	30	24	–	–	–	Healthy
				48	–	–	–	
30	M. H.	F	34	24	–	–	–	Healthy
				48	–	–	–	
31	Y. F.	F	40	24	–	–	–	Healthy
				48	–	–	–	
32	K. F.	F	24	24	–	–	–	Healthy
				48	–	–	–	
33	A. M.	F	30	24	–	–	–	Healthy
				48	–	–	–	
34	T. M.	F	36	24	–	–	–	Healthy
				48	–	–	–	
35	T. M.	M	27	24	–	–	–	Healthy
				48	–	–	–	
36	R. M.	F	37	24	–	–	–	Healthy
				48	–	–	–	
37	T. M.	F	30	24	–	–	–	Healthy
				48	–	–	–	
38	H. M.	F	53	24	–	–	–	Healthy
				48	–	–	–	
39	T. M.	M	38	24	–	–	–	Healthy
				48	–	–	–	
40	S. M.	F	43	24	–	–	–	Healthy
				48	–	–	–	
41	Y. Y.	M	39	24	–	–	–	Healthy
				48	–	–	–	
42	M. W.	F	28	24	–	–	–	Healthy
				48	–	–	–	
43	K. W.	F	21	24	–	–	–	Healthy
				48	–	–	–	

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SafePharm Laboratories

S-Face BC-810:

Polyglyceryl-8 Decabehenate /Caprate

ACUTE EYE IRRITATION IN THE RABBIT

SPL PROJECT NUMBER: 0432/0103

AUTHOR: A Pooles

STUDY SPONSOR:

Sakamoto Yakuhin Kogyo Co., Ltd.
1-20, Rinkai-cho
Izumiotsu-shi
Osaka 595-0075
JAPAN

TEST FACILITY:

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

Telephone: +44 (0) 1332 792896

Facsimile: +44 (0) 1332 799018

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QUALITY ASSURANCE REPORT

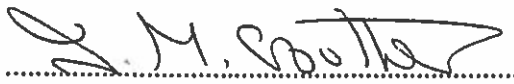
This study type is classed as short-term. The standard test method for this study type ("General Study Plan" in OECD terminology) was reviewed for compliance once only on initial production. Inspection of the routine and repetitive procedures that constitute the study is carried out as a continuous process designed to encompass the major phases at or about the time this study was in progress. In addition, inspection of general facilities not specifically related to this study are done monthly or annually in accordance with QA Standard Procedure.

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
	10 March 2008	Test Material Preparation
	03 March 2008	Animal Preparation
	04 March 2008	Dosing
	03 March 2008	Assessment of Response
§	01 May 2008	Draft Report Audit
§	Date of QA Signature	Final Report Audit

§ Evaluation specific to this study



DATE: 28 MAY 2008

For Safepharm Quality Assurance Unit*

*Authorised QA Signatures:

Head of Department:	JR Pateman CBiol MIBiol DipRQA ACQI FRQA
Deputy Head of Department:	JM Crowther MIScT MRQA
Senior Audit Staff:	G Wren ONC MRQA

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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.

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

A. Pooles DATE: 28/5/08

A Pooles BA (Hons)
Study Director

This report may be presented in final form as a digital (pdf) document. Such documents produced by Safepharm are prepared by scanning the paper original, and are considered of equivalent integrity and authenticity to versions produced by optical photocopy. However, in all cases the hand-signed paper original, held in secure archives, is the definitive document.

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S-Face BC-810:

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 iridial inflammation and moderate conjunctival irritation. All treated eyes appeared normal at the 48-hour observation.

Conclusion. The test material produced a maximum group mean score of 13.0 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.

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S-Face BC-810:

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 03 April 2008 and 12 April 2008.

2. TEST MATERIAL

2.1 Description, Identification and Storage Conditions

Sponsor's identification	:	S-Face BC-810
Description	:	extremely pale yellow solid
Batch number	:	80121
Date received	:	25 February 2008
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.

A Certificate of Analysis supplied by the Sponsor is given in Appendix 4.

2.2 Preparation of Test Material

For the purpose of the study the test material was ground to a powder before use.

The absorption of the test material was not determined.

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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
10% w/w aqueous preparation of the test material	6.6	6.7

3. METHODS

3.1 Animals and Animal Husbandry

Three New Zealand White rabbits were supplied by Harlan France SARL, 03800 Gannat, France. 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.

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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, which was found to weigh approximately 63 mg (as measured by gently compacting the required volume into an adapted syringe) 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:

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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.

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5. RESULTS

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

No corneal effects were noted during the study.

Iridial inflammation was noted in all treated eyes one hour after treatment.

Moderate conjunctival irritation was noted in all treated eyes one hour after treatment with minimal conjunctival irritation at the 24-hour observation.

All treated eyes appeared normal at the 48-hour observation.

6. CONCLUSION

The test material produced a maximum group mean score of 13.0 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	67344 Male				67362 Male				67364 Male			
	IPR = 2				IPR = 2				IPR = 3			
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	1	0	0	0	1	0	0	0	1	0	0	0
Score (D x 5)	5	0	0	0	5	0	0	0	5	0	0	0
CONJUNCTIVAE												
A = Redness	2	1	0	0	2	1	0	0	2	1	0	0
B = Chemosis	1	0	0	0	1	0	0	0	1	0	0	0
C = Discharge	1	0	0	0	1	0	0	0	1	0	0	0
Score (A + B + C) x 2	8	2	0	0	8	2	0	0	8	2	0	0
Total Score	13	2	0	0	13	2	0	0	13	2	0	0

IPR = Initial pain reaction

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S-Face BC-810 : 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
67344 Male	13	2	0	0
67362 Male	13	2	0	0
67364 Male	13	2	0	0
Group Total	39	6	0	0
Group Mean Score	13.0	2.0	0.0	0.0

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S-Face BC-810 : 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.

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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

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S-Face BC-810 : 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)

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Appendix 4 Certificate of Analysis

SAKAMOTO YAKUHIN KOGYO CO.,LTD.

HEAD OFFICE : 1-2-8 AWAJIMACHI,CHUO-KU,OSAKA CITY 541-0047,JAPAN
TEL (81)6-6231-1851 FAX (81)6-6222-0631
TOKYO BRANCH : 1-6-2 MARUNOUCHI,CHIYODA-KU,TOKYO 100-0005,JAPAN
TEL (81)3-3211-3813 FAX (81)3-3215-1789

date : Feb. 20, 2008

Product : S-Face BC-810
Lot. No. : 80121
Quantity : 100g × 1

Certificate of analysis / Quality

	Result
Description	A pale yellow solid with a faint characteristic odor
Color (Gardner)	3 ~ 4
Acid value (mgKOH/g)	3.9
Saponification value (mgKOH/g)	150.6
Heavy metal (ppm)	less than 20
Arsenic (ppm)	less than 2
Residue on ignition (%)	less than 0.5

Y. Noguchi
Yasunori Noguchi
Quality Control Manager

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Appendix 5 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

TEST FACILITY	TEST TYPE
SafePharm Laboratories Ltd. Shardlow Business Park Shardlow Derbyshire DE72 2GD	Analytical Chemistry Environmental Fate Environmental Toxicity Mutagenicity Phys/Chem testing Toxicology

DATE OF INSPECTION

21st August 2007

A general inspection for compliance with the Principles of Good Laboratory Practice was carried out at the above test facility 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.

15/10/07

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

MHRA

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S-FACE BC-810
Polyglyceryl-8 Decabehenate/Caprate
SKIN SENSITISATION IN THE GUINEA PIG - MAGNUSSON AND KLIGMAN MAXIMISATION METHOD

SPL PROJECT NUMBER: 0432/0104

Study n°: SMK-PH-08/0099

AUTHOR: F. Richeux

STUDY SPONSOR:

Sakamoto Yakuhin Kogyo Co., Ltd
1-20, Rinkai-cho
Izumiotsu-shi
Osaka 595-0075
JAPAN

STUDY MONITOR:

A. Pooles
Safepharm Laboratories Limited
Shardlow Business Park
Shardlow
Derbyshire
DE72 2GD
United Kingdom

TEST FACILITY:

Phycher Bio Développement
ZI de Toctoucau
18 Chemin de Lou Tribail
33611 CESTAS Cedex
FRANCE

Telephone: (33) 5 57 97 16 80

Facsimile: (33) 5 57 97 16 81

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QUALITY ASSURANCE REPORT

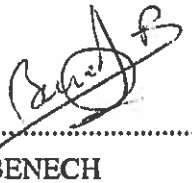
The routine inspections of the toxicity tests performed at Phycher Bio Développement are carried out as a continuous process designed to encompass the major phases of at least one similar test at or about the time this study was in progress.

This report has been audited by Phycher Bio Développement 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 within twenty-four hours of evaluation. Audits of study documentation, and process inspections appropriate to the type and schedule of this study were as follows:

§ 25 February 2008	Protocol Compliance Audit
30 January 2008	Main study: macroscopic examination at the reading time 24 hours
§ 06 May 2008	Draft Report Audit
§ 28 May 2008	Final Report Audit

§ Evaluation specific to this study



..... DATE: 28 May 2008

Bernard BENECH
Quality Assurance Manager

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GLP COMPLIANCE STATEMENT

I, the undersigned, François Richeux, Study director, certify that the study SMK-PH-08/0099 was performed on the premises of the Laboratory PHYCHER Bio Développement.

I, certify that the objectives laid down in the protocol were achieved and no undesirable event occurred to affect the quality or the integrity of the study. I consider the data generated to be valid. This report fully and accurately reflects the operating procedures used and data generated.

The entire work was devised and performed in compliance with the principles of the Good Laboratory Practice (G.L.P.), as defined in *the directive 2004/10/EC, the O.E.C.D. ruling relative to the mutual acceptance of data in the evaluation of chemical substances (C(81) 30 (final) Appendix 2 – May 12th , 1981; C (97) 186, November 26th, 1987),* and in the French decree n° 2006-1523 dated December 04th, 2006 of the Journal Officiel de la République Française (see certificate of G.L.P. in appendix 9).

..... DATE: 28 May 2008

François Richeux
Study Director
Expert Toxicologist (PhD, ERT)

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S-FACE BC-810:
SKIN SENSITISATION IN THE GUINEA PIG - MAGNUSSON AND KLIGMAN MAXIMISATION METHOD

SUMMARY

Introduction. The study was performed to assess the contact sensitisation potential of the test material in the albino guinea pig. The method was designed to meet the requirements of the following:

- OECD Guidelines for the Testing of Chemicals No. 406 "Skin Sensitisation" (adopted 17 July 1992)
- Method B6 Acute Toxicity (Skin Sensitisation) of Commission Directive 96/54/EC

Method. Ten test and five control animals were used for the main study. Two phases were involved in the main test; an induction of a response by intradermal injection and topical application and a topical challenge of that response.

Based on the results of sighting tests, the concentrations of test material for the topical induction and topical challenge phases were selected as:

Intradermal Induction	:	5% in olive oil
Topical Induction	:	60% in liquid paraffin
Topical Challenge	:	60% and 30% in liquid paraffin

Conclusion. Under the conditions of the test, the test material produced a 0% sensitisation rate and was classified as a non-sensitiser to guinea pig skin. The test material did not meet the criteria for classification as a sensitiser according to EU labelling regulations Commission Directive 2001/59/EC. No symbol and risk phrase are required.

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S-FACE BC-810:

SKIN SENSITISATION IN THE GUINEA PIG - MAGNUSSON AND KLIGMAN MAXIMISATION METHOD

1. INTRODUCTION

The study was performed to assess the contact sensitisation potential of the test material in the albino guinea pig. The method was designed to meet the requirements of the following:

- OECD Guidelines for the Testing of Chemicals No. 406 "Skin Sensitisation" (adopted 17 July 1992)
- Method B6 Acute Toxicity (Skin Sensitisation) of Commission Directive 96/54/EC

The albino guinea pig has been shown to be a suitable species for this type of study and is recommended in the test method. The strain used in these laboratories has been shown to produce satisfactory sensitisation responses using known positive sensitisers (see Appendix 8). The results of the study are believed to be of value in predicting the likely contact sensitisation potential of the test material to man.

The study was performed between 25 March 2008 and 24 April 2008.

2. TEST MATERIAL

2.1 Description, Identification and Storage Conditions

Sponsor's identification	:	S-FACE BC-810
Code number	:	PH-08/0099
Description	:	pale yellow solid
CAS number	:	-
Batch number	:	80121
Date received	:	12 March 2008
Storage conditions	:	room temperature

The integrity of supplied data relating to the identity, purity and stability of the test material is the responsibility of the Sponsor. A certificate of analysis is given in Appendix 10.

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2.2 Preparation of Test Material

For the purpose of this study the test material was freshly prepared in olive oil and liquid paraffin. The concentrations used are stated in the procedure section.

The test material has been reduced in fine powder and heated at 50°C.

The absorption of the test material was not determined.

Determination by analysis of the concentration, homogeneity and stability of the test material preparations was not appropriate because it was not specified in the Study Plan and is not a requirement of the Test Guideline.

3. METHODS

3.1 Animals and Animal Husbandry

Female albino Dunkin Hartley guinea pigs were supplied by Charles River (F-69592 L'Arbresle). Females were nulliparous and non-pregnant. After an acclimatisation period of at least five days, each animal was selected at random and identified individually by marking with picric acid and an ear tattoo. At the start of the main study the animals were in the weight range of 255 to 284 g and were four weeks old.

The animals were housed in groups of two or three in makrolon cages furnished with woodflakes. Free access to mains tap water and food (SDS, Essex UK, CM8 3AB) was allowed throughout the study. The diet, drinking water and bedding were routinely analysed and were considered not to contain any contaminant that could reasonably be expected to affect the purpose or integrity of the study.

The temperature and relative humidity were set to achieve limits of 20 to 23°C and 42 to 60% 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 ten changes per hour and the lighting was controlled by a time switch to give twelve hours continuous light (07:00 to 19:00) and twelve hours darkness.

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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

The method used for assessing the sensitising properties of the test material was based on the Guinea Pig Maximisation Test of Magnusson B & Kligman A M, (1969) J. Invest. Dermatol. **52**: 268 - 276.

3.2.1 Selection of Concentrations for Main Study (Sighting Tests)

The concentrations of test material to be used at each stage of the main study were determined by 'sighting tests' in which groups of guinea pigs were treated with various concentrations of test material. The procedures were as follows:

3.2.1.1 Selection of Concentration for Intradermal Induction

Intradermal injections (0.1 ml/injection site) were made on the clipped shoulder of two guinea pigs, at the concentrations of 5%, 2.5%, 1.25%, 0.625%, 0.3125% and 0.1562% by successive dilution (v/v) in olive oil. A macroscopic evaluation of the injection sites was conducted approximately 24 hours after injection to determine whether these concentrations caused necrosis. Any evidence of systemic toxicity was also recorded. The highest concentration which did not cause necrosis was selected for the intradermal induction stage of the main study. The results are given in Appendix 1.

The concentrations higher than 5% were not administrable by intradermal injection.

3.2.1.2 Selection of Concentration for Topical Induction

Two guinea pigs were treated with preparations of the test material diluted at 60% (w/w), 30% (w/w), 15% (w/w) and 7.5% (v/v) by successive dilution in liquid paraffin. Applications were made to the clipped flanks under occlusive dressings for an exposure period of 24 hours. The degree of erythema and oedema was evaluated approximately 24 hours after dressing removal. The highest concentration producing only moderate dermal irritation was selected for the topical induction stage of the main study. The results are given in Appendix 2.

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3.2.1.3 Selection of Concentration for Topical Challenge

Four preparations of the test material diluted at 60% (w/w), 30% (w/w), 15% (w/w) and 7.5% (v/v) by successive dilution in liquid paraffin were applied to the clipped flanks of three guinea pigs under occlusive dressings for an exposure period of 24 hours. These guinea pigs did not form part of the main study but had been treated identically to the control animals of the main study, up to Day 14. The degree of erythema and oedema was evaluated approximately 24 hours after dressing removal. The highest non-irritant concentration of the test material and one lower concentration were selected for the topical challenge stage of the main study. The results are given in Appendix 3.

3.2.2 Main Study

A group of fifteen guinea pigs was used for the main study, ten tests and five controls. The bodyweight of each animal was recorded at the start and the end of the study and are presented in Appendix 4.

Two phases were involved in the main study; (a) an induction of a response and (b) a challenge of that response.

3.2.2.1 Induction

Induction of the Test Animals: Shortly before treatment on Day 0 the hair was removed from an area approximately 40 mm x 60 mm on the shoulder region of each animal with veterinary clippers. A row of three injections (0.1 ml each) was made on each side of the spine. The injections were:

a) Freund's Complete Adjuvant plus isotonic sodium chloride in the ratio 1:1

b) 5% of the test material

c) a 5% formulation of the test material in a 1:1 preparation of Freund's Complete Adjuvant plus olive oil

On Day 6, the scapular region of all test and control animals was shaved and was brushed with a solution of sodium lauryl sulfate at 10% in thick vaseline, in order to create a local irritation.

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On Day 7 the same area on the shoulder region used previously for intradermal injections was clipped again and treated with a topical application of the test material formulation. A topical application of the test material at 60% under occlusive dressing was made. The occlusive dressing was kept in place for 48 hours.

The degree of erythema and oedema was evaluated approximately 24 hours after dressing removal. The results are given in Appendix 5

Induction of the Control Animals: On Day 0, the intradermal induction was performed using an identical procedure to that used for the test animals except that the test material was omitted from the intradermal injections. Injection b) was therefore the vehicle alone, injection c) was a 50% formulation of the vehicle in a 1:1 preparation of Freund's Complete Adjuvant plus isotonic sodium chloride. Similarly, on Day 7, the topical induction procedure was identical to that used for the test animals except that the test material was omitted.

The degree of erythema and oedema was evaluated approximately 24 hours after dressing removal. The results are given in Appendix 6.

3.2.2.2 Challenge

The flanks of each animal were previously clipped free of hair with veterinary clippers.

The test material formulation at the maximum non-irritant concentration (60% in liquid paraffin) was applied to one side of the shorn flank of each animal under an occlusive dressing. To ensure that the maximum non-irritant concentration was used at challenge, the test material at a concentration of 30% (v/v) in liquid paraffin was similarly applied under an occlusive dressing to the opposite skin site on the shorn flank.

After 24 hours, the dressing was carefully removed and discarded. The topical challenge sites were cleaned if required.

Prior to the 24-hour observation the flanks were clipped to remove regrown hair.

Approximately 24 and 48 hours after challenge dressing removal, the degree of erythema and oedema was quantified using the scale shown in Appendix 7.

Any other reactions were also recorded.

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3.3 Interpretation of Results

The percentage of test animals that showed a more severe reaction at the test material challenge site than the most severe reaction seen in the control animals, was compared with the following scale:

PERCENTAGE OF ANIMALS SENSITISED	CLASSIFICATION OF SENSITISATION POTENTIAL
0	Non sensitiser
> 0 - 8	Weak sensitiser
> 8 - 28	Mild sensitiser
>28 – 64	Moderate sensitiser
>64 - 80	Strong sensitiser
>80 - 100	Extreme sensitiser

Barely perceptible erythema (grade 1) is often a non-specific response to the dosing procedure and is not considered to be a significant or conclusive indication of delayed contact hypersensitivity. Furthermore, transient challenge reactions (those which do not persist for at least 48 hours) will not be attributed to contact sensitisation.

The results were evaluated according to Commission Directive 2001/59/EC for classification and labelling of dangerous substances.

4. ARCHIVES

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

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5. RESULTS

5.1 Preliminary Sighting Tests

5.1.1 Skin Reactions Observed After Intradermal Induction

Individual skin reactions at the intradermal induction sites of the preliminary sighting test animals are presented in Appendix 1.

No skin reactions were noted at the intradermal induction sites of the preliminary sighting test animals following treatment with the test material at the concentrations of 5%, 2.5%, 1.25%, 0.625%, 0.312% and 0.156%.

The concentration chosen for use in the Intradermal Induction phase of the main test was 5% (maximal non necrotising concentration (MNNC)).

5.1.2 Skin Reactions Observed After Topical Induction

Individual skin reactions at the topical induction sites of the preliminary sighting test animals are presented in Appendix 2.

No skin reactions were noted at the topical induction sites of the preliminary sighting test animals following treatment with the test material at concentrations of 60%, 30%, 15% and 7.5%.

The concentration chosen for use in the 2nd topical Induction of the main test was 60% and the 1st concentration chosen for the MNIC (maximal non irritant concentration) determination was 60%.

5.1.3 Skin Reactions Observed After Topical Challenge

Individual skin reactions at the topical challenge sites of the preliminary sighting test animals are presented in Appendix 3.

No skin reactions were noted at the topical challenge sites of the preliminary sighting test animals following treatment with the test material at concentrations of 60%, 30%, 15% and 7.5%.

The concentrations chosen for use in the Topical Challenge phase of the main test were 60% (maximal non irritant concentration (MNIC)) and 30% (1/2 MNIC) in liquid paraffin.

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5.2 Skin Reactions Observed After Topical Challenge

Individual skin reactions at the topical challenge sites of the test and control group animals are given in Table 1 and overall results are presented in Table 2.

60% in liquid paraffin

No skin reactions were noted at the topical challenge site of the test or control group animals at the 24 and 48-hour observations.

30% in liquid paraffin

No skin reactions were noted at the topical challenge site of the test or control group animals at the 24 and 48-hour observations.

6. CONCLUSION

Under the conditions of the test, the test material produced a 0% sensitisation rate and was classified as a non-sensitiser to guinea pig skin.

The test material did not meet the criteria for classification as a sensitiser according to EU labelling regulations Commission Directive 2001/59/EC. No symbol and risk phrase are required.

Table 1 Individual Skin Reactions at Topical Challenge

CHALLENGE CONCENTRATIONS: 60% and 30%

VEHICLE: liquid paraffin

Group	Animal Number	Skin Reactions (Hours after Removal of Dressings)											
		60%						30%					
		24 Hours			48 Hours			24 Hours			48 Hours		
		Er	Oe	Other	Er	Oe	Other	Er	Oe	Other	Er	Oe	Other
TEST	C9820	0	0	-	0	0	-	0	0	-	0	0	-
	C9821	0	0	-	0	0	-	0	0	-	0	0	-
	C9822	0	0	-	0	0	-	0	0	-	0	0	-
	C9823	0	0	-	0	0	-	0	0	-	0	0	-
	C9824	0	0	-	0	0	-	0	0	-	0	0	-
	C9825	0	0	-	0	0	-	0	0	-	0	0	-
	C9826	0	0	-	0	0	-	0	0	-	0	0	-
	C9827	0	0	-	0	0	-	0	0	-	0	0	-
	C9828	0	0	-	0	0	-	0	0	-	0	0	-
	C9829	0	0	-	0	0	-	0	0	-	0	0	-

Er = Erythema Oe = Oedema - = No other reactions noted

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Table 1 (continued) Individual Skin Reactions at Topical Challenge

CHALLENGE CONCENTRATIONS: **60% and 30%**

VEHICLE: **liquid paraffin**

Group	Animal Number	Skin Reactions (Hours after Removal of Dressings)											
		60%						30%					
		24 Hours			48 Hours			24 Hours			48 Hours		
		Er	Oe	Other	Er	Oe	Other	Er	Oe	Other	Er	Oe	Other
CONTROL	C9815	0	0	-	0	0	-	0	0	-	0	0	-
	C9816	0	0	-	0	0	-	0	0	-	0	0	-
	C9817	0	0	-	0	0	-	0	0	-	0	0	-
	C9818	0	0	-	0	0	-	0	0	-	0	0	-
	C9819	0	0	-	0	0	-	0	0	-	0	0	-

Er = Erythema Oe = Oedema - = No other reactions noted

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Table 2 Assessment of the Sensitising Potential following Topical Challenge

Group	Observation (Hours after Removal of Dressings)	Concentration	Number of Animals Showing Sensitisation Reactions				Positive Responses	Animals Sensitised
			0	1	2	3 or >		
Test	24	60%	10	0	0	0	0%	0%
	48	60%	10	0	0	0	0%	0%
	24	30%	10	0	0	0	0%	0%
	48	30%	10	0	0	0	0%	0%
Control	24	60%	5	0	0	0	0%	
	48	60%	5	0	0	0	0%	
	24	30%	5	0	0	0	0%	
	48	30%	5	0	0	0	0%	

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Appendix 1 Intradermal Sighting Test – Summary of Results

VEHICLE: **Olive oil**

Animal Identification	Concentration of Test Material (%)	Time of Observation	Grade of Erythema at Injection Sites
C9806	5	24 Hours	0
	2.5	24 Hours	0
	1.25	24 Hours	0
	0.625	24 Hours	0
	0.312	24 Hours	0
	0.156	24 Hours	0
C9807	5	24 Hours	0
	2.5	24 Hours	0
	1.25	24 Hours	0
	0.625	24 Hours	0
	0.312	24 Hours	0
	0.156	24 Hours	0

The concentration of the test material selected for the intradermal induction test of the main test was 5%

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Appendix 2 Topical Sighting Test for Topical Induction Application (24-Hour Exposure) – Individual Skin Reactions

VEHICLE: liquid paraffin

Animal Identification	Concentration of Test Material (%)	Skin Reactions (24 Hours After Removal of Patches)		
		Er	Oe	Other
C9806	60	0	0	-
	30	0	0	-
	15	0	0	-
	7.5	0	0	-
C9807	60	0	0	-
	30	0	0	-
	15	0	0	-
	7.5	0	0	-

The concentration of the test material selected for the main test topical induction was 60%

Er = Erythema Oe = Oedema - = No other reactions noted

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Appendix 3 Topical Sighting Test for Topical Challenge Application (24-Hour Exposure) – Individual Skin Reactions

VEHICLE: liquid paraffin

Animal Identification	Concentration of Test Material (%)	Skin Reactions (24 Hours After Removal of Patches)		
		Er	Oe	Other
C9809	60	0	0	-
	30	0	0	-
	15	0	0	-
	7.5	0	0	-
C9810	60	0	0	-
	30	0	0	-
	15	0	0	-
	7.5	0	0	-
C9811	60	0	0	-
	30	0	0	-
	15	0	0	-
	7.5	0	0	-

The concentrations of the test material selected for the main test topical challenge were 60% and 30% in liquid paraffin

Er = Erythema Oe = Oedema - = No other reactions noted

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Appendix 4 Individual Bodyweights and Bodyweight Gains

Group	Animal Number	Bodyweight (g)		Bodyweight (g) Increase
		Day 0	Day 23	
TEST	C9820	270	430	160
	C9821	280	440	160
	C9822	284	489	205
	C9823	255	416	161
	C9824	269	479	210
	C9825	259	425	166
	C9826	256	397	141
	C9827	256	384	128
	C9828	255	431	176
	C9829	257	431	174
	Mean	264.1	432.2	168.1
	Standard Deviation	11.0	32.3	25.3

Bodyweight increases of the guinea pigs in the test group between Day 0 and Day 23 were comparable to those noted in the control group over the same period.

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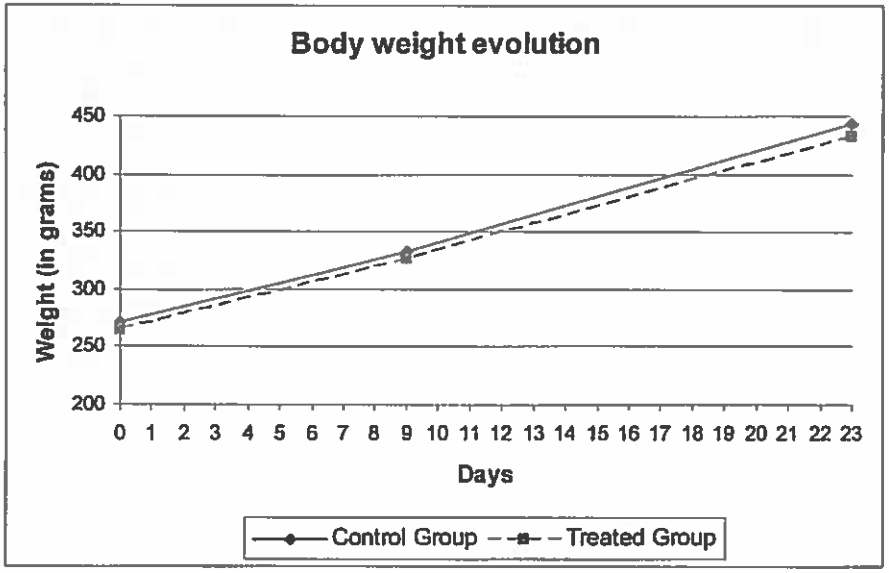
Appendix 4 (continued) Individual Bodyweights and Bodyweight Gains

Group	Animal Number	Bodyweight (g)		Bodyweight (g) Increase
		Day 0	Day 23	
CONTROL	C9815	273	439	166
	C9816	275	487	212
	C9817	276	420	144
	C9818	266	429	163
	C9819	266	445	179
	Mean	271.2	444.0	172.8
	Standard Deviation	4.9	25.9	25.2



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Appendix 5 Individual Skin Reactions in Test Animals during Induction Phase

INDUCTION CONCENTRATION: **1st induction: 5%**

2nd induction: 60%

Animal Number	Evaluation of Induction Sites on Day					
	1			10		
	Er	Oe	Other	Er	Oe	Other
C9820	0	0	-	0	0	-
C9821	0	0	-	0	0	-
C9822	0	0	-	0	0	-
C9823	0	0	-	0	0	-
C9824	0	0	-	0	0	-
C9825	0	0	-	0	0	-
C9826	0	0	-	0	0	-
C9827	0	0	-	0	0	-
C9828	0	0	-	0	0	-
C9829	0	0	-	0	0	-

Er = Erythema Oe = Oedema - = No other reactions noted

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Appendix 6 Individual Skin Reactions in Control Animals during Induction Phase

VEHICLE: **1st induction: olive oil**

2nd induction: liquid paraffin

Animal Number	Evaluation of Induction Sites on Day					
	1			10		
	Er	Oe	Other	Er	Oe	Other
C9815	0	0	-	0	0	-
C9816	0	0	-	0	0	-
C9817	0	0	-	0	0	-
C9818	0	0	-	0	0	-
C9819	0	0	-	0	0	-

Er = Erythema Oe = Oedema - = No other reactions noted

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S-FACE BC-810: SKIN SENSITISATION IN THE GUINEA PIG -MAGNUSSON AND KLIGMAN MAXIMISATION METHOD

Appendix 7 Scales for Evaluation of Skin Reactions

EVALUATION OF ERYTHEMA #	VALUE
No visible modification	0
Slight or patches of erythema	1
Moderate confluent erythema	2
Intense erythema and swelling	3

EVALUATION OF OEDEMA †	VALUE
No visible modification	0
Slight oedema	1
Moderate oedema	2
Severe oedema	3

From: Modified OECD Test Guideline 406, 1992 and Method B6 Skin Sensitisation of Commission Directive 96/54/EC.

† 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, p31.

Appendix 8 Summary of Positive Control Data for the Magnusson and Kligman Maximisation Study

Date Start	Test	Positive Control Material	1st induction	2nd Induction	Challenge Concentration	Observation (Hours after Removal of Dressings)	QUOTATION				Incidence of Sensitisation
							0	1	2	3	
16/01/2007	13	α-Hexylcinnamaldehyde CAS [101-86-0]	100%*	100%	50%	24	0	1	9	0	100%
						48	1	6	3	0	90%
					25%	24	0	2	8	0	100%
						48	3	7	0	0	70%
16/10/2007	14	α-Hexylcinnamaldehyde CAS [101-86-0]	50%	100%	50%	24	0	0	0	10	100%
						48	1	7	2	0	90%
					25%	24	0	0	1	9	100%
						48	0	7	3	0	100%
12/02/2008	15	α-Hexylcinnamaldehyde CAS [101-86-0]	0%	0%	50%	24	5	0	0	0	0%
						48	5	0	0	0	0%
					25%	24	5	0	0	0	0%
						48	5	0	0	0	0%
			50%	100%	50%	24	0	7	1	2	100%
						48	5	4	1	0	50%
					25%	24	1	3	4	2	90%
						48	4	5	1	0	60%

Conclusion: Based on these results, under the conditions of the test, the Positive control material, α-Hexylcinnamaldehyde, was classified sensitisers according to EU labelling regulations Commission Directive 2001/59/EC. The symbol "Xi", the indication of danger 'irritant' and risk phrase R 43 "May Cause Sensitisation by Skin Contact" are therefore required.

* Topical induction

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Appendix 9 Statement of GLP Compliance in Accordance with Directive 88/320/EEC

FRENCH REPUBLIC
INTERMINISTERIAL GROUP OF CHEMICALS (GIPC)

Paris, 21 February 2007

(Translated from the original version in French)

Subject: Evaluation of conformity to GLP according to the directive 88/320/EEC.

In the application of the 81-278 decree dated 25th March 1981 modified by the 90-206 decree dated 7th March 1990 and the 98-1312 decree dated 31 December 1998 concerning the Good Laboratory Practice, I confirm that, following the inspection visit made by the Comité Français d'Accréditation (COFRAC – Section Essais) in accordance with its usual procedures and upon your request, the GIPC was able to establish that the assays perform in your test facility comply with GLP.

Comptiance with the GLP principles.
Areas of expertise
- o **2 Toxicity studies**
- o **3 Mutagenicity studies**

Inspection date: 11 January 2007

- o **Periodical inspection (i.p.)**

Date of GIPC decision: 15 February 2007
Date of validity: 11 January 2007
Year of 1st validity: 1998
Period of validity: 18 months

PHYCHER BIO Developpement
ZI de Toctoucau
18, chemin de Lou Tribail
33611 CESTAS Cedex

President of the Interministerial
Group of Chemicals
Mr Pierre CREYSSEL

General Secretary of GIPC – DiGITIP – Simap – 12, rue Viliot – 75572 Paris Cedex 12
Tel: 01 53 44 96 10 Telecopy: 01 53 44 91 72

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Appendix 10 Certificate of analysis of the test material

SAKAMOTO YAKUHIN KOGYO CO.,LTD.

HEAD OFFICE : 1-2-8 AWAJIMACHI,CHUO-KU,OSAKA CITY 541-0047,JAPAN
TEL (81)6-6231-1851 FAX (81)6-6222-0631
TOKYO BRANCH : 1-8-2 MARUNOUCHI,CHIYODA-KU,TOKYO 100-0005,JAPAN
TEL (81)3-3211-3612 FAX (81)3-3218-1789

date : Feb. 20, 2008

Product : S-Face BC-810
Lot. No. : 80121
Quantity : 100g × 1

Certificate of analysis / Quality

	Result
Description	A pale yellow solid with a faint characteristic odor
Color (Gardner)	3 ~ 4
Acid value (mgKOH/g)	3.9
Saponification value (mgKOH/g)	150.6
Heavy metal (ppm)	less than 20
Arsenic (ppm)	less than 2
Residue on ignition (%)	less than 0.5

Y. Noguchi
Yasunori Noguchi
Quality Control Manager

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SafePharm Laboratories

S-Face BC-810:

Polyglyceryl-8 Decabehenate / Caprate

ACUTE ORAL TOXICITY IN THE RAT - FIXED DOSE METHOD

SPL PROJECT NUMBER: 0432/0101

AUTHOR: A Pooles

STUDY SPONSOR:

Sakamoto Yakuhin Kogyo Co., Ltd.
1-20, Rinkai-cho
Izumiotsu-shi
Osaka 595-0075
JAPAN

TEST FACILITY:

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

Telephone: +44 (0) 1332 792896

Facsimile: +44 (0) 1332 799018

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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. In addition, inspection of general facilities not specifically related to this study are done monthly or annually in accordance with QA Standard Procedure.

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:

	12 June 2007	Standard Test Method Compliance Audit
	02 April 2008	Test Material Preparation
	03 April 2008	Animal Preparation
	08 April 2008	Dosing
	08 April 2008	Assessment of Response
	08 April 2008	Necropsy
§	27 May 2008	Draft Report Audit
§	Date of QA Signature	Final Report Audit

§ Evaluation specific to this study

..... DATE: 27 JUN 2008

For Safepharm Quality Assurance Unit*

---

*Authorised QA Signatures:

Head of Department:	JR Pateman CBiol MIBiol DipRQA ACQI FRQA
Deputy Head of Department:	JM Crowther MIScT MRQA
Senior Audit Staff:	G Wren ONC MRQA

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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.

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

A. Poob DATE: 27/6/08

A Pooles BA (Hons)
Study Director

This report may be presented in final form as a digital (pdf) document. Such documents produced by Safepharm are prepared by scanning the paper original, and are considered of equivalent integrity and authenticity to versions produced by optical photocopy. However, in all cases the hand-signed paper original, held in secure archives, is the definitive document.

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S-Face BC-810:

ACUTE ORAL TOXICITY IN THE RAT - FIXED DOSE METHOD

SUMMARY

Introduction. The study was performed to assess the acute oral toxicity of the test material in the Sprague-Dawley CD strain rat. The method was designed to meet the requirements of the following:

- OECD Guidelines for Testing of Chemicals No 420 "Acute Oral Toxicity - Fixed Dose Method" (adopted 17 December 2001)
- Method B1 *bis* Acute Toxicity (Oral) of Commission Directive 2004/73/EC

Method. Following a sighting test at a dose level of 2000 mg/kg, an additional four fasted female animals were given a single oral dose of test material, as a suspension in arachis oil BP, at a dose level of 2000 mg/kg bodyweight. Clinical signs and bodyweight development were monitored during the study. All animals were subjected to gross necropsy.

Mortality. There were no deaths.

Clinical Observations. There were no signs of systemic toxicity.

Bodyweight. All animals showed expected gains in bodyweight.

Necropsy. No abnormalities were noted at necropsy.

Conclusion. The acute oral median lethal dose (LD_{50}) of the test material in the female Sprague-Dawley CD strain rat was estimated to be greater than 2000 mg/kg bodyweight (Globally Harmonised Classification System – Unclassified).

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S-Face BC-810:

ACUTE ORAL TOXICITY IN THE RAT - FIXED DOSE METHOD

1. INTRODUCTION

The study was performed to assess the acute oral toxicity of the test material in the Sprague-Dawley CD strain rat. The method was designed to meet the requirements of the following:

- OECD Guidelines for Testing of Chemicals No 420 "Acute Oral Toxicity - Fixed Dose Method" (adopted 17 December 2001)
- Method B1 *bis* Acute Toxicity (Oral) of Commission Directive 2004/73/EC

The test system was chosen because the rat 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 toxicity of the test material to man.

The study was performed between 03 April 2008 and 24 April 2008.

2. TEST MATERIAL AND EXPERIMENTAL PREPARATION

2.1 Description, Identification and Storage Conditions

Sponsor's identification	:	S-Face BC-810
Description	:	extremely pale yellow solid
Batch number	:	80121
Date received	:	25 February 2008
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.

A Certificate of Analysis supplied by the Sponsor is given in Appendix 3.

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2.2 Experimental Preparation

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

Determination by analysis of the concentration, homogeneity and stability of the test material preparations was not appropriate because it was not specified in the Study Plan and is not a requirement of the Test Guideline.

3. METHODS

3.1 Animals and Animal Husbandry

Female Sprague-Dawley CD (Crl : CD® (SD) IGS BR) strain rats were supplied by Charles River (UK) Ltd, Margate, Kent, UK. On receipt the animals were randomly allocated to cages. The females were nulliparous and non-pregnant. After an acclimatisation period of at least five days the animals were selected at random and given a number unique within the study by indelible ink-marking on the tail and a number written on a cage card. At the start of the study the animals were eight to twelve weeks of age. The bodyweight variation did not exceed ± 20% of the initial/mean bodyweight of any previously dosed animal(s).

The animals were housed in groups of up to four 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 (Certified Rat and Mouse Diet) was allowed throughout the study. The diet, drinking water and bedding were routinely analysed 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. 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.

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3.2 Procedure

Using available information on the toxicity of the test material, 2000 mg/kg was chosen as the starting dose.

Dose Level (mg/kg)	Concentration (mg/ml)	Dose Volume (ml/kg)	Number of Rats
			Female
2000	200	10	t

In the absence of toxicity at a dose level of 2000 mg/kg, an additional group of animals was treated as follows:

Dose Level (mg/kg)	Concentration (mg/ml)	Dose Volume (ml/kg)	Number of Rats
			Female
2000	200	10	4

A total of five animals were therefore treated at a dose level of 2000 mg/kg in the study.

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 its fasted bodyweight at the time of dosing.

Clinical observations were made ½, 1, 2, and 4 hours after dosing and subsequently once daily for fourteen days. Morbidity and mortality checks were made twice daily.

Individual bodyweights were recorded on Day 0 (the day of dosing) and on Days 7 and 14.

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

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3.3 Evaluation of Data

The test material will be classified according to Annex 3 of the OECD Guidelines for Testing of Chemicals No. 420 "Acute Oral Toxicity - Fixed Dose Method" (adopted 17 December 2001) as shown in the Flow Chart in Appendix 2.

Evaluation of data included identification of the number of animals that died during the study (or that were killed for humane reasons), and determination of the nature, severity, onset and duration of the toxic effects. If possible, the signs of evident toxicity were described. Evident toxicity refers to the toxic effects of sufficient severity that administration of the next higher dose level could result in development of severe signs of toxicity and probable mortality. Effects on bodyweights and abnormalities noted at necropsy were also identified.

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

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.

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5. RESULTS

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

5.1 Mortality

There were no deaths.

5.2 Clinical Observations

No signs of systemic toxicity were noted.

5.3 Bodyweight

Individual bodyweights and bodyweight changes are given in Table 2.

All animals showed expected gains in bodyweight over the observation period.

5.4 Necropsy

Individual necropsy findings are given in Table 3.

No abnormalities were noted at necropsy.

6. CONCLUSION

The acute oral median lethal dose (LD_{50}) of the test material in the female Sprague-Dawley CD strain rat was estimated to be greater than 2000 mg/kg bodyweight (Globally Harmonised Classification System – Unclassified).

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
2000	1-0 Female	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2-0 Female	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2-1 Female	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2-2 Female	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2-3 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

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Table 2 Individual Bodyweights and Bodyweight Changes

Dose Level mg/kg	Animal Number and Sex	Bodyweight (g) at Day			Bodyweight Gain (g) During Week	
		0	7	14	1	2
2000	1-0 Female	202	223	251	21	28
	2-0 Female	215	236	259	21	23
	2-1 Female	215	236	273	21	37
	2-2 Female	227	254	278	27	24
	2-3 Female	208	252	282	44	30

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Table 3 Individual Necropsy Findings

Dose Level mg/kg	Animal Number and Sex	Time of Death	Macroscopic Observations
2000	1-0 Female	Killed Day 14	No abnormalities detected
	2-0 Female	Killed Day 14	No abnormalities detected
	2-1 Female	Killed Day 14	No abnormalities detected
	2-2 Female	Killed Day 14	No abnormalities detected
	2-3 Female	Killed Day 14	No abnormalities detected

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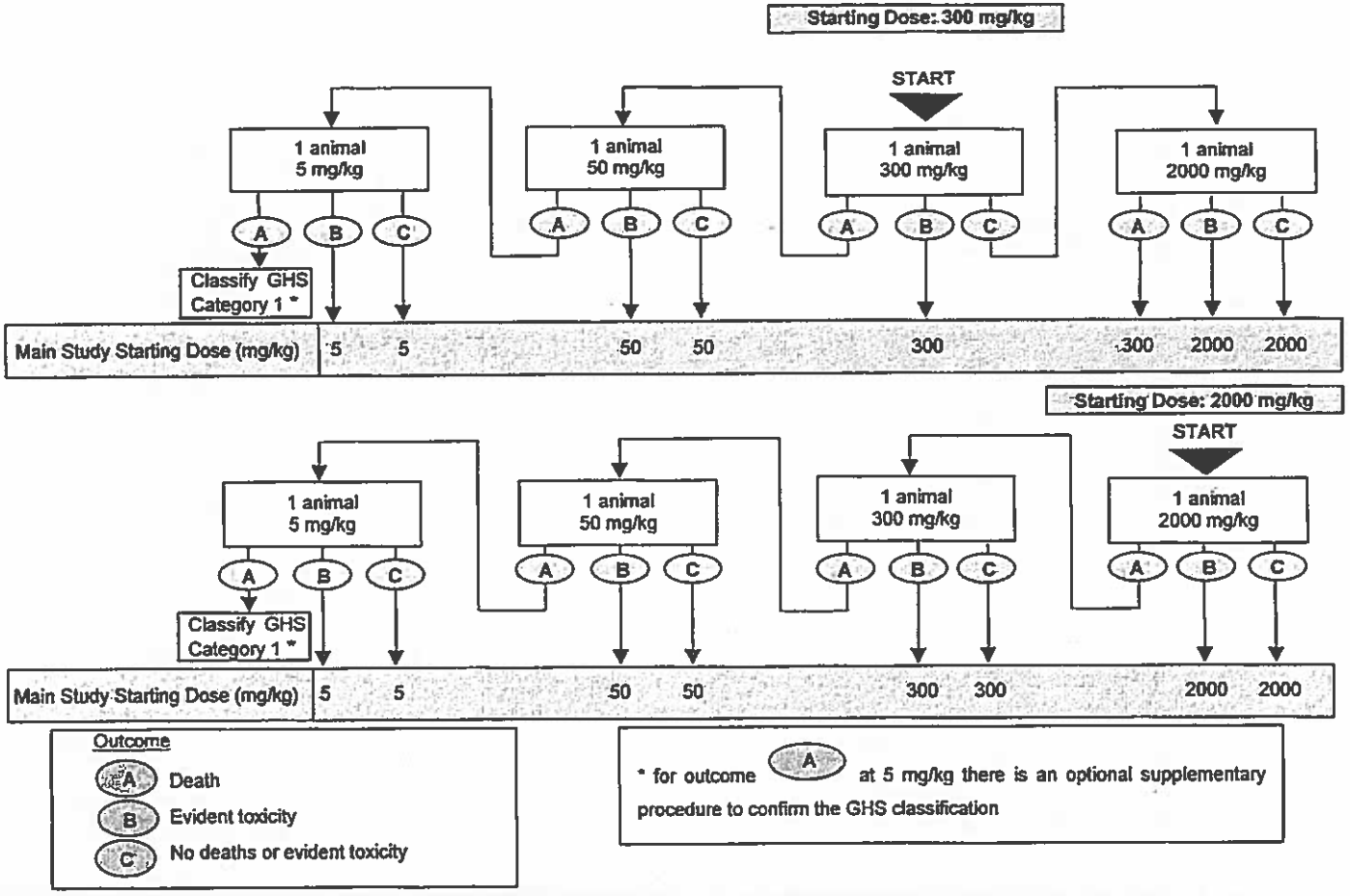
Appendix 1 Flow Chart for the Sighting Test



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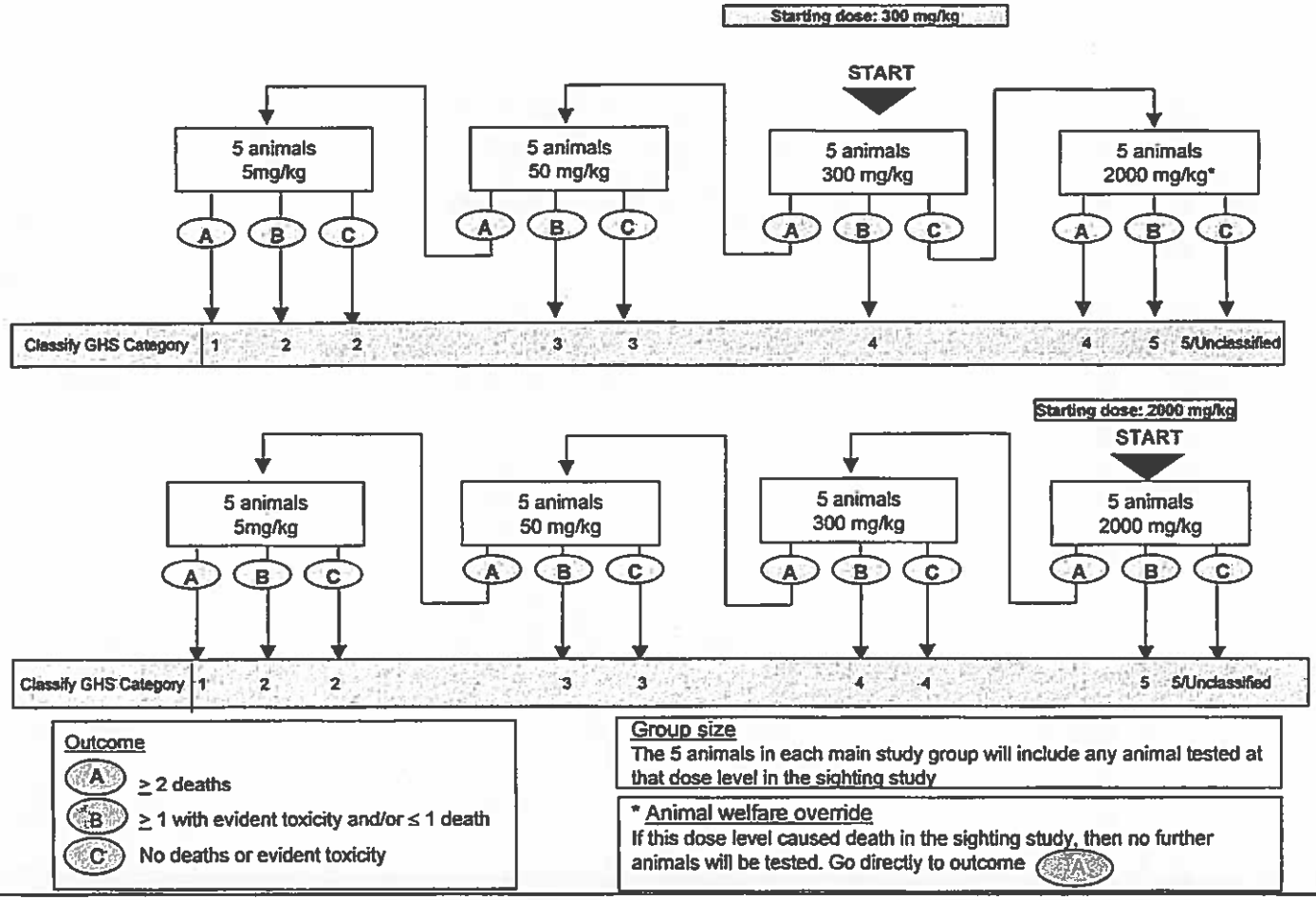
Appendix 2 Flow Chart for the Main Test



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Appendix 3 Certificate of Analysis

SAKAMOTO YAKUHIN KOGYO CO.,LTD.

HEAD OFFICE : 1-2-8 AWAJIMACHI,CHUO-KU,OSAKA CITY 541-0047,JAPAN
TEL (81)6-6231-1851 FAX (81)6-6222-0631
TOKYO BRANCH : 1-8-2 MARUNOUCHI,CHIYODA-KU,TOKYO 100-0005,JAPAN
TEL (81)3-3211-3613 FAX (81)3-3211-1789

date : Feb. 20, 2008

Product : S-Face BC-810
Lot. No. : 80121
Quantity : 100g × 1

Certificate of analysis / Quality	Result
Description	A pale yellow solid with a faint characteristic odor
Color (Gardner)	3 ~ 4
Acid value (mgKOH/g)	3.9
Saponification value (mgKOH/g)	150.6
Heavy metal (ppm)	less than 20
Arsenic (ppm)	less than 2
Residue on ignition (%)	less than 0.5

Y. Noguchi
Yasunori Noguchi
Quality Control Manager

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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

TEST FACILITY	TEST TYPE
SafePharm Laboratories Ltd. Shardlow Business Park Shardlow Derbyshire DE72 2GD	Analytical Chemistry Environmental Fate Environmental Toxicity Mutagenicity Phys/Chem testing Toxicology

DATE OF INSPECTION

21st August 2007

A general inspection for compliance with the Principles of Good Laboratory Practice was carried out at the above test facility 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.

15/10/07

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

MHRA

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Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: Lillian Gill, D.P.A.
Director - COSMETIC INGREDIENT REVIEW (CIR)

FROM: Beth A. Lange, Ph.D.
Industry Liaison to the CIR Expert Panel

DATE: March 25, 2016

SUBJECT: Information on Polyglyceryl-8 Decaerucate/Decaisostearate/Decaricinoleate (SLR posted January 28, 2016)

SafePharm Laboratories. 2003. Acute dermal irritation in the rabbit (Polyglyceryl-8 Decaerucate/Decaisostearate/Decaricinoleate).

SafePharm Laboratories. 2003. Skin senistization in the guinea pig - Magnusson and Kligman maximisation method (Polyglyceryl-8 Decaerucate/Decaisostearate/Decaricinoleate).

SafePharm Laboratories. 2003. Acute eye irritation in the rabbit (Polyglyceryl-8 Decaerucate/Decaisostearate/Decaricinoleate).

SafePharm Laboratories. 2003. Acute oral toxicity in the rat - acute toxic class method (Polyglyceryl-8 Decaerucate/Decaisostearate/Decaricinoleate).

SafePharm Laboratories. 2003. Reverse mutation assy "Ames test" using *Salmonella typhimurium* and *Escherichia coli* (Polyglyceryl-8 Decaerucate/Decaisostearate/Decaricinoleate).

1620 L Street, N.W., Suite 1200 | Washington, D.C. 20036 | 202.331.1770 | 202.331.1969 (fax) | www.personalcarecouncil.org

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SafePharm Laboratories

S-FACE MX-10:
Polyglyceryl-8 Decaerucate / Deca isostearate / Deca ricinol
ACUTE DERMAL IRRITATION
IN THE RABBIT

SPL PROJECT NUMBER: 432/053

AUTHOR: R Driscoll

STUDY SPONSOR:

Sakamoto Yakuhin Kogyo Co., Ltd.
1-20, Rinkai-cho
Izumiotsu-city
OSAKA 595-0075
JAPAN

TEST FACILITY:

Safepharm Laboratories Limited
P.O. Box No. 45
DERBY
DE1 2BT
U.K.

Telephone: (01332) 792896

Facsimile: (01332) 799018

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QUALITY ASSURANCE REPORT

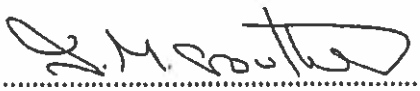
This study type is classed as short-term. The standard test method for this study type ("General Study Plan" in OECD terminology) was reviewed for compliance once only on initial production. Inspection of the routine and repetitive procedures that constitute the study is carried out as a continuous process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

	28 March 2002	Standard Test Method Compliance Audit
	22 October 2002	Test Material Preparation
	14 October 2002	Animal Preparation
	03 October 2002	Dosing
	11 October 2002	Assessment of Response
§	27 November 2002	Draft Report Audit
§	Date of QA Signature	Final Report Audit

§ Evaluation specific to this study



..... DATE: 06 JAN 2003

For Safepharm Quality Assurance Unit*

*Authorised QA Signatures:

Head of Department:	JR Pateman CBiol MIBiol DipRQA FRQA
Deputy Head of Department:	JM Crowther MIScT MRQA
Senior Audit Staff:	JV Johnson BSc MRQA; G Wren ONC MRQA

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GLP COMPLIANCE STATEMENT

The work described was performed in compliance with UK GLP standards (Schedule 1, Good Laboratory Practice Regulations 1999 (SI 1999/3106)). 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 87/18/EEC (as amended by Directive 1999/11/EC) and 88/320/EEC (as amended by Directive 1999/12/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, 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: 03 JAN 2003

R Driscoll BTech (Hons)
Study Director

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S-FACE MX-10:

ACUTE DERMAL IRRITATION IN THE RABBIT

SUMMARY

Introduction. The study was performed to assess the irritancy potential of the test material to the skin 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. 404 "Acute Dermal Irritation/Corrosion" (adopted 17 July 1992)
- Commission Directive 92/69/EEC Method B4 Acute Toxicity (Skin Irritation)

Results. A single 4-hour, semi-occluded application of the test material to the intact skin of three rabbits produced no evidence of skin irritation.

Conclusion. The test material produced a primary irritation index of 0.0 and was classified as non-irritant to rabbit skin according to the Draize classification scheme. No corrosive effects were noted.

The test material did not meet the criteria for classification as irritant or corrosive according to the EU labelling regulations Commission Directive 2001/59/EC.

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S-FACE MX-10:

ACUTE DERMAL IRRITATION IN THE RABBIT

1. INTRODUCTION

The study was performed to assess the irritancy potential of the test material following a single, 4-hour, semi-occluded application to the intact rabbit skin. The method was designed to meet the requirements of the following:

- OECD Guidelines for the Testing of Chemicals No. 404 "Acute Dermal Irritation/Corrosion" (adopted 17 July 1992)
- Commission Directive 92/69/EEC Method B4 Acute Toxicity (Skin Irritation)

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 skin irritancy potential of the test material to man.

The study was performed between 01 October 2002 and 04 October 2002.

2. TEST MATERIAL

2.1 Description, Identification and Storage Conditions

Sponsor's identification	:	S-FACE MX-10
Description	:	pale yellow viscous liquid
Batch number	:	206103
Date received	:	02 August 2002
Storage conditions	:	room temperature in the dark

Data relating to the identity, purity and stability of the test material are the responsibility of the Sponsor.

A Certificate of Analysis supplied by the Sponsor is given in Appendix 2.

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.

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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
Undiluted as Supplied	approximately 5.0
90% v/v aqueous preparation of the test material	approximately 5.5

3. METHODS

3.1 Animals and Animal Husbandry

Three New Zealand White rabbits were supplied by David Percival Ltd, Moston, Sandbach, Cheshire, UK. At the start of the study the animals were in the weight range of 2.0 to 3.5 kg and were twelve to sixteen 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 metal cages. Free access to mains drinking water and food (Certified Rabbit Diet (Code 5322) supplied by International Product Supplies Limited, Wellingborough, Northants, UK) 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

On the day before the test each of a group of three rabbits was clipped free of fur from the dorsal/flank area using veterinary clippers. Only animals with a healthy intact epidermis by gross observation were selected for the study.

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On the day of the test a suitable test site was selected on the back of each rabbit. A quantity of 0.5 ml of the test material was introduced under a 2.5 cm x 2.5 cm cotton gauze patch and placed in position on the shorn skin. The patch was secured in position with a strip of surgical adhesive tape. To prevent the animals interfering with the patches, the trunk of each rabbit was wrapped in an elasticated corset and the animals were returned to their cages for the duration of the exposure period.

Four hours after application the corset and patches were removed from each animal and any residual test material removed by gentle swabbing with cotton wool soaked in 74% Industrial Methylated Spirits.

Approximately one hour following the removal of the patches, and 24, 48 and 72 hours later, the test sites were examined for evidence of primary irritation and scored according to the following scale:

EVALUATION OF SKIN REACTIONS

Erythema and Eschar Formation	**Value**
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	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

Any other skin reactions, if present, were also recorded.

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3.3 Interpretation of Results

Calculation of Primary Irritation Index and Grading of Irritancy Potential Using the Draize Scheme

The scores for erythema and oedema at the 24 and 72-hour readings were totalled for the three test rabbits (12 values) and this total was divided by six to give the primary irritation index of the test material. The test material was classified according to the following scheme devised by Draize J H (1959) "Dermal Toxicity" In: Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics. Association of Food and Drug Officials of the United States, Austin, Texas, p.47:

Primary Irritation Index	Classification of Irritancy
0	Non-irritant
> 0 to 2	Mild irritant
> 2 to 5	Moderate irritant
> 5 to 8	Severe irritant

If irreversible alteration of the dermal tissue is noted in any rabbit, as judged by the Study Director, which include ulceration and clear necrosis or signs of scar tissue, the test material is classified as corrosive to rabbit skin. Classification according to Draize may, therefore, not be applicable.

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.

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5. RESULTS

The individual scores for erythema/eschar and oedema, are given in Table 1.

No evidence of skin irritation was noted during the study.

6. CONCLUSION

The test material produced a primary irritation index of 0.0 and was classified as NON-IRRITANT to rabbit skin according to the Draize classification scheme. No corrosive effects were noted.

The test material did not meet the criteria for classification as irritant or corrosive according to the EU labelling regulations Commission Directive 2001/59/EC.

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S-FACE MX-10 : ACUTE DERMAL IRRITATION IN THE RABBIT

Table 1 Individual Skin Reactions

Skin Reaction	Observation Time	Individual Scores – Rabbit Number and Sex			Total
		50 Male	51 Male	52 Male	
Erythema/Eschar Formation	1 Hour	0	0	0	(0)
	24 Hours	0	0	0	0
	48 Hours	0	0	0	(0)
	72 Hours	0	0	0	0
Oedema Formation	1 Hour	0	0	0	(0)
	24 Hours	0	0	0	0
	48 Hours	0	0	0	(0)
	72 Hours	0	0	0	0
Sum of 24 and 72-hour Readings (S)	:	0			
Primary Irritation Index (S/6)	:	0/6 = 0.0			
Classification	:	NON-IRRITANT			

( ) = Total values not used for calculation of primary irritation index

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Appendix 1 Statement of GLP Compliance in Accordance with Directive 88/320/EEC

THE DEPARTMENT OF HEALTH OF THE GOVERNMENT OF THE UNITED KINGDOM

GOOD LABORATORY PRACTICE

STATEMENT OF COMPLIANCE
IN ACCORDANCE WITH DIRECTIVE 88/320 EEC

LABORATORY

SafePharm Laboratories Ltd
Shardlow Business Park
London Road
Shardlow
Derbyshire
DE72 2GD

TEST TYPE

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

DATE OF INSPECTION
28 February 2000

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

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

Roger G. Alexander
26/4/00

Dr. Roger G. Alexander
Head, UK GLP Monitoring Authority

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Appendix 2 Certificate of Analysis

SAKAMOTO YAKUHIN KOGYO CO.,LTD.

HEAD OFFICE : 1-2-4 AWAJIMACHI,CHUO-KU,OSAKA CITY 541-0047,JAPAN
TEL (81)6-6231-1851 FAX (81)6-6222-0631
TOKYO BRANCH : 1-6-2 MARUNOUCHI,CHIYODA-KU,TOKYO 100-0005,JAPAN
TEL (81)3-3211-3813 FAX (081)3-3215-1788

date : Jul. 30 , 2002

Product : S-Face MX-10
Lot. No. : 206103
Quantity : 100g × 2

Certificate of analysis / Quality

	Result
Description	A pale yellow viscous liquid with a faint characteristic odor
Color (Gardner)	1
Acid value (mgKOH/g)	1.3
Saponification value (mgKOH/g)	157.0
Heavy metal (ppm)	less than 20
Arsenic (ppm)	less than 2
Residue on ignition (%)	less than 0.2

J. Noguchi
Yasunori Noguchi
Quality Control Chief

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SAFEPHARM LABORATORIES LTD

S-FACE MX-10:

ACUTE DERMAL IRRITATION IN THE RABBIT

SPL PROJECT NUMBER: 432/053

I verify that this is an exact copy of the original report which is located in the Archives of Safepharm Laboratories Ltd., Derby, UK.

..... DATE: 09 JAN 2003

R Driscoll BTech (Hons)
Study Director

Distributed for Comment Only -- Do Not Cite or Quote

SafePharm Laboratories

S-FACE MX-10:
Polyglyceryl-8 Decaerucate/Decaisostearate/Decaricinolea
SKIN SENSITISATION IN THE GUINEA PIG - MAGNUSSON AND KLIGMAN MAXIMISATION METHOD

SPL PROJECT NUMBER: 432/055

AUTHOR: R Driscoll

STUDY SPONSOR:

Sakamoto Yakuhin Kogyo Co., Ltd.
1-20, Rinkai-cho
Izumiotsu-city
OSAKA 595-0075
JAPAN

TEST FACILITY:

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

Telephone: +44 (0) 1332 792896

Facsimile: +44 (0) 1332 799018

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QUALITY ASSURANCE REPORT

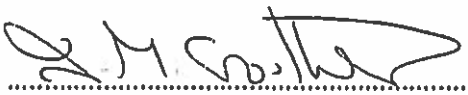
This study type is classed as short-term. The standard test method for this study type ("General Study Plan" in OECD terminology) was reviewed for compliance once only on initial production. Inspection of the routine and repetitive procedures that constitute the study is carried out as a continuous process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

	28 March 2002	Standard Test Method Compliance Audit
	23 October 2002	Test Material Preparation
	24 October 2002	Animal Preparation
	14 October 2002	Dosing
	30 October 2002	Assessment of Response
§	09 December 2002	Draft Report Audit
§	Date of QA Signature	Final Report Audit

§ Evaluation specific to this study



..... DATE: 06 JAN 2003 .....

For Safepharm Quality Assurance Unit*

*Authorised QA Signatures:

Head of Department:	JR Pateman CBiol MIBiol DipRQA FRQA
Deputy Head of Department:	JM Crowther MIScT MRQA
Senior Audit Staff:	JV Johnson BSc MRQA; G Wren ONC MRQA

Distributed for Comment Only -- Do Not Cite or Quote

GLP COMPLIANCE STATEMENT

The work described was performed in compliance with UK GLP standards (Schedule 1, Good Laboratory Practice Regulations 1999 (SI 1999/3106)). 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 87/18/EEC (as amended by Directive 1999/11/EC) and 88/320/EEC (as amended by Directive 1999/12/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, 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.

R Driscoll ..... DATE: 03 JAN 2003

R Driscoll BTech (Hons)
Study Director

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S-FACE MX-10:

SKIN SENSITISATION IN THE GUINEA PIG - MAGNUSSON AND KLIGMAN MAXIMISATION METHOD

SUMMARY

Introduction. The study was performed to assess the contact sensitisation potential of the test material in the albino guinea pig. The method was designed to meet the requirements of the following:

- OECD Guidelines for the Testing of Chemicals No. 406 "Skin Sensitisation" (adopted 17 July 1992)
- Commission Directive 96/54/EC Method B6 Acute Toxicity (Skin Sensitisation)

Method. Ten test and five control animals were used for the study. Two phases were involved in the main study; an induction of a response by intradermal injection and topical application and a topical challenge of that response.

Based on the results of sighting tests, the concentrations of test material for the induction and challenge phases were selected as:

Intradermal Induction	:	5% v/v in arachis oil BP
Topical Induction	:	undiluted as supplied
Topical Challenge	:	undiluted as supplied and 75% v/v in arachis oil BP

Conclusion. Under the conditions of the test, the test material produced a 0% (0/10) sensitisation rate and was classified as a non-sensitiser to guinea pig skin.

The test material did not meet the criteria for classification as a sensitiser according to EU labelling regulations Commission Directive 2001/59/EC. No symbol and risk phrase are required.

Distributed for Comment Only -- Do Not Cite or Quote

S-FACE MX-10:

SKIN SENSITISATION IN THE GUINEA PIG - MAGNUSSON AND KLIGMAN MAXIMISATION METHOD

1. INTRODUCTION

The study was performed to assess the contact sensitisation potential of the test material in the albino guinea pig. The method was designed to meet the requirements of the following:

- OECD Guidelines for the Testing of Chemicals No. 406 "Skin Sensitisation" (adopted 17 July 1992)
- Commission Directive 96/54/EC Method B6 Acute Toxicity (Skin Sensitisation)

The albino guinea pig has been shown to be a suitable species for this type of study and is recommended in the test method. The strain used in these laboratories has been shown to produce satisfactory sensitisation responses using known positive sensitisers (see Appendix 8). The results of the study are believed to be of value in predicting the likely contact sensitisation potential of the test material to man.

The study was performed between 19 September 2002 and 02 November 2002.

2. TEST MATERIAL

2.1 Description, Identification and Storage Conditions

Sponsor's identification	:	S-FACE MX-10
Description	:	pale yellow viscous liquid
Batch number	:	206103
Date received	:	02 August 2002
Storage conditions	:	room temperature in the dark

Data relating to the identity, purity and stability of the test material are the responsibility of the Sponsor.

A Certificate of Analysis supplied by the Sponsor is given in Appendix 10.

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2.2 Preparation of Test Material

For the purpose of this study the test material was used undiluted and freshly prepared in arachis oil BP. The concentrations used are stated in the procedure section.

The absorption of the test material was not determined.

Determination by analysis of the concentration, homogeneity and stability of the test material preparations was not appropriate because it was not specified in the Study Plan and is not a requirement of the Test Guideline.

3. METHODS

3.1 Animals and Animal Husbandry

Twenty-two male albino Dunkin Hartley guinea pigs were supplied by David Hall Limited, Burton-on-Trent, Staffordshire, UK. After an acclimatisation period of at least five days, each animal was selected at random and given a number unique within the study which was written on a small area of clipped rump using a black indelible marker-pen. At the start of the main study the animals were in the weight range of 261 to 403g, and were eight to twelve weeks old. All control group animals were below the weight specified in the Standard Test Method (300g). This deviation was considered not to affect the purpose or integrity of the study.

The animals were housed singly or in pairs in solid-floor polypropylene cages furnished with woodflakes. Free access to mains tap water and food (Certified Guinea Pig Diet (Code 5026) supplied by International Product Supplies Limited, Wellingborough, Northants, UK) was allowed throughout the study. The diet, drinking water and bedding were routinely analysed and were considered not to contain any contaminant that could reasonably be expected to affect 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.

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3.2 Procedure

The method used for assessing the sensitising properties of the test material was based on the Guinea Pig Maximisation Test of Magnusson B & Kligman A M, J. Invest. Dermatol. (1969) 52: 268 - 276.

3.2.1 Selection of Concentrations for Main Study (Sighting Tests)

The concentrations of test material to be used at each stage of the main study were determined by 'sighting tests' in which groups of guinea pigs were treated with various concentrations of test material. The procedures were as follows:

3.2.1.1 Selection of Concentration for Intradermal Induction

Intradermal injections (0.1 ml/injection site) were made on the clipped shoulder of two guinea pigs, using concentrations of 1% and 5% v/v in arachis oil BP. The degree of erythema at the injection sites was assessed approximately 24, 48, 72 hours and 7 days after injection according to the scale shown in Appendix 7. The degree of oedema was not evaluated. Any evidence of systemic toxicity was also recorded. The highest concentration that caused only mild to moderate skin irritation, and which was well tolerated systemically, was selected for the intradermal induction stage of the main study. The results are given in Appendix 1.

3.2.1.2 Selection of Concentration for Topical Induction

Two guinea pigs (intradermally injected with Freund's Complete Adjuvant fifteen days earlier) were treated with the undiluted test material and three preparations of the test material (75%, 50% and 25% v/v in arachis oil BP). Applications were made to the clipped flanks under occlusive dressings for an exposure period of 48 hours. The degree of erythema and oedema was evaluated approximately 1, 24 and 48 hours after dressing removal. The highest concentration producing only mild to moderate dermal irritation was selected for the topical induction stage of the main study. The results are given in Appendix 2.

3.2.1.3 Selection of Concentration for Topical Challenge

The undiluted test material and three preparations of the test material (75%, 50% and 25% v/v in arachis oil BP) were applied to the clipped flanks of two guinea pigs under occlusive dressings for an exposure period of 24 hours. These guinea pigs did not form part of the main study but had been treated identically to the control animals of the main study, up to Day 14. The degree of

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erythema and oedema was evaluated approximately 1, 24 and 48 hours after dressing removal. The highest non-irritant concentration of the test material and one lower concentration were selected for the topical challenge stage of the main study. The results are given in Appendix 3.

3.2.2 Main Study

A group of fifteen guinea pigs was used for the main study, ten test and five control. The bodyweight of each animal was recorded at the start and end of the study and are presented in Appendix 6.

Two phases were involved in the main study; (a) an induction of a response and (b) a challenge of that response.

3.2.2.1 Induction

Induction of the Test Animals: Shortly before treatment on Day 0 the hair was removed from an area approximately 40 mm x 60 mm on the shoulder region of each animal with veterinary clippers. A row of three injections (0.1 ml each) was made on each side of the mid-line into a 20 mm x 40 mm area. The injections were:

a) Freund's Complete Adjuvant plus distilled water in the ratio 1:1

b) a 5% v/v formulation of the test material in arachis oil BP

c) a 5% v/v formulation of the test material in a 1:1 preparation of Freund's Complete Adjuvant plus distilled water

Approximately 24 and 48 hours after intradermal injection the degree of erythema at the test material injection sites (ie. injection site b) was evaluated according to the scale shown in Appendix 7.

On Day 7 the same area on the shoulder region used previously for intradermal injections was clipped again and treated with a topical application of the undiluted test material. A filter paper patch (WHATMAN No. 4: approximate size 40 mm x 20 mm), loaded with the undiluted test material was applied to the prepared skin and held in place with a strip of surgical adhesive tape covered with an overlapping length of aluminium foil. The patch and foil were further secured with a strip of elastic adhesive bandage wound in a double layer around the torso of each animal. This occlusive dressing was kept in place for 48 hours.

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The degree of erythema and oedema was quantified one and twenty-four hours following removal of the patches using the scale shown in Appendix 7.

Any other reactions were also recorded.

Induction of the Control Animals: The intradermal induction was performed using an identical procedure to that used for the test animals except that the test material was omitted from the intradermal injections. Injection b) was therefore the vehicle alone, injection c) was a 50% formulation of the vehicle in a 1:1 preparation of Freund's Complete Adjuvant plus distilled water. Similarly, the topical induction procedure was identical to that used for the test animals except that the test material was omitted.

3.2.2.2 Challenge

Shortly before treatment on Day 21, an area of approximately 50 mm x 70 mm on both flanks of each animal, was clipped free of hair with veterinary clippers.

A square filter paper patch (WHATMAN No. 4: approximate size 20 mm x 20 mm), loaded with the undiluted test material was applied to the shorn right flank of each animal and was held in place with a strip of surgical adhesive tape. To ensure that the maximum non-irritant concentration was used at challenge, the test material at a concentration of 75% v/v in arachis oil BP was similarly applied to a skin site on the left shorn flank. The patches were occluded with an overlapping length of aluminium foil and secured with a strip of elastic adhesive bandage wound in a double layer around the torso of each animal.

After 24 hours, the dressing was carefully removed and discarded. The challenge sites were swabbed with cotton wool soaked in diethyl ether to remove residual material. The position of the treatment sites was identified by using a black indelible marker-pen.

Prior to the 24-hour observation the flanks were clipped using veterinary clippers to remove regrown hair.

Approximately 24 and 48 hours after challenge dressing removal, the degree of erythema and oedema was quantified using the scale shown in Appendix 7.

Any other reactions were also recorded.

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3.3 Interpretation of Results

Skin reactions noted at the challenge sites of the test group animals will be attributed to skin sensitisation, providing that reactions of equal severity are not seen at the corresponding challenge sites of the control group animals.

If skin reactions are seen at the challenge sites of the control group animals, these will be due to skin irritation, and therefore only skin reactions of greater severity in the test group animals will be attributed to skin sensitisation.

Barely perceptible erythema (grade ±) is often a non-specific response to the dosing procedure and is not considered to be a significant or conclusive indication of delayed contact hypersensitivity. Furthermore, transient challenge reactions (those which do not persist for at least 48 hours) will not be attributed to contact sensitisation.

The sensitisation potential of the test material will be classified as follows:

Percentage of sensitised animals	Classification
0	non-sensitiser
>0 – 8	weak sensitiser
>8 – 28	mild sensitiser
>28 – 64	moderate sensitiser
>64 – 80	strong sensitiser
>80 – 100	extreme sensitiser

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.

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5. RESULTS

5.1 Skin Reactions Observed After Intradermal Induction

Individual skin reactions at the intradermal induction sites of the test and control group animals are presented in Appendix 4.

Discrete or patchy to moderate and confluent erythema was noted at the intradermal induction sites of test and control group animals.

5.2 Skin Reactions Observed After Topical Induction

Individual skin reactions at the topical induction sites of the test and control group animals are presented in Appendix 5.

Discrete or patchy erythema was noted at the topical induction sites of two test group animals at the 24-hour observation. Bleeding from the intradermal injection sites was noted in eight test group animals at the 1-hour observation.

No skin reactions were noted at the topical induction sites of control group animals.

5.3 Skin Reactions Observed After Topical Challenge

Individual skin reactions at the challenge sites of the test and control group animals are given in Table 1.

Undiluted as Supplied

No skin reactions were noted at the challenge sites of the test or control group animals at the 24 or 48-hour observations.

75% v/v in Arachis Oil BP

No skin reactions were noted at the challenge sites of the test or control group animals at the 24 or 48-hour observations.

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6. CONCLUSION

The test material produced a 0% (0/10) sensitisation rate and was classified as a NON-SENSITISER to guinea pig skin under the conditions of the test.

The test material did not meet the criteria for classification as a sensitiser according to EU labelling regulations Commission Directive 2001/59/EC. No symbol and risk phrase are required.

Table 1 Individual Skin Reactions at Challenge

CHALLENGE CONCENTRATIONS: Undiluted as Supplied and 75% v/v

VEHICLE: Arachis Oil BP

Group	Animal Number	Skin Reactions (Hours after Removal of Dressings)											
		100%						75%					
		24 Hours			48 Hours			24 Hours			48 Hours		
		Er	Oe	Other	Er	Oe	Other	Er	Oe	Other	Er	Oe	Other
TEST	1	0	0	-	0	0	-	0	0	-	0	0	-
	2	0	0	-	0	0	-	0	0	-	0	0	-
	3	0	0	-	0	0	-	0	0	-	0	0	-
	4	0	0	-	0	0	-	0	0	-	0	0	-
	5	0	0	-	0	0	-	0	0	-	0	0	-
	6	0	0	-	0	0	-	0	0	-	0	0	-
	7	0	0	-	0	0	-	0	0	-	0	0	-
	8	0	0	-	0	0	-	0	0	-	0	0	-
	9	0	0	-	0	0	-	0	0	-	0	0	-
	10	0	0	-	0	0	-	0	0	-	0	0	-
CONTROL	11	0	0	-	0	0	-	0	0	-	0	0	-
	12	0	0	-	0	0	-	0	0	-	0	0	-
	13	0	0	-	0	0	-	0	0	-	0	0	-
	14	0	0	-	0	0	-	0	0	-	0	0	-
	15	0	0	-	0	0	-	0	0	-	0	0	-

Er = Erythema
Oe = Oedema
- = No other reactions noted

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Appendix 1 Intradermal Sighting Test – Summary of Results

VEHICLE: **Arachis Oil BP**

Animal Identification	Concentration of Test Material (% v/v)	Time of Observation	Grade of Erythema at Injection Sites	Evidence of Systemic Toxicity
B	1	24 Hours	3R	None
		48 Hours	2	None
		72 Hours	2	None
		7 Days	1	None
C	5	24 Hours	2R	None
		48 Hours	2	None
		72 Hours	2	None
		7 Days	1	None

The concentration of the test material selected for the intradermal induction stage of the main study was 5% v/v in arachis oil BP

R = Reaction extends beyond treatment site

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Appendix 2 Topical Sighting Test for Induction Application (48-Hour Exposure) – Individual Skin Reactions

VEHICLE: Arachis Oil BP

Animal Identification	Concentration of Test Material (% v/v)	Skin Reactions (Hours After Removal of Patches)								
		1			24			48		
		Er	Oe	Other	Er	Oe	Other	Er	Oe	Other
D	100	2	0	-	0	0	-	0	0	-
	75	2	0	-	0	0	-	0	0	-
	50	2	0	-	0	0	-	0	0	-
	25	2	0	-	0	0	-	0	0	-
E	100	2	0	-	0	0	-	0	0	-
	75	2	0	-	0	0	-	0	0	-
	50	2	0	-	0	0	-	0	0	-
	25	1	0	-	0	0	-	0	0	-

The undiluted test material was selected for the main study topical induction

Er = Erythema
Oe = Oedema
- = No other reactions noted

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Appendix 3 Topical Sighting Test for Challenge Application (24-Hour Exposure) – Individual Skin Reactions

VEHICLE: **Arachis Oil BP**

Animal Identification	Concentration of Test Material (% v/v)	Skin Reactions (Hours After Removal of Patches)								
		1			24			48		
		Er	Oe	Other	Er	Oe	Other	Er	Oe	Other
F	100	2	0	-	0	0	-	0	0	-
	75	1	0	-	0	0	-	0	0	-
	50	1	0	-	0	0	-	0	0	-
	25	1	0	-	0	0	-	0	0	-
G	100	1	0	-	0	0	-	0	0	-
	75	1	0	-	0	0	-	0	0	-
	50	1	0	-	0	0	-	0	0	-
	25	1	0	-	0	0	-	0	0	-

The undiluted test material and a 75% v/v concentration of the test material in arachis oil BP were selected for the main study topical challenge

Er = Erythema
Oe = Oedema
- = No other reactions noted

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Appendix 4 Intradermal Induction – Individual Skin Reactions

Group	Animal Number	Grade of Erythema at Observation Site			
		24 Hours		48 Hours	
		Left Side	Right Side	Left Side	Right Side
TEST	1	2	2	2	2
	2	2	2	2	2
	3	2	2	2	2
	4	2	2	2	2
	5	2	2	2	2
	6	2	2	2	2
	7	1	1	1	2
	8	1	2	2	2
	9	2	2	2	2
	10	2	2	2	2
CONTROL	11	1	1	0	0
	12	1	1	0	0
	13	1	1	0	1
	14	1	1	0	0
	15	1	2	0	1

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Appendix 5 Topical Induction – Individual Skin Reactions

Group	Animal Number	Skin Reactions (Hours After Removal of Dressing)					
		1 Hour			24 Hours		
		Er	Oe	Other	Er	Oe	Other
TEST	1	0	0	-	0	0	-
	2	0	0	Bs	0	0	-
	3	0	0	Bs	0	0	-
	4	0	0	Bs	0	0	-
	5	0	0	Bs	1	0	-
	6	0	0	Bs	0	0	-
	7	0	0	Bs	1	0	-
	8	0	0	-	0	0	-
	9	0	0	Bs	0	0	-
	10	0	0	Bs	0	0	-
CONTROL	11	0	0	-	0	0	-
	12	0	0	-	0	0	-
	13	0	0	-	0	0	-
	14	0	0	-	0	0	-
	15	0	0	-	0	0	-

Er = Erythema
Oe = Oedema
- = No other reactions noted
Bs = Bleeding from intradermal injection sites

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Appendix 6 Individual Bodyweights and Bodyweight Gains

Group	Animal Number	Bodyweight (g)		Bodyweight (g) Increase
		Day 0	Day 24	
TEST	1	403	570	167
	2	324	543	219
	3	320	467	147
	4	309	484	175
	5	389	571	182
	6	398	541	143
	7	401	522	121
	8	341	457	116
	9	326	512	186
	10	333	508	175
CONTROL	11	292	472	180
	12	262	462	200
	13	261	415	154
	14	282	379	97
	15	269	378	109

Bodyweight increases of the guinea pigs in the test group between Day 0 and Day 24 were comparable to those noted in the control group over the same period.

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S-FACE MX-10 : SKIN SENSITISATION IN THE GUINEA PIG - MAGNUSSON AND KLIGMAN MAXIMISATION METHOD

Appendix 7 Scales For Evaluation of Skin Reactions

EVALUATION OF ERYTHEMA #	VALUE
No erythema	0
Barely perceptible erythema	±
Discrete or patchy erythema	1
Moderate and confluent erythema	2
Intense erythema and swelling	3

EVALUATION OF OEDEMA †	VALUE
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 extending beyond the area of exposure)	4

From: Modified OECD Test Guideline 406, 1992 and Method B6 Skin Sensitisation of Commission Directive 96/54/EC.

† 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, p31.

Appendix 8 Summary of Positive Control Data for the Magnusson and Kligman Maximisation Study

Project Number	Date Start	Date End	Number of Animals and Sex*		Positive Control Material	Concentration			Incidence of Sensitisation
						Induction		Challenge	
			Test	Control		Intradermal	Topical		
039/422	12/01/00	05/02/00	10 Female	5 Female	2-Mercaptobenzothiazole	5% in arachis oil BP	50% in acetone:PEG 400 (70:30)	50% and 25% in acetone:PEG 400 (70:30)	100% (10/10)
039/444	29/06/00	22/07/00	10 Male	5 Male	2-Mercaptobenzothiazole	5% in arachis oil BP	50% in acetone:PEG 400 (70:30)	50% and 25% in acetone:PEG 400 (70:30)	100% (9/9)
039/446	28/06/00	06/08/00	10 Male	5 Male	α-Hexylcinnamaldehyde	5% in arachis oil BP	100%	100% and 75% in arachis oil BP	50% (5/10)
039/458	25/01/01	25/02/01	10 Male	5 Male	α-Hexylcinnamaldehyde	5% in arachis oil BP	100%	100% and 75% in arachis oil BP	40% (4/10)
039/540	26/09/01	29/10/01	10 Male	5 Male	α-Hexylcinnamaldehyde	5% in arachis oil BP	100%	100% and 75% in arachis oil BP	20% (2/10)
039/576	22/05/02	15/06/02	10 Male	5 Male	2-Mercaptobenzothiazole	5% in arachis oil BP	50% in acetone:PEG 400 (70:30)	50% and 25% in acetone:PEG 400 (70:30)	80% (8/10)

* All animals supplied by David Hall Ltd, Burton-on-Trent, Staffordshire, UK

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Appendix 9 Statement of GLP Compliance in Accordance with Directive 88/320/EEC

THE DEPARTMENT OF HEALTH OF THE GOVERNMENT OF THE UNITED KINGDOM

GOOD LABORATORY PRACTICE

STATEMENT OF COMPLIANCE
IN ACCORDANCE WITH DIRECTIVE 88/320 EEC

LABORATORY

SafePharm Laboratories Ltd
Shardlow Business Park
London Road
Shardlow
Derbyshire
DE72 2GD

TEST TYPE

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

DATE OF INSPECTION
28 February 2000

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

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

Roger G. Alexander
26/4/00

Dr. Roger G. Alexander
Head, UK GLP Monitoring Authority

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Appendix 10 Certificate of Analysis

SAKAMOTO YAKUHIN KOGYO CO.,LTD.

HEAD OFFICE : 1-2-8 AWAJIMACHI,CHUO-KU,OSAKA CITY 541-0047,JAPAN
TEL (81)6-6231-1851 FAX (81)6-6222-0631
TOKYO BRANCH : 1-8-2 MARUNOUCHI,CHIYODA-KU,TOKYO 100-0005,JAPAN
TEL (81)3-3211-3813 FAX (081)3-3215-1788

date : Jul. 30 , 2002

Product : S-Face MX-10
Lot No. : 206103
Quantity : 100g × 2

Certificate of analysis / Quality

	Result
Description	A pale yellow viscous liquid with a faint characteristic odor
Color (Gardner)	1
Acid value (mgKOH/g)	1.3
Saponification value (mgKOH/g)	157.0
Heavy metal (ppm)	less than 20
Arsenic (ppm)	less than 2
Residue on ignition (%)	less than 0.2

Y. Noguchi
Yasunori Noguchi
Quality Control Chief

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SAFEPHARM LABORATORIES LTD

S-FACE MX-10:

**SKIN SENSITISATION IN THE GUINEA PIG -
MAGNUSSON AND KLIGMAN MAXIMISATION METHOD**

SPL PROJECT NUMBER: 432/055

I verify that this is an exact copy of the original report which is located in the Archives of Safepharm Laboratories Ltd., Derby, UK.

..... DATE: 09 JAN 2003

R Driscoll BTech (Hons)
Study Director

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SafePharm Laboratories

S-FACE MX-10:
ACUTE EYE IRRITATION IN THE RABBIT

Polyglyceryl-8 Decaerucate/Decaisostearate/Decaricinoleate

SPL PROJECT NUMBER: 432/054

AUTHOR: R Driscoll

STUDY SPONSOR:

Sakamoto Yakuhin Kogyo Co., Ltd.
1-20, Rinkai-cho
Izumiotsu-city
OSAKA 595-0075
JAPAN

TEST FACILITY:

Safepharm Laboratories Limited
P.O. Box No. 45
DERBY
DE1 2BT
U.K.

Telephone: (01332) 792896

Facsimile: (01332) 799018

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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:

	28 March 2002	Standard Test Method Compliance Audit
	28 October 2002	Test Material Preparation
	09 October 2002	Animal Preparation
	09 October 2002	Dosing
	14 October 2002	Assessment of Response
§	27 November 2002	Draft Report Audit
§	Date of QA Signature	Final Report Audit

§ Evaluation specific to this study

..... DATE: 06 JAN 2003

For Safepharm Quality Assurance Unit*

*Authorised QA Signatures:
Head of Department: JR Pateman CBiol MIBiol DipRQA FRQA
Deputy Head of Department: JM Crowther MIScT MRQA
Senior Audit Staff: JV Johnson BSc MRQA; G Wren ONC MRQA

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GLP COMPLIANCE STATEMENT

The work described was performed in compliance with UK GLP standards (Schedule 1, Good Laboratory Practice Regulations 1999 (SI 1999/3106)). 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 87/18/EEC (as amended by Directive 1999/11/EC) and 88/320/EEC (as amended by Directive 1999/12/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, 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: 03 JAN 2003

R Driscoll BTech (Hons)
Study Director

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S-FACE MX-10:

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 February 1987)
- Commission Directive 92/69/EEC Method B5 Acute Toxicity (Eye Irritation)

Result. A single application of the test material to the non-irrigated eye of three rabbits produced moderate conjunctival irritation. Treated eyes appeared normal at the 48 or 72-hour observations.

Conclusion. The test material produced a maximum group mean score of 10.0 and was classified as a mild irritant (Class 4 on a 1 to 8 scale) to the rabbit eye according to a modified Kay and Calandra classification system.

The test material did not meet the criteria for classification as irritant according to EU labelling regulations Commission Directive 2001/59/EC.

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S-FACE MX-10:

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 February 1987)
- Commission Directive 92/69/EEC Method B5 Acute Toxicity (Eye Irritation)

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 07 October 2002 and 17 October 2002.

2. TEST MATERIAL

2.1 Description, Identification and Storage Conditions

Sponsor's identification	:	S-FACE MX-10
Description	:	pale yellow viscous liquid
Batch number	:	206103
Date received	:	02 August 2002
Storage conditions	:	room temperature in the dark

Data relating to the identity, purity and stability of the test material are the responsibility of the Sponsor.

A Certificate of Analysis supplied by the Sponsor is given in Appendix 5.

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.

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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
Undiluted as Supplied	approximately 5.0
90% v/v aqueous preparation of the test material	approximately 5.5

3. METHODS

3.1 Animals and Animal Husbandry

Three New Zealand White rabbits were supplied by David Percival Ltd, Moston, Sandbach, Cheshire, UK. 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 metal cages. Free access to mains drinking water and food (Certified Rabbit Diet (Code 5322) supplied by International Product Supplies Limited, Wellingborough, Northants, UK) 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.

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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.

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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.

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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.

Moderate conjunctival irritation was noted in all treated eyes one hour after treatment with minimal conjunctival irritation noted in all treated eyes at the 24-hour observation and in one treated eye at the 48-hour observation.

Two treated eyes appeared normal at the 48-hour observation and the remaining treated eye appeared normal at the 72-hour observation.

6. CONCLUSION

The test material produced a maximum group mean score of 10.0 and was classified as a MILD IRRITANT (CLASS 4 ON A 1 TO 8 SCALE) to the rabbit eye according to a modified Kay and Calandra classification system.

The test material did not meet the criteria for classification as irritant according to EU labelling regulations Commission Directive 2001/59/EC.

Table 1 Individual Scores and Individual Total Scores for Ocular Irritation

Rabbit Number and Sex	IPR = 1				IPR = 1				IPR = 1			
	4 Male				184 Male				185 Male			
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	2	1	1	0	2	1	0	0	2	1	0	0
B = Chemosis	1	1	0	0	1	0	0	0	1	0	0	0
C = Discharge	2	0	0	0	2	1	0	0	2	1	0	0
Score (A + B + C) x 2	10	4	2	0	10	4	0	0	10	4	0	0
Total Score	10	4	2	0	10	4	0	0	10	4	0	0

IPR = Initial pain reaction

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S-FACE MX-10 : 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
4 Male	10	4	2	0
184 Male	10	4	0	0
185 Male	10	4	0	0
Group Total	30	12	2	0
Group Mean Score	10.0	4.0	0.7	0.0

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S-FACE MX-10 : 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.

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S-FACE MX-10 : 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

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S-FACE MX-10 : 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		More than half of the individual total scores at 7 days 10 or less	Moderate irritant	(5)
	Group mean total score at 7 days 20 or less	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		More than half of the individual total scores at 7 days 30 or less	Severe irritant	(6)
	Group mean total score at 7 days 40 or less	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		More than half of the individual total scores at 7 days 60 or less	Very severe irritant	(7)
	Group mean total score at 7 days 80 or less	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)

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Appendix 4 Statement of GLP Compliance in Accordance with Directive 88/320/EEC

THE DEPARTMENT OF HEALTH OF THE GOVERNMENT OF THE UNITED KINGDOM

GOOD LABORATORY PRACTICE

STATEMENT OF COMPLIANCE IN ACCORDANCE WITH DIRECTIVE 88/320 EEC

LABORATORY

SafePharm Laboratories Ltd
Shardlow Business Park
London Road
Shardlow
Derbyshire
DE72 2GD

TEST TYPE

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

DATE OF INSPECTION
28 February 2000

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

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

Roger G. Alexander
26/4/00

Dr. Roger G. Alexander
Head, UK GLP Monitoring Authority

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Appendix 5 Certificate of Analysis

SAKAMOTO YAKUHIN KOGYO CO.,LTD.

HEAD OFFICE : 1-2-4 AWAJIMACHI,CHUO-KU,OSAKA CITY 541-0047,JAPAN
TEL (81)6-6231-1851 FAX (81)6-6222-0631
TOKYO BRANCH : 1-6-2 MARUNOUCHI,CHIYODA-KU,TOKYO 100-0005,JAPAN
TEL (81)3-3211-3813 FAX (081)3-3215-1789

date : Jul. 30 , 2002

Product : S-Face MX-10
Lot. No. : 206103
Quantity : 100g × 2

Certificate of analysis / Quality

	Result
Description	A pale yellow viscous liquid with a faint characteristic odor
Color (Gardner)	1
Acid value (mgKOH/g)	1.3
Saponification value (mgKOH/g)	157.0
Heavy metal (ppm)	less than 20
Arsenic (ppm)	less than 2
Residue on ignition (%)	less than 0.2

J. Noguchi
Yasunori Noguchi
Quality Control Chief

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SAFEPHARM LABORATORIES LTD

S-FACE MX-10:

ACUTE EYE IRRITATION IN THE RABBIT

SPL PROJECT NUMBER: 432/054

I verify that this is an exact copy of the original report which is located in the Archives of Safepharm Laboratories Ltd., Derby, UK.

..... DATE: 09 JAN 2003

R Driscoll BTech (Hons)
Study Director

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SafePharm Laboratories

S-FACE MX-10:

Polyglyceryl-8 Decaercate / Decaisostearate / Decaricinoleate

ACUTE ORAL TOXICITY IN THE RAT
- ACUTE TOXIC CLASS METHOD

SPL PROJECT NUMBER: 432/057

AUTHOR: R Driscoll

STUDY SPONSOR:

Sakamoto Yakuhin Kogyo Co., Ltd.
1-20, Rinkai-cho
Izumiotsu-city
OSAKA 595-0075
JAPAN

TEST FACILITY:

Safepharm Laboratories Limited
Shardlow Business Park
Shardlow
Derbyshire
DE72 2GD

Telephone: +44 (0) 1332 792896

Facsimile: +44 (0) 1332 799018

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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:

	07 June 2002	Standard Test Method Compliance Audit
	10 February 2003	Test Material Preparation
	25 February 2003	Animal Preparation
	10 February 2003	Dosing
	13 February 2003	Assessment of Response
	11 February 2003	Necropsy
§	20 March 2003	Draft Report Audit
§	Date of QA Signature	Final Report Audit

§ Evaluation specific to this study

..... G Wren ..... DATE: 22 APR 2003 .....

For Safepharm Quality Assurance Unit*

*Authorised QA Signatures:

Head of Department:	JR Pateman CBiol MIBiol DipRQA FRQA
Deputy Head of Department:	JM Crowther MIScT MRQA
Senior Audit Staff:	JV Johnson BSc MRQA; G Wren ONC MRQA

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GLP COMPLIANCE STATEMENT

The work described was performed in compliance with UK GLP standards (Schedule 1, Good Laboratory Practice Regulations 1999 (SI 1999/3106)). 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 87/18/EEC (as amended by Directive 1999/11/EC) and 88/320/EEC (as amended by Directive 1999/12/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, 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: 17 APR 2003

R Driscoll BTech (Hons)
Study Director

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S-FACE MX-10:

ACUTE ORAL TOXICITY IN THE RAT – ACUTE TOXIC CLASS METHOD

SUMMARY

Introduction. The study was performed to assess the acute oral toxicity of the test material following a single oral administration in the Sprague-Dawley CD strain rat. The method was designed to meet the requirements of the following:

- OECD Guidelines for the Testing of Chemicals No. 423 "Acute Oral Toxicity – Acute Toxic Class Method" (adopted 17 December 2001)

Method. A group of three fasted females was treated with the test material at a dose level of 2000 mg/kg bodyweight. This was followed by a further group of three fasted females at the same dose level.

The test material was administered orally undiluted. Clinical signs and bodyweight development were monitored during the study. All animals were subjected to gross necropsy.

Mortality. There were no deaths.

Clinical Observations. There were no signs of systemic toxicity.

Bodyweight. All animals showed expected gains in bodyweight over the study period.

Necropsy. No abnormalities were noted at necropsy.

Conclusion. The acute oral median lethal dose (LD_{50}) of the test material in the female Sprague-Dawley CD strain rat was estimated from the flow chart in Appendix 1 as being greater than 2500 mg/kg bodyweight.

The test material does not meet the criteria for classification according to EU labelling regulations Commission Directive 2001/59/EC.

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S-FACE MX-10:

ACUTE ORAL TOXICITY IN THE RAT - ACUTE TOXIC CLASS METHOD

1. INTRODUCTION

The study was performed to assess the acute oral toxicity of the test material following a single oral administration in the Sprague-Dawley CD strain rat. The method was designed to meet the requirements of the following:

- OECD Guidelines for the Testing of Chemicals No. 423 "Acute Oral Toxicity – Acute Toxic Class Method" (adopted 17 December 2001)

The rat was selected for this study as it is a readily available rodent species, historically used in safety evaluation studies, and is acceptable to appropriate regulatory authorities. The oral route was selected as the most appropriate route of exposure and the results are believed to be of value in predicting the likely toxicity of the test material to man.

The study was performed between 05 February 2003 and 04 March 2003.

2. TEST MATERIAL AND EXPERIMENTAL PREPARATION

2.1 Description, Identification and Storage Conditions

Sponsor's identification	:	S-FACE MX-10
Description	:	pale yellow viscous liquid
Batch number	:	206103
Date received	:	02 August 2002
Storage conditions	:	room temperature in the dark

Data relating to the identity, purity and stability of the test material are the responsibility of the Sponsor.

A Certificate of Analysis supplied by the Sponsor is given in Appendix 3.

2.2 Preparation of Test Material

For the purpose of the study the test material was used as supplied. The specific gravity was determined and used to calculate the appropriate dose volume for the required dose level.

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3. METHODS

3.1 Animals and Animal Husbandry

Female Sprague-Dawley CD (Hsd:Sprague Dawley™ (CD™)) strain rats were supplied by Harlan UK Limited, Bicester, Oxon, UK. On receipt the animals were randomly allocated to cages. The animals were nulliparous and non-pregnant. After an acclimatisation period of at least five days the animals were selected at random and given a number unique within the study by indelible ink-marking on the tail and a number written on a cage card. At the start of the study the animals were eight to twelve weeks of age. The bodyweights fell within an interval of ± 20% of the mean initial bodyweight of the first treated group.

The animals were housed in groups of three 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 (Certified Rat and Mouse Diet (Code 5LF2) supplied by International Product Supplies Limited, Wellingborough, Northants, UK) was allowed throughout the study. The diet, drinking water and bedding were routinely analysed 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. 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

In the absence of data suggesting the test material was toxic, 2000 mg/kg was chosen as the starting dose.

Groups of fasted animals were treated as follows:

Dose Level (mg/kg)	Specific Gravity	Dose Volume (ml/kg)	Number of Rats
			Female
2000	0.947	2.12	3
2000	0.947	2.12	3

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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 group 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 fourteen 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.

3.3 Evaluation of Data

Data evaluations included the relationship, if any, between the exposure of the animal to the test material and the incidence and severity of all abnormalities including behavioural and clinical observations, gross lesions, bodyweight 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 material was made as shown in the schematic diagram in Appendix 1.

The results were evaluated according to Commission Directive 2001/59/EC for classification and labelling of dangerous substances and preparations.

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.

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5. RESULTS

5.1 Mortality Data

Individual mortality data are given in Table 1.

There were no deaths.

5.2 Clinical Observations

Individual clinical observations are given in Table 2.

There were no signs of systemic toxicity.

5.3 Bodyweight

Individual bodyweights and weekly bodyweight changes are given in Table 3.

All animals showed expected gains in bodyweight over the study period.

5.4 Necropsy

Individual necropsy findings are given in Table 4.

No abnormalities were noted at necropsy.

6. CONCLUSION

The acute oral median lethal dose (LD_{50}) of the test material in the female Sprague-Dawley CD strain rat was estimated from the flow chart in Appendix 1 as being greater than 2500 mg/kg bodyweight.

The test material does not meet the criteria for classification according to EU labelling regulations Commission Directive 2001/59/EC.

Table 1 Mortality Data

Dose Level mg/kg	Sex	Number of Animals Treated	Deaths During Day of Dosing (Hours)				Deaths During Period After Dosing (Days)								Deaths
			½	1	2	4	1	2	3	4	5	6	7	8-14	
2000	Female	3	0	0	0	0	0	0	0	0	0	0	0	0	0/3
	Female	3	0	0	0	0	0	0	0	0	0	0	0	0	0/3

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Table 2 Individual Clinical Observations

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
2000	1-0 Female	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	1-1 Female	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	1-2 Female	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2-0 Female	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2-1 Female	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2-2 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

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Table 3 Individual Bodyweights and Weekly Bodyweight Changes

Dose Level mg/kg	Animal Number and Sex	Bodyweight (g) at Day			Bodyweight Gain (g) During Week	
		0	7	14	1	2
2000	1-0 Female	203	210	216	7	6
	1-1 Female	199	218	228	19	10
	1-2 Female	190	204	221	14	17
	2-0 Female	189	212	221	23	9
	2-1 Female	189	216	224	27	8
	2-2 Female	186	210	223	24	13

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Table 4 Individual Necropsy Findings

Dose Level mg/kg	Animal Number and Sex	Time of Death	Macroscopic Observations
2000	1-0 Female	Killed Day 14	No abnormalities detected
	1-1 Female	Killed Day 14	No abnormalities detected
	1-2 Female	Killed Day 14	No abnormalities detected
	2-0 Female	Killed Day 14	No abnormalities detected
	2-1 Female	Killed Day 14	No abnormalities detected
	2-2 Female	Killed Day 14	No abnormalities detected

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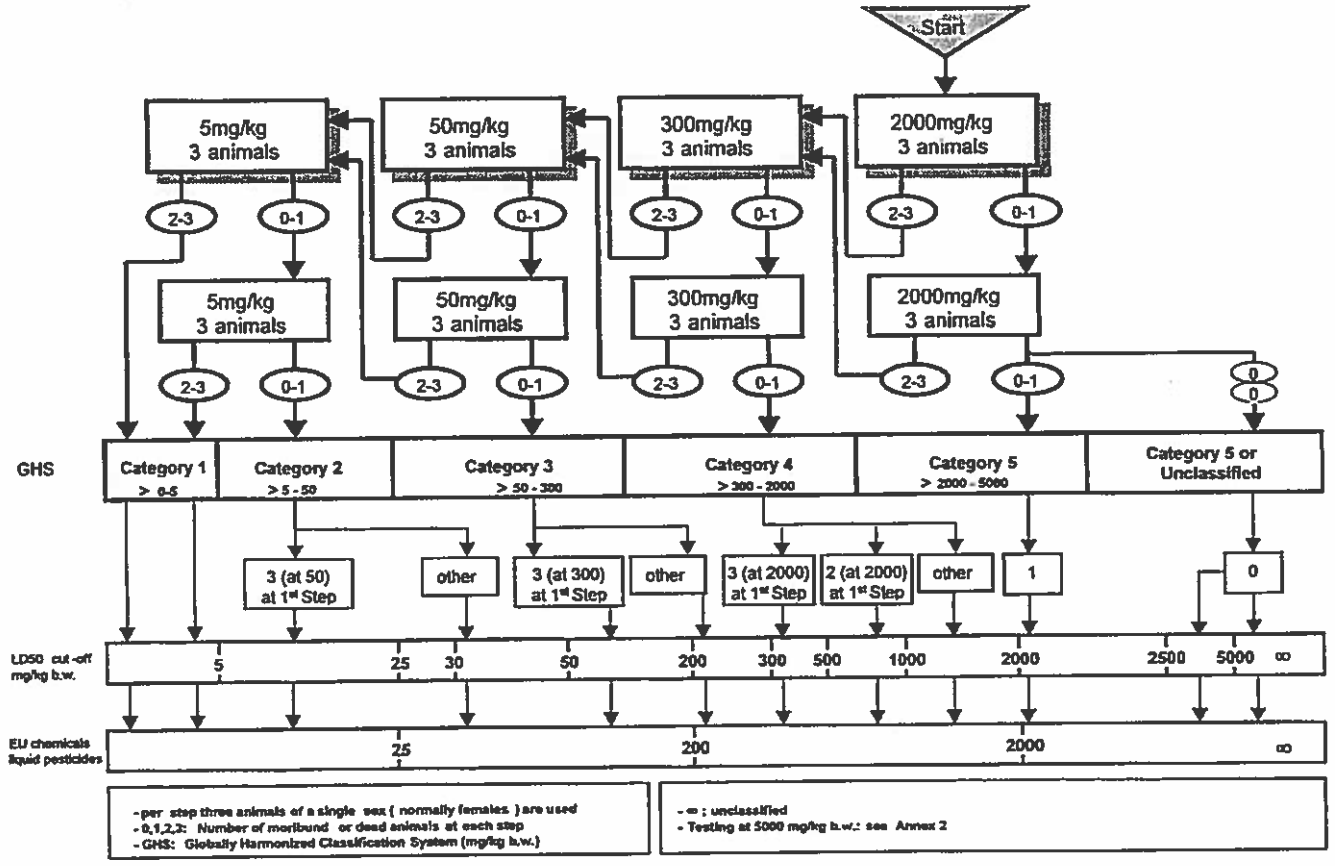
Appendix 1 Test Procedure with a Starting Dose of 2000 mg/kg Bodyweight



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Appendix 2 Statement of GLP Compliance in Accordance with Directive 88/320/EEC

THE DEPARTMENT OF HEALTH OF THE GOVERNMENT OF THE UNITED KINGDOM

GOOD LABORATORY PRACTICE

STATEMENT OF COMPLIANCE IN ACCORDANCE WITH DIRECTIVE 88/320 EEC

LABORATORY
SafePharm Limited
Shardlow Business Park,
London Road,
Shardlow,
Derbyshire,
DE72 2GD

TEST TYPE
Analytical/Clinical Chemistry
Environmental tox.
Environmental fate
Mutagenicity
Phys./Chem. tests
Toxicology

DATE OF INSPECTION

2nd December 2002

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

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

13/2/03

Dr. Roger G. Alexander
Head, UK GLP Monitoring Authority

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Appendix 3 Certificate of Analysis

SAKAMOTO YAKUHIN KOGYO CO.,LTD.

HEAD OFFICE : 1-2-6 AWAJIMACHI,CHUO-KU,OSAKA CITY 541-0047,JAPAN
TEL (81)6-6231-1851 FAX (81)6-6222-0631
TOKYO BRANCH : 1-6-2 MARUNOUCHI,CHIYODA-KU,TOKYO 100-0005,JAPAN
TEL (81)3-3211-3813 FAX (081)3-3215-1789

date : Jul. 30 , 2002

Product : S-Face MX-10
Lot. No. : 206103
Quantity : 100g × 2

Certificate of analysis / Quality

	Result
Description	A pale yellow viscous liquid with a faint characteristic odor
Color (Gardner)	1
Acid value (mgKOH/g)	1.3
Saponification value (mgKOH/g)	157.0
Heavy metal (ppm)	less than 20
Arsenic (ppm)	less than 2
Residue on ignition (%)	less than 0.2

Y. Noguchi
Yasunori Noguchi
Quality Control Chief

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SAFEPHARM LABORATORIES LTD

S-FACE MX-10:

ACUTE ORAL TOXICITY IN THE RAT
– ACUTE TOXIC CLASS METHOD

SPL PROJECT NUMBER: 432/057

I verify that this is an exact copy of the original report which is located in the Archives of Safepharm Laboratories Ltd., Derby, UK.

.....R Driscoll..... DATE: 30 APR 2003

R Driscoll BTech (Hons)
Study Director

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SafePharm Laboratories

S-Face MX-10:
Polyglyceryl-8 Decaerucate/ Decaisostearate/ Deca ricinoleate

REVERSE MUTATION ASSAY "AMES TEST" USING *SALMONELLA TYPHIMURIUM* AND *ESCHERICHIA COLI*

SPL PROJECT NUMBER: 432/056

AUTHOR: P W Thompson

STUDY SPONSOR:

Sakamoto Yakuhin Kogyo Co., Ltd.
1-20, Rinkai-cho
Izumiotsu-city
OSAKA 595-0075
JAPAN

TEST FACILITY:

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

Telephone: +44 (0) 1332 792896

Facsimile: +44 (0) 1332 799018

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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:

	19 August 2002	Standard Test Method Compliance Audit
	25 October 2002	Test Material Preparation
	24 October 2002	Test System Preparation
	17 October 2002	Exposure
	28 October 2002	Assessment of Response
§	17 December 2002	Draft Report Audit
§	Date of QA Signature	Final Report Audit

§ Evaluation specific to this study

..... DATE: 13 MAY 2003

For Safepharm Quality Assurance Unit*

*Authorised QA Signatures:

Head of Department:	JR Pateman CBiol MIBiol DipRQA FRQA
Deputy Head of Department:	JM Crowther MIScT MRQA
Senior Audit Staff:	JV Johnson BSc MRQA; G Wren ONC MRQA

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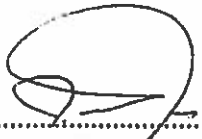
GLP COMPLIANCE STATEMENT

The work described was performed in compliance with UK GLP standards (Schedule 1, Good Laboratory Practice Regulations 1999 (SI 1999/3106)). 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 87/18/EEC (as amended by Directive 1999/11/EC) and 88/320/EEC (as amended by Directive 1999/12/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, 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: 13 MAY 2003

P W Thompson HNC
Study Director

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S-Face MX-10:

REVERSE MUTATION ASSAY "AMES TEST" USING *SALMONELLA TYPHIMURIUM* AND *ESCHERICHIA COLI*

SUMMARY

Introduction. The method was designed to conform to the guidelines for bacterial mutagenicity testing published by the major Japanese Regulatory Authorities including METI, MHLW and MAFF. It also meets the requirements of the OECD Guidelines for Testing of Chemicals No. 471 "Bacterial Reverse Mutation Test", Method B13/14 of Commission Directive 2000/32/EC and the USA, EPA (TSCA) OPPTS harmonised guidelines.

Methods. Salmonella typhimurium strains TA1535, TA1537, TA98 and TA100 and *Escherichia coli* strain WP2uvrA⁻ were treated with solutions of the test material using the Ames plate incorporation method at five dose levels, in triplicate, both with and without the addition of a rat liver homogenate metabolising system (10% liver S9 in standard co-factors). The dose range was determined in a preliminary toxicity assay and was 50 to 5000 µg/plate in the range-finding study. The experiment was repeated on a separate day using the same dose range as the range-finding study, fresh cultures of the bacterial strains and fresh test material formulations.

Results. The vehicle (tetrahydrofuran) control plates gave counts of revertant colonies within the normal range. All of the positive control chemicals used in the test induced marked increases in the frequency of revertant colonies, both with and without metabolic activation. Thus, the sensitivity of the assay and the efficacy of the S9-mix were validated.

The test material caused no visible reduction in the growth of the bacterial background lawn at any dose level. The test material was, therefore, tested up to the maximum recommended dose level of 5000 µg/plate. An oily precipitate was observed at and above 1500 µg/plate, this did not prevent the scoring of revertant colonies.

No significant increases in the frequency of revertant colonies were recorded for any of the bacterial strains, with any dose of the test material, either with or without metabolic activation.

Conclusion. The test material was considered to be non-mutagenic under the conditions of this test.

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S-Face MX-10:

REVERSE MUTATION ASSAY "AMES TEST" USING *SALMONELLA TYPHIMURIUM* AND *ESCHERICHIA COLI*

1. INTRODUCTION

This study was designed to assess the mutagenic potential of the test material using a bacterial/microsome test system. The study was based on the *in vitro* technique described by Ames and his co-workers (1, 2, 3) and Garner *et al* (4) in which mutagenic activity is assessed by exposing histidine auxotrophs of *Salmonella typhimurium* and tryptophan auxotrophs of *Escherichia coli* to various concentrations of the test material. The method conforms to the guidelines for bacterial mutagenicity testing published by the major Japanese Regulatory Authorities including METI, MHLW and MAFF. This method also conforms with the OECD Guidelines for the Testing of Chemicals, Protocol No. 471, Method B13/14 in EC Commission Directive 2000/32/EC and the USA, EPA (TSCA) OPPTS harmonised guidelines. A copy of the Certificate of Compliance with GLP, issued by the UK Department of Health, is included as Appendix 3.

The mutant strains of *Salmonella* are incapable of synthesising histidine and are, therefore, dependent for growth on an external source of this particular amino acid. When exposed to a mutagenic agent these bacteria may undergo a reverse mutation to histidine independent forms which are detected by their ability to grow on a histidine deficient medium. Using various strains of this organism, revertants produced after exposure to a chemical mutagen may arise as a result of base-pair substitution in the genetic material (miscoding) or frame-shift mutation in which genetic material is either added or deleted. In order to make the bacteria more sensitive to mutation by chemical and physical agents, several additional traits have been introduced. These include a deletion through the excision repair gene (uvrB$^-$ *Salmonella* strains) which renders the organism incapable of DNA excision repair and deep rough mutation (rfa) which increases the permeability of the cell wall. A mutant strain of *Escherichia coli* (WP2uvrA$^-$), which requires tryptophan and which can be reverse mutated by base substitution to tryptophan independence, was used to complement the *Salmonella* strains. This strain also has a deletion in an excision repair gene (uvrA$^-$).

Since many compounds do not exert a mutagenic effect until they have been metabolised by enzyme systems not available in the bacterial cell, the test material and the bacteria are also

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incubated in the presence of a liver microsomal preparation (S9-mix) prepared from rats pre-treated with a compound known to induce an elevated level of these enzymes.

The experimental phase of this study was performed between 23 October 2002 and 08 November 2002.

2. TEST MATERIAL

Sponsor's identification	:	S-Face MX-10
Description	:	Pale yellow viscous liquid
Chemical name	:	Polyglyceryl-8 Deca Erucate/Isostearate/Ricinoleate
Purity	:	>99%
Batch number	:	206103
Date received	:	02 August 2002
Storage conditions	:	room temperature, in the dark

Data relating to the identity, purity and stability of the test material are the responsibility of the Sponsor.

3. METHODS

3.1 Tester Strains

Salmonella typhimurium	TA1535, TA1537, TA98 and TA100
Escherichia coli	WP2uvrA$^-$

The *Salmonella typhimurium* strains were obtained from the University of California at Berkeley on culture discs on 4 August 1995 whilst *Escherichia coli* strain WP2uvrA$^-$ was obtained from the British Industrial Biological Research Association on 17 August 1987. All of the strains were stored at -196°C in a Statebourne liquid nitrogen freezer, model SXR 34. Prior to the master strains being used, characterisation checks were carried out to confirm the amino-acid requirement, presence of rfa, R factors, uvrB or uvrA mutation and the spontaneous reversion rate.

In this assay, overnight sub-cultures of the appropriate coded stock cultures were prepared in nutrient broth and incubated at 37°C for approximately 10 hours. Each culture was monitored spectrophotometrically for turbidity with titres determined by viable count analysis on nutrient agar plates.

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3.2 Preparation of Test and Control Materials

The test material was accurately weighed and approximate half-log dilutions prepared in tetrahydrofuran by mixing on a vortex mixer on the day of each experiment. Tetrahydrofuran is toxic to all of the tester strains at and above 50 µl, therefore, all of the test material formulations were prepared at concentrations four times greater than required on Vogel-Bonner agar plates. To compensate, each formulation (and the vehicle control) were dosed using 25 µl aliquots. Tetrahydrofuran is an acceptable vehicle for use in this test system (6). Analysis for concentration, homogeneity and stability of the test material formulations is not a requirement of the test guidelines and was, therefore, not determined. Prior to use, the solvent was dried using molecular sieves (sodium alumino-silicate) *ie* 2 mm pellets with a nominal pore diameter of 4×10^{-4} microns.

Vehicle and positive controls were used in parallel with the test material. A solvent treatment group was used as the vehicle control and the positive control materials were as follows:

N-ethyl-N'-nitro-N-nitrosoguanidine (ENNG):	2 µg/plate for WP2uvrA$^-$, 3 µg/plate for TA100 and 5 µg/plate for TA1535
9-Aminoacridine (9AA):	80 µg/plate for TA1537
4-Nitroquinoline-1-oxide (4NQO):	0.2 µg/plate for TA98

In addition, 2-Aminoanthracene (2AA) and Benzo(a)pyrene (BP), which are non-mutagenic in the absence of metabolising enzymes, were used in the series of plates with S9-mix at the following concentrations:

2AA at 1 µg/plate for TA100
2AA at 2 µg/plate for TA1535 and TA1537
2AA at 10 µg/plate for WP2uvrA$^-$
BP at 5 µg/plate for TA98

3.3 Microsomal Enzyme Fraction

S9 was prepared in-house on 07 September 2002 from the livers of male Sprague-Dawley rats weighing ~ 250g. These had each orally received three consecutive daily doses of phenobarbitone/ β-naphthoflavone (80/100 mg per kg per day) prior to S9 preparation on Day 4. Before use, each batch of S9 was assayed for its ability to metabolize the indirect mutagens 2-Aminoanthracene and Benzo(a)pyrene. The S9 was stored at -196°C.

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3.4 S9-Mix and Agar

The S9-mix was prepared immediately before use using sterilised co-factors and maintained on ice for the duration of the test.

S9	5.0 ml
1.65 M KCl/0.4 M $MgCl_2$	1.0 ml
0.1 M Glucose-6-phosphate	2.5 ml
0.1 M NADPH	2.0 ml
0.1 M NADH	2.0 ml
0.2 M Sodium phosphate buffer (pH 7.4)	25.0 ml
Sterile distilled water	12.5 ml

A 0.5 ml aliquot of S9-mix and 2 ml of molten, trace histidine or tryptophan supplemented, top agar was overlaid onto a sterile Vogel-Bonner Minimal agar plate in order to assess the sterility of the S9-mix. This procedure was repeated, in triplicate, on the day of each experiment.

Top agar was prepared using 0.6% Difco Bacto agar and 0.5% sodium chloride with 5 ml of 1.0 mM histidine and 1.0 mM biotin or 1.0 mM tryptophan solution added to each 100 ml of top agar. Vogel-Bonner Minimal agar plates were purchased from Fred Baker Scientific.

3.5 Test Procedure

3.5.1 Preliminary Toxicity Study

In order to select appropriate dose levels for use in the main study, a preliminary test was carried out to determine the toxicity of the test material. The concentrations tested were 0, 0.15, 0.5, 1.5, 5, 15, 50, 150, 500, 1500 and 5000 µg/plate. The study was performed by mixing 0.1 ml of bacterial culture (TA100 or WP2uvrA$^-$), 0.025 ml of test material formulation, 0.5 ml of S9-mix or phosphate buffer and 2 ml of molten, trace histidine or tryptophan supplemented, top agar and overlaying onto sterile plates of Vogel-Bonner Minimal agar (30 ml/plate). Ten concentrations of the test material and a vehicle control (tetrahydrofuran) were tested. In addition, 0.025 ml of the maximum concentration of the test material and 2 ml of molten, trace histidine or tryptophan supplemented, top agar was overlaid onto a sterile Nutrient agar plate in order to assess the sterility of the test material. After approximately 48 hours incubation at 37°C the plates were assessed for numbers of revertant colonies using a Domino colony counter and examined for effects on the growth of the bacterial background lawn.

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3.5.2 Mutation Study – Experiment 1 (Range-finding Study)

Five concentrations of the test material (50, 150, 500, 1500 and 5000 µg/plate) were assayed in triplicate against each tester strain, using the direct plate incorporation method.

Measured aliquots (0.1 ml) of one of the bacterial cultures were dispensed into sets of test tubes followed by 2.0 ml of molten, trace histidine or tryptophan supplemented, top agar, 0.025 ml of the vehicle and test material formulation or 0.1 ml of positive control and either 0.5 ml of S9-mix or phosphate buffer. The contents of each test tube were mixed and equally distributed onto the surface of Vogel-Bonner Minimal agar plates (one tube per plate). This procedure was repeated, in triplicate, for each bacterial strain and for each concentration of test material both with and without S9-mix.

All of the plates were incubated at 37°C for approximately 48 hours and the frequency of revertant colonies assessed using a Domino colony counter.

3.5.3 Mutation Study – Experiment 2 (Main Study)

The second experiment was performed using methodology as described for the range-finding study, using fresh bacterial cultures, test material and control solutions. The test material dose range was the same as the range-finding study (50 to 5000 µg/plate).

3.6 Acceptance Criteria

The reverse mutation assay may be considered valid if the following criteria are met:

All tester strain cultures exhibit a characteristic number of spontaneous revertants per plate in the vehicle and untreated controls. Acceptable ranges are presented in the standard test method section 3 with historical control ranges for 2000 and 2001 presented in Appendix 2.

The appropriate characteristics for each tester strain have been confirmed, eg rfa cell-wall mutation and pkM101 plasmid R-factor etc.

All tester strain cultures should be in the approximate range of 1 to 9.9 x 10^9 bacteria per ml.

Each mean positive control value should be at least two times the respective vehicle control value for each strain, thus demonstrating both the intrinsic sensitivity of the tester strains to mutagenic

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exposure and the integrity of the S9-mix. The positive control historical ranges for 2000 and 2001 are presented in Appendix 2.

There should be a minimum of four non-toxic test material dose levels.

There should not be an excessive loss of plates due to contamination.

3.7 Evaluation Criteria

The test material may be considered positive in this test system if the following criteria are met:

The test material should have induced a reproducible, dose-related and statistically (Dunnett's method of linear regression(5)) significant increase in the revertant count in at least one strain of bacteria.

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.

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5. RESULTS

5.1 Preliminary Toxicity Study

The test material was non-toxic to the strains of bacteria used (TA100 and WP2uvrA⁻). The test material formulation and S9-mix used in this experiment were both shown to be effectively sterile.

The number of revertant colonies for the toxicity assay were:

With (+) or without (-) S9-mix	Strain	Dose (μg/plate)										
		0	0.15	0.5	1.5	5	15	50	150	500	1500	5000
-	TA100	119	123	107	109	117	87	130	113	102	106P	75P
+	TA100	113	150	136	136	133	142	107	126	134	125P	129P
-	WP2uvrA⁻	18	19	26	18	20	22	14	12	22	22P	18P
+	WP2uvrA⁻	29	31	28	26	26	22	24	19	43	29P	16P

P = oily precipitate

5.2 Mutation Study

Prior to use, the master strains were checked for characteristics, viability and spontaneous reversion rate (all were found to be satisfactory). These data are not given in the report. The S9-mix used in both experiments of the main study was shown to be sterile.

Results for the negative controls (spontaneous mutation rates) are presented in Table 1 and were considered to be acceptable. These data are for concurrent untreated control plates performed on the same day as the Mutation Study.

The individual plate counts, the mean number of revertant colonies and the standard deviations for the test material, vehicle and positive controls both with and without metabolic activation, are presented in Table 2 to Table 5 with the results also expressed graphically in Figure 1 to Figure 4.

Information regarding the equipment and methods used in these experiments as required by the Japanese Ministry of Economy, Trade and Industry and Japanese Ministry of Health, Labour and Welfare are presented in Appendix 1.

A history profile of vehicle and positive control values is presented in Appendix 2.

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The test material caused no visible reduction in the growth of the bacterial background lawn at any dose level. The test material was, therefore, tested up to the maximum recommended dose level of 5000 µg/plate. An oily precipitate was observed at and above 1500 µg/plate, this did not prevent the scoring of revertant colonies.

No significant increases in the frequency of revertant colonies were recorded for any of the bacterial strains, at any dose level either with or without metabolic activation.

All of the positive control chemicals used in the test induced marked increases in the frequency of revertant colonies thus confirming the activity of the S9-mix and the sensitivity of the bacterial strains.

6. CONCLUSION

The test material was considered to be non-mutagenic under the conditions of this test.

7. REFERENCES

1. Ames B N, Durston W E, Yamasaki E and Lee F D (1973b) *Proc. Natl. Acad. Sci. (USA)*, **70**, 2281-2285.

2. Ames B N, McCann J and Yamasaki E (1975b) *Mutation Research*, **31**, 347- 364.

3. Maron DM and Ames BN (1983) *Mutation Research*, **113**, 173-215.

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5. Kirkland D J (Ed) (1989) Statistical Evaluation of Mutagenicity Test Data. *UKEMS Sub-committee on Guidelines for Mutagenicity Testing*, **Report - Part III**, Cambridge University Press.

6. Maron D et al (1981) *Mutation Research*, **88**, 343-350.

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S-Face MX-10: REVERSE MUTATION ASSAY "AMES TEST"
USING *SALMONELLA TYPHIMURIUM* AND *ESCHERICHIA COLI*

Table 1 Spontaneous Mutation Rates (Concurrent Negative Controls)

Range-finding Study

Number of revertants (mean number of colonies per plate)									
Base-pair substitution type						Frameshift type			
TA100		TA1535		WP2uvrA⁻		TA98		TA1537	
93		11		31		23		19	
101	(94)	20	(14)	21	(26)	22	(25)	3	(9)
89		11		25		31		5	

Main Study

Number of revertants (mean number of colonies per plate)									
Base-pair substitution type						Frameshift type			
TA100		TA1535		WP2uvrA⁻		TA98		TA1537	
79		18		27		25		13	
73	(77)	14	(15)	31	(29)	32	(25)	16	(12)
79		12		28		18		7	

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S-Face MX-10: REVERSE MUTATION ASSAY "AMES TEST"
USING *SALMONELLA TYPHIMURIUM* AND *ESCHERICHIA COLI*

Table 2 Test Results: Range-Finding Study – Without Metabolic Activation

Test Period		From: 01 November 2002						To: 04 November 2002			
With or without S9-Mix	Test substance concentration (µg/plate)	Number of revertants (mean number of colonies per plate)									
		Base-pair substitution type						Frameshift type			
		TA100		TA1535		WP2uvrA-		TA98		TA1537	
-	0	107 92 109	(103) 9.3#	19 12 14	(15) 3.6	26 24 20	(23) 3.1	23 35 29	(29) 6.0	5 3 8	(5) 2.5
-	50	104 110 80	(98) 15.9	8 16 17	(14) 4.9	32 29 29	(30) 1.7	26 22 29	(26) 3.5	5 6 14	(8) 4.9
-	150	101 101 100	(101) 0.6	21 11 12	(15) 5.5	29 28 28	(28) 0.6	23 21 18	(21) 2.5	11 9 11	(10) 1.2
-	500	93 88 103	(95) 7.6	13 14 17	(15) 2.1	29 16 27	(24) 7.0	26 23 15	(21) 5.7	15 11 4	(10) 5.6
-	1500	110 P 96 P 80 P	(95) 15.0	18 P 14 P 19 P	(17) 2.6	11 P 27 P 23 P	(20) 8.3	27 P 26 P 27 P	(27) 0.6	14 P 11 P 5 P	(10) 4.6
-	5000	CP 102 P 76 P	(89) 18.4	13 P 13 P 31 P	(19) 10.4	20 P 24 P 26 P	(23) 3.1	24 P 25 P 20 P	(23) 2.6	8 P 8 P 13 P	(10) 2.9
Positive controls	Name	ENNG		ENNG		ENNG		4NQO		9AA	
S9-Mix	Concentration (µg/plate)	3		5		2		0.2		80	
-	No. colonies per plate	493 454 484	(477) 20.4	160 225 194	(193) 32.5	574 591 530	(565) 31.5	98 112 102	(104) 7.2	2577 2488 2382	(2482) 97.6

ENNG N-ethyl-N'-nitro-N-nitrosoguanidine
4NQO 4-Nitroquinoline-1-oxide
9AA 9-Aminoacridine
C Contaminated
P Precipitate
Standard deviation

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S-Face MX-10: REVERSE MUTATION ASSAY "AMES TEST" USING *SALMONELLA TYPHIMURIUM* AND *ESCHERICHIA COLI*

Table 4 Test Results: Main Study – Without Metabolic Activation

Test Period		From: 05 November 2002						To: 08 November 2002			
With or without S9-Mix	Test substance concentration (µg/plate)	Number of revertants (mean number of colonies per plate)									
		Base-pair substitution type						Frameshift type			
		TA100		TA1535		WP2uvrA-		TA98		TA1537	
-	0	89 72 93	(85) 11.2#	25 27 20	(24) 3.6	46 47 25	(39) 12.4	34 33 36	(34) 1.5	2 29 30	(20) 15.9
-	50	73 75 87	(78) 7.6	21 15 14	(17) 3.8	18 24 33	(25) 7.5	23 22 23	(23) 0.6	7 8 8	(8) 0.6
-	150	73 72 74	(73) 1.0	16 9 13	(13) 3.5	29 22 20	(24) 4.7	20 24 21	(22) 2.1	8 27 C	(18) 13.4
-	500	73 82 69	(75) 6.7	26 29 18	(24) 5.7	39 28 15	(27) 12.0	31 32 25	(29) 3.8	4 8 9	(7) 2.6
-	1500	101 93 102	(99) 4.9	21 15 21	(19) 3.5	18 24 C	(21) 4.2	21 22 26	(23) 2.6	11 15 12	(13) 2.1
-	5000	78 67 94	(80) 13.6	13 14 9	(12) 2.6	33 25 32	(30) 4.4	31 34 20	(28) 7.4	13 12 12	(12) 0.6
Positive controls	Name	ENNG		ENNG		ENNG		4NQO		9AA	
	Concentration (µg/plate)	3		5		2		0.2		80	
S9-Mix -	No. colonies per plate	373 569 761	(568) 194.0	221 183 210	(205) 19.6	700 635 626	(654) 40.4	131 125 144	(133) 9.7	1441 1849 1129	(1473) 361.1

ENNG N-ethyl-N'-nitro-N-nitrosoguanidine
4NQO 4-Nitroquinoline-1-oxide
9AA 9-Aminoacridine
C Contaminated
Standard deviation

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S-Face MX-10: REVERSE MUTATION ASSAY "AMES TEST"
USING *SALMONELLA TYPHIMURIUM* AND *ESCHERICHIA COLI*

Table 5 Test Results: Main Study – With Metaboiic Activation

Test Period		From: 05 November 2002						To: 08 November 2002			
With or without S9-Mix	Test substance concentration (µg/plate)	Number of revertants (mean number of colonies per plate)									
		Base-pair substitution type						Frameshift type			
		TA100		TA1535		WP2uvrA-		TA98		TA1537	
+	0	105 113 97	(105) 8.0#	21 20 21	(21) 0.6	23 32 32	(29) 5.2	54 46 34	(45) 10.1	18 9 17	(15) 4.9
+	50	99 114 115	(109) 9.0	22 17 20	(20) 2.5	28 28 21	(26) 4.0	32 33 50	(38) 10.1	13 14 9	(12) 2.6
+	150	106 118 100	(108) 9.2	24 20 13	(19) 5.6	35 22 32	(30) 6.8	36 31 27	(31) 4.5	21 6 6	(11) 8.7
+	500	98 93 94	(95) 2.6	7 12 16	(12) 4.5	31 26 23	(27) 4.0	34 45 31	(37) 7.4	12 9 8	(10) 2.1
+	1500	98 107 105	(103) 4.7	14 17 18	(16) 2.1	28 21 22	(24) 3.8	45 45 49	(46) 2.3	12 14 20	(15) 4.2
+	5000	117 88 94	(100) 15.3	14 16 11	(14) 2.5	C 35 33	(34) 1.4	39 36 33	(36) 3.0	12 8 16	(12) 4.0
Positive controls	Name	2AA		2AA		2AA		BP		2AA	
	Concentration (µg/plate)	1		2		10		5		2	
S9-Mix +	No. colonies per plate	872 1054 1033	(986) 99.6	559 521 448	(509) 56.4	869 804 787	(820) 43.3	466 445 429	(447) 18.6	619 637 673	(643) 27.5

BP Benzo(a)pyrene
2AA 2-Aminoanthracene
C Contaminated
Standard deviation

Figure 1 **Dose Response Curve: Range-Finding Study – Without Metabolic Activation**

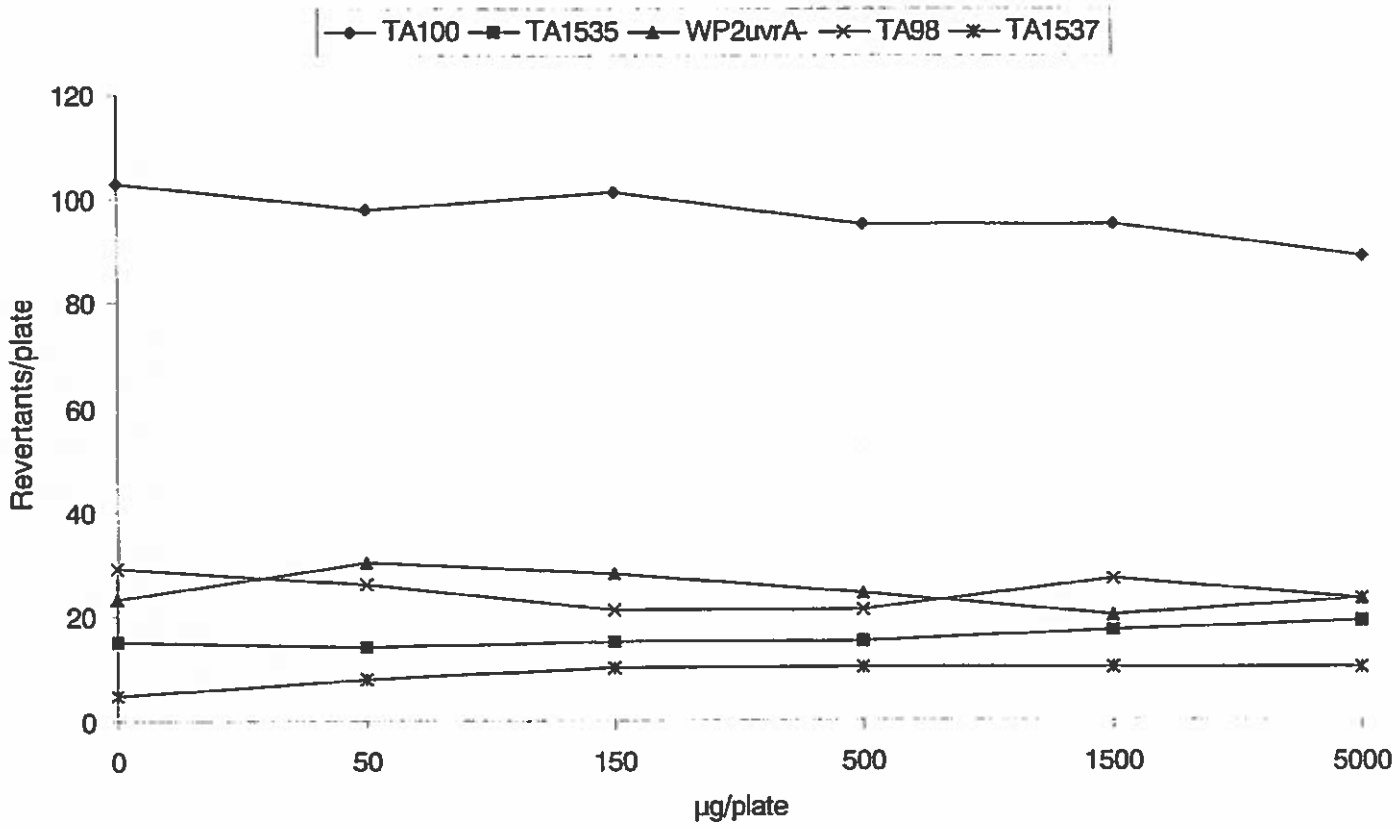

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Figure 2 **Dose Response Curve: Range-Finding Study – With Metabolic Activation**

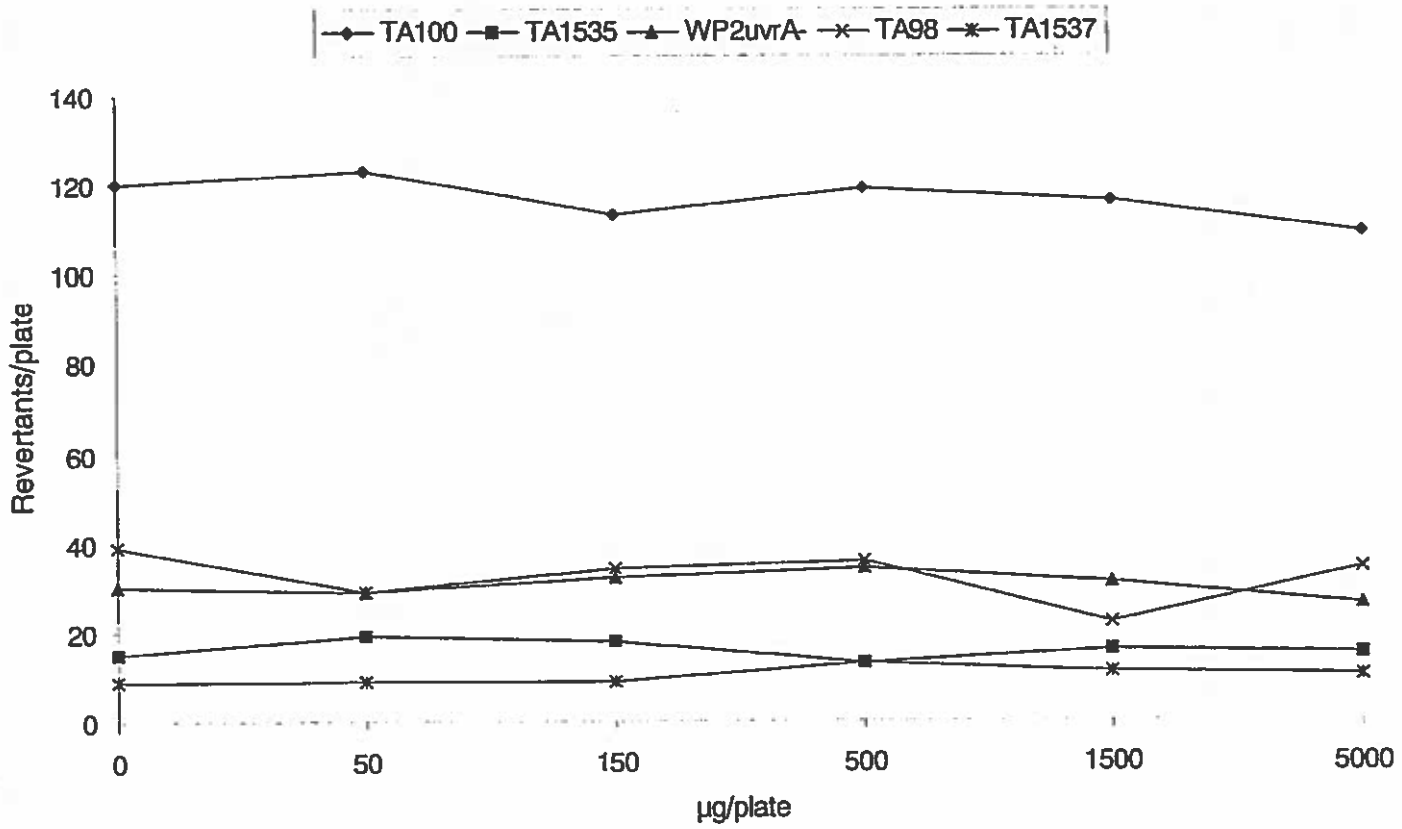

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Figure 3 **Dose Response Curve: Main Study – Without Metabolic Activation**

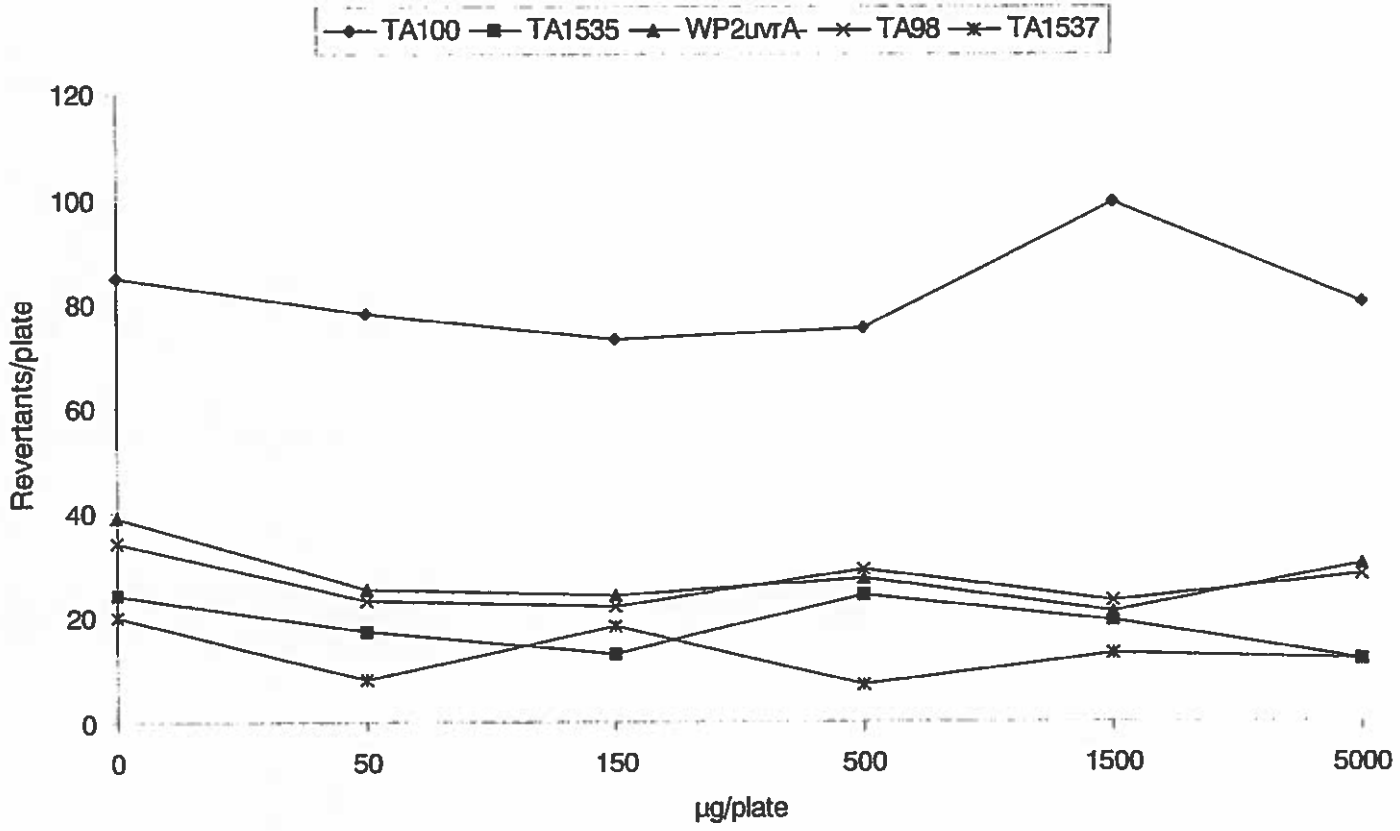

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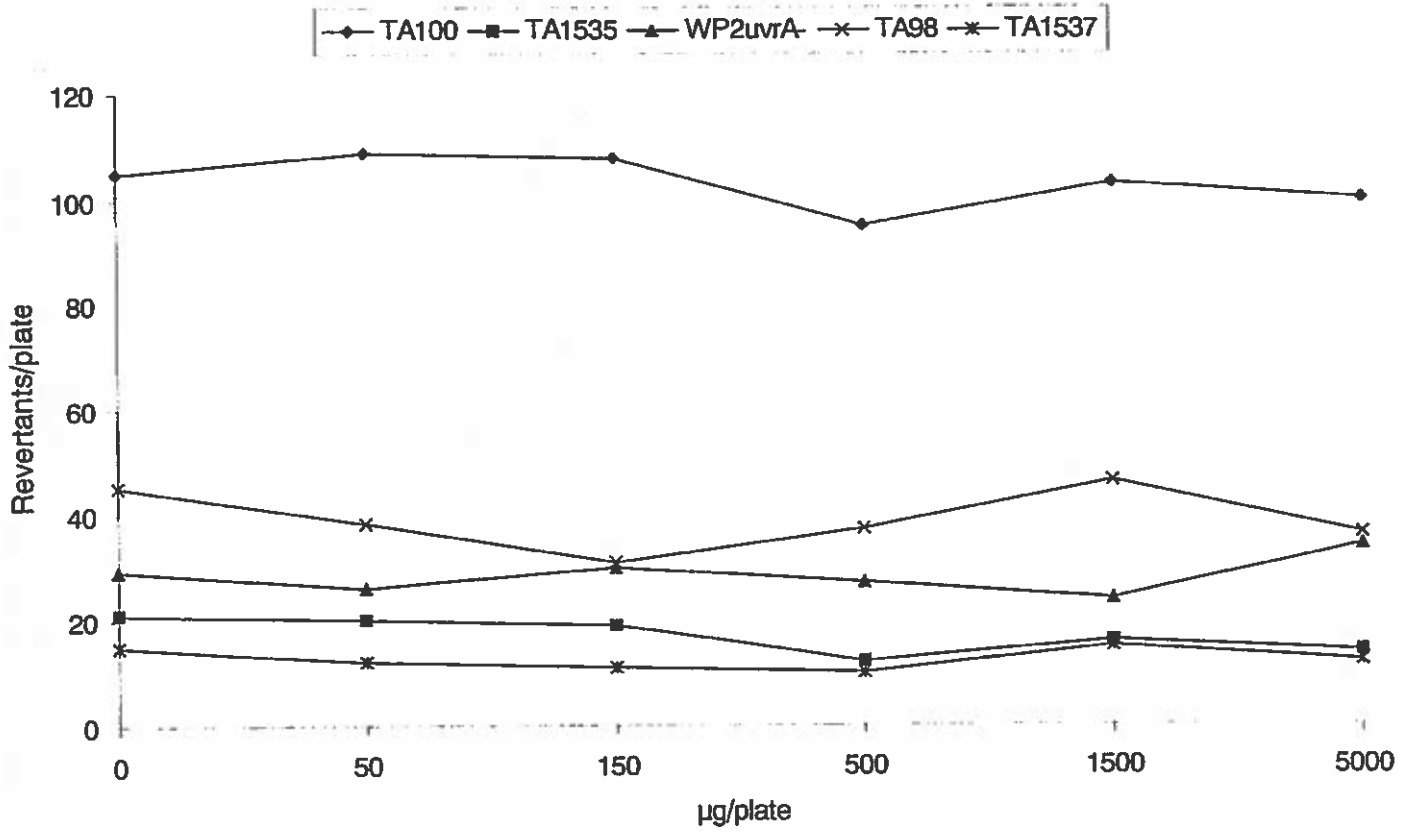
Figure 4 **Dose Response Curve: Main Study – With Metabolic Activation**



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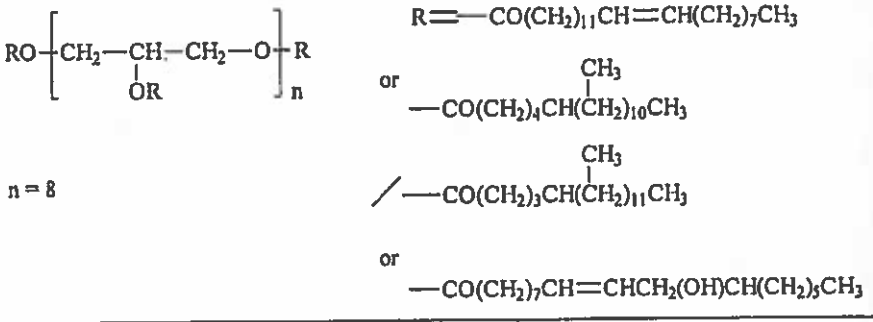
S-Face MX-10: REVERSE MUTATION ASSAY "AMES TEST"
USING *SALMONELLA TYPHIMURIUM* AND *ESCHERICHIA COLI*

Appendix 1 Report of Results in Mutagenicity Test using Micro-organisms

1. GENERAL ITEMS (to be completed by the sponsor)

Name of the new chemical substance (IUPAC nomenclature)	Polyglyceryl-8 Deca Erucate/Isostearate/Ricinoleate					
Other name	S-Face MX-10					
Structural formula or rational formula (or outline of manufacturing method, in case both are unknown)	 n = 8					
Purity of the new chemical substance tested	>99%		Lot No. of the new chemical substance tested	206103		
Name of impurities and concentration	Under 1%					
CAS No.	-		Vapour pressure	-		
Molecular weight	3560		Partition coefficient	-		
Melting point (°C)	-20		Appearance at ordinary temperature	Pale yellow viscous liquid		
Boiling point (°C)	>350					
Stability	Stable under normal temperatures and pressure					
Degree of solubility in solvent	Solvent	Degree of solubility	Stability in solvent	Solvent	Degree of solubility	Stability in solvent
	Water	Insoluble at 50mg/ml	-	DMSO	Insoluble at 50mg/ml	-
	Acetone	Insoluble at 50mg/ml	-	Dimethyl formamide	Insoluble at 50mg/ml	-
	Ethanol	Insoluble at 50mg/ml	-	Acetonitrile	Insoluble at 50mg/ml	-
	Tetrahydrofuran	Soluble at 200mg/ml	*			

Solubility data generated by the testing facility
* No visible change in test material formulations throughout each experiment, therefore, assumed stable

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S-Face MX-10: REVERSE MUTATION ASSAY "AMES TEST"
USING *SALMONELLA TYPHIMURIUM* AND *ESCHERICHIA COLI*

2. TESTER STRAINS

Tester strain	Obtained from	Date obtained
TA100	UCB*	4 August 1995
TA1535	UCB*	4 August 1995
WP2uvrA⁻	BIBRA**	17 August 1987
TA98	UCB*	4 August 1995
TA1537	UCB*	4 August 1995

3. S-9 MIX

(1) Source of S9

(encircle the applicable number and fill in the relevant entries)

Made in-house or purchase	(1.) Made in-house	2. Purchase (Supplier)
Prepared on	07 September 2002	
Lot No. (in case of purchase)	-	
Storage temperature	-196°C	

(2) Preparation of S9

Animal used		Inducing substance	
Species strain	Rat Sprague-Dawley	Name	Phenobarbitone/β-naphthoflavone
Sex	Male	Administration method	Oral
Age (in weeks)	7 weeks	Administration period and amount (mg/kg bodyweight/day)	3 days 80/100 mg per kg per day
Weight	~ 250g		

(3) Composition of S9-Mix

Constituents	Amount in 1 ml S9-Mix	Constituents	Amount in 1 ml S9-Mix
S9	0.1 ml	NADPH	4.0 µmol
$MgCl_2$	8.0 µmol	NADH	4.0 µmol
KCl	33.0 µmol	Na-phosphate buffer	100.0 µmol
Glucose-6-phosphate	5.0 µmol	Others ( )	
Glucose-6-phosphate dehydrogenase			

* UCB: University of California at Berkeley
** BIBRA: British Industrial Biological Research Association

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4. PREPARATION OF THE TEST MATERIAL IN SOLUTION

(encircle the applicable response)

	Name	Supplier	Lot No.	Grade	Purity (%)
Solvent used	Tetrahydrofuran	Flsher Scientific	0102658	Dry solvent	99.97%
Reason for selection of solvent	Readily soluble at 200 mg/ml				
Appearance of the test material preparation	(Solution) Suspension Others ( )				
Suspension and other methods when test substance difficult to dissolve	Solution achieved by action on an autovortex mixer				
Storage time and temperature of solution from preparation until use	35 minutes at room temperature (range-finding study) 1 hour and 20 minutes at room temperature (main study)				
Conversion of purity	Yes (No)				

5. CONDITIONS OF PRE-CULTURE ETC.

(1) Conditions

Nutrient Broth	Name	Manufacturer	Lot No.
	Oxoid Nutrient Broth No.2	Oxoid Ltd.	276098 07/07
Period of pre-culture	10 hr		
Culture flask (form, size)	Costar 75 cm^2 tissue culture flasks		
Amount of culture medium	5 ml	Amount of strain inoculated	20µl

(2) Cell viability at the end of pre-culture

		Base-pair substitution type				Frameshift type		
		TA100	TA1535	WP2uvrA⁻		TA98	TA1537	
Cell number (x 10^9/ml)	Range-finding study	1.8	1.0	2.6		1.9	1.1	
	Main study	1.1	1.5	3.0		1.8	1.5	
Count method (encircle the applicable number)	1. Conversion from O.D. value (2.) Stepwise dilution method 3. Others ( )							

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USING *SALMONELLA TYPHIMURIUM* AND *ESCHERICHIA COLI*

6. MINIMUM GLUCOSE AGAR PLATE MEDIUM

(encircle the applicable number and fill in the relevant entries)

Made in-house or purchase	1. Made in-house (2.) Purchase from Fred Baker Scientific
Prepared on	01 October 2002 (range-finding study) 14 September 2002 (main study)
Lot No. in case of purchase	340407 04/03 (range-finding study) 337373 03/03 (main study)
Name of supplier and lot no. of the used agar	-

7. TEST METHOD (encircle the applicable number and fill in the relevant entries)

(1) Test Method and the reason why it was selected

Test method used	1. Pre-incubation method (2.) Plate incorporation method 3. Others ( )
Reason in case of others	-

(2) Test Conditions

		Pre-incubation	Plate Method
Composition	Bacterial suspension	ml	0.1 ml
	Test substance solution	ml	0.025 ml
	Na-phosphate buffer	ml	0.5 ml
	S9-mix (in case of metabolic activation method)	ml	0.5 ml
	Top agar solution	ml	2.0 ml
	Others ( )	ml	-
Pre-incubation	Temperature	°C	-
	Time	min	-
Incubation	Temperature	°C	37°C
	Time	hours	48 hours

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S-Face MX-10: REVERSE MUTATION ASSAY "AMES TEST"
USING *SALMONELLA TYPHIMURIUM* AND *ESCHERICHIA COLI*

8. COUNT METHOD OF COLONIES (Encircle 1 and 2 if both methods used)

Count method	1. By hand	(2.) Colony counter
Conduction of correction	1. No	(2.) Yes (Correction method: Area and miscount correction)

9. TEST RESULTS

(1) The results should be reported on the attached form

(2) Judgement of the results

Judgement (encircle one)	Positive	(Negative)
Reason for judgement: No significant increase in the frequency of revertant colonies was recorded for any bacterial strain used with any dose of the test material in two separate experiments either with or without metabolic activation.		

(when judged as positive, attach a table for relative activity)

(3) Referential matters

An oily precipitate was observed at and above 1500 µg/plate, this did not prevent the scoring of revertant colonies. Tetrahydrofuran was selected as the vehicle for this study because the test material was soluble at 200 mg/ml (the most concentrated stock dilution) and was non-soluble at 50 mg/ml in a range of Ames recommended vehicles in solubility checks performed in-house (see page 23). Tetrahydrofuran is an acceptable vehicle for use in this test system (6). Prior to use, the solvent was dried using molecular sieves (sodium alumino-silicate) ie 2 mm pellets with a nominal pore diameter of 4×10^{-4} microns.

For a substance to be considered positive in this test system, it should have induced a reproducible, dose-related and statistically (Dunnett's method of linear regression(5)) significant increase in the revertant count in at least one strain of bacteria.

Phenobarbitone/β-naphthoflavone was used to induce higher enzyme levels in the rat liver S9. The S9-mix used in each experiment of this study was shown to be sterile. The test material formulations showed no evidence of contamination when tested for sterility during the preliminary toxicity test. The use of approximate half-log dilutions instead of twofold dilutions was agreed with the JMOL in January 1995.

[REMARKS] "Referential matters" – (fill in the view etc of the Study Director on the test results)

10. OTHERS

	Name	Safepharm Laboratories Ltd.		
	Address	P.O. Box 45, Derby, United Kingdom		Tel No: 0044 1332 792896 Fax No: 0044 1332 799018
Study Director	Title	Senior Genetic Toxicologist	Name	P W Thompson HNC
	Years of experience: 15			
Study number	432/056			
Test dates	22 October 2002 Protocol authorised by Study Director		13 MAY 2003 Final report authorised by Study Director	

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S-Face MX-10: REVERSE MUTATION ASSAY "AMES TEST"
USING *SALMONELLA TYPHIMURIUM* AND *ESCHERICHIA COLI*

Appendix 2 History Profile of Vehicle and Positive Control Values

COMBINED VEHICLE AND UNTREATED CONTROL VALUES 2000

Strain	TA100		TA1535		WP2uvrA⁻		TA102		TA98		TA1537		TA1538		WP2uvrA⁻ pkm101		TA97a	
S9-Mix	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Mean	115	118	22	16	22	24	284	312	23	32	12	16	14	31	222	198	102	125
SD	22	22	5	4	5	6	41	35	6	6	4	4	6	3	99	42	14	4
Min	63	63	8	9	8	10	193	224	11	13	4	8	8	29	113	168	82	122
Max	198	181	40	31	45	47	381	377	55	63	25	27	21	33	377	228	123	127
Values	801	621	761	588	706	540	149	81	764	594	749	575	6	2	5	2	6	2

POSITIVE CONTROL VALUES 2000

Strain	TA100		TA1535		WP2uvrA⁻		TA102		TA98		TA1537		TA1538		WP2uvrA⁻ pkm101		TA97a	
S9-Mix	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Mean	488	1620	382	278	663	664	877	794	138	259	1071	466	225	607	2089	1389	261	685
SD	137	490	260	89	243	212	137	123	34	70	327	121	28	40	175	967	1	141
Min	234	443	125	128	203	185	522	388	89	129	290	191	193	568	1965	705	260	585
Max	1190	3079	1843	630	1520	1534	1161	1090	262	506	2160	768	245	648	2213	2073	261	785
Values	172	172	172	170	166	166	69	70	173	172	171	170	3	3	2	2	2	2

COMBINED VEHICLE AND UNTREATED CONTROL VALUES 2001

Strain	TA100		TA1535		WP2uvrA⁻		TA102		TA98		TA1537		TA1538		WP2uvrA⁻ pkm101		TA97a		TA104	
S9-Mix	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Mean	106	116	16	15	22	26	278	305	23	32	11	13	14	no data available	144	147	116	131	512	no data available
SD	23	23	4	4	5	6	45	39	7	8	4	4	5		61	14	21	24	93	
Min	58	62	8	7	11	13	195	201	10	13	2	4	6		74	131	84	114	389	
Max	178	178	37	37	41	52	405	421	56	58	23	29	20		292	164	134	134	616	
Values	817	658	790	621	656	501	244	157	814	662	793	630	5		10	4	5	2	5	

POSITIVE CONTROL VALUES 2001

Strain	TA100		TA1535		WP2uvrA⁻		TA102		TA98		TA1537		TA1538		WP2uvrA⁻ pkm101		TA97a		TA104	
S9-Mix	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Mean	505	1582	431	336	685	756	849	830	140	303	1415	428	153	501	1946	2102	618	750	825	1445
SD	190	541	412	110	323	243	179	159	43	96	713	146	60	246	794	844	62	142	209	202
Min	259	454	98	113	248	242	461	485	72	136	197	139	113	322	672	956	547	649	644	1224
Max	1829	2738	2039	990	1960	1340	1541	1303	362	679	3616	792	133	413	2790	3100	655	850	1125	1621
Values	165	163	163	161	156	155	92	93	166	164	162	160	4	4	7	7	3	2	4	3

SD = Standard deviation
Min = Minimum value
Max = Maximum value

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Appendix 3 Statement of GLP Compliance in Accordance with Directive 88/320/EEC

THE DEPARTMENT OF HEALTH OF THE GOVERNMENT OF THE UNITED KINGDOM

GOOD LABORATORY PRACTICE

STATEMENT OF COMPLIANCE IN ACCORDANCE WITH DIRECTIVE 88/320 EEC

LABORATORY	TEST TYPE
SafePharm Laboratories Ltd Shardlow Business Park London Road Shardlow Derbyshire DE72 2GD	Analytical Chemistry Environmental Fate Environmental Toxicity Mutagenicity Phys/Chem Tests Toxicology

DATE OF INSPECTION
28 February 2000

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

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

Roger G. Alexander 26/4/00

Dr. Roger G. Alexander
Head, UK GLP Monitoring Authority

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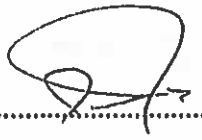
SAFEPHARM LABORATORIES LIMITED

S-Face MX-10

SPL PROJECT NUMBER: 432/056

I verify that this is an exact copy of the original report which is located in the Archives of SafePharm Laboratories Limited, Derby, UK.

P W Thompson HNC
Study Director ..... 

DATE ..... 14 MAY 2003

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Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: Lillian Gill, D.P.A.
Director - COSMETIC INGREDIENT REVIEW (CIR)

FROM: Beth A. Lange, Ph.D.
Industry Liaison to the CIR Expert Panel

DATE: March 25, 2016

SUBJECT: Information on Polyglyceryl-10 Decaethylhexanoate (SLR posted January 28, 2016)

CiTox LAB Hungary Ltd. 2012. The testing of Polyglyceryl-10 Decaethylhexanoate with bacterial reverse mutation assay.

Consumer Product Testing Co. 2012. The MatTek Corporation EpiOcular™ tissue model *in vitro* toxicity testing system (Polyglyceryl-10 Decaethylhexanoate).

Japan Hair Science Association. 2011. Patch test on the cosmetic ingredient (Polyglyceryl-10 Decaethylhexanoate).

Research Institute for Organic Syntheses Inc. 2011. In vitro skin irritation test (EpiDerm™ Model) (Polyglyceryl-10 Decaethylhexanoate).

Dermscan Asia. 2012. Assessment of the sensitizing potential of a cosmetic product: final clinical safety test under dermatological control (Polyglyceryl-10 Decaethylhexanoate).

1620 L Street, N.W., Suite 1200 | Washington, D.C. 20036 | 202.331.1770 | 202.331.1969 (fax) | www.personalcarecouncil.org

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CiToxLAB Hungary Ltd.
Veszprém - Szabadságpuszta

FINAL REPORT

The Testing of Polyglyceryl-10 Decaethylhexanoate with Bacterial Reverse Mutation Assay

Study code: 11/342-007M

Study Director: Judit Hargitai, PhD.

23 March 2012

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STATEMENT OF THE STUDY DIRECTOR

This study has been performed in accordance with the study plan agreed upon by the Sponsor, the OECD Guidelines for Testing of Chemicals (No. 471, 21 July 1997), Commission Regulation (EC) No.440/2008, B.13/14., EPA Health Effects Test Guidelines, OPPTS 870.5100 (EPA 712-C-98-247, 1998 and EPA 712-C-96-247, 1996), and the Principles of Good Laboratory Practice (Hungarian GLP Regulations: 9/2001 (III.30.) EüM-FVM joint decree of the Minister of Health and the Minister of Agriculture and Regional Development, which corresponds to the OECD GLP, ENV/MC/CHEM(98)17).

I, the undersigned, declare that this report constitutes a true record of the actions undertaken and the results obtained in this study. By virtue of my dated signature I accept the responsibility for the validity of the data and the following conclusion drawn from them:

In conclusion, the test item Polyglyceryl-10 Decaethylhexanoate (Batch Number: 91015) had no mutagenic activity in the applied bacterium tester strains under the test conditions used in this study.

Signature: Hargitai Judit Date: 23 March 2012

Judit Hargitai, PhD.
Study Director

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STATEMENT OF THE MANAGEMENT

According to the conditions of the research and development agreement between SAKAMOTO YAKUHIN KOGYO CO., LTD. (as Sponsor) and CiToxLAB Hungary Ltd. [formerly: LAB Research Ltd.] (as Test Facility) the study titled "The Testing of Polyglyceryl-10 Decaethylhexanoate with Bacterial Reverse Mutation Assay" has been performed in compliance with the study plan and the Principles of Good Laboratory Practice.

Signature: _____ Date: 23 Nov 2012

Christopher Banks, DABT
Managing Director

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QUALITY ASSURANCE STATEMENT

Study Code: 11/342-007M

Study Title: The Testing of Polyglyceryl-10 Decaethylhexanoate with Bacterial Reverse Mutation Assay

Test Item: Polyglyceryl-10 Decaethylhexanoate

In order to enable the results of this study to meet legal requirements including regulatory submission and/or safety assessment , this study has been inspected and this report audited by the Quality Assurance Unit in compliance with the Principles of Good Laboratory Practice. As far as it can be reasonably established the methods described and the results incorporated in this report accurately reflect the raw data produced during this study.

All inspections, data reviews and the report audit were reported in written form to the study director and to management. The dates of such inspections and of the report audit are given below:

Date	Phase(s) Inspected/Audited	Date of report to	
		Management	Study Director
09 January 2012	Study Plan	09 January 2012	09 January 2012
24 January 2012	Test item formulation	24 January 2012	24 January 2012
13 March 2012	Draft Report	14 March 2012	13 March 2012
23 March 2012	Final Report	23 March 2012	23 March 2012

Signature: Czuczay Diána Date: 23 March 2012

Diána Czuczay, M.Sc.
On behalf of QA

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GENERAL INFORMATION

STUDY TITLE	:	The Testing of Polyglyceryl-10 Decaethylhexanoate with Bacterial Reverse Mutation Assay
TEST ITEM	:	Polyglyceryl-10 Decaethylhexanoate
SPONSOR	:	SAKAMOTO YAKUHIN KOGYO CO., LTD. Address: 1-20, Rinkaicho, Izumiotsu-City, Osaka, 595-0075, Japan Phone: +81 725 33 6981 Fax: +81 725 23 1198
TEST FACILITY	:	CiToxLAB Hungary Ltd. (Formerly known as LAB Research Ltd. before 1st September 2011) Address: H-8200 Veszprém, Szabadságpuszta, Hungary Phone: +36 88 545 300 Fax: +36 88 545 301
BASIS OF STUDY	:	OECD Guidelines for Testing of Chemicals, No.471 EPA Health Effects Test Guidelines, OPPTS 870.5100 Commission Regulation (EC) No 440/2008, B.13/14.

STUDY PLAN	: 09 January 2012
AMENDMENT 1 TO THE STUDY PLAN	: 20 January 2012
RANGE FINDING TEST	: 13-15 January 2012
EXPERIMENTAL PERIOD I.	: 24-26 January 2012
EXPERIMENTAL PERIOD II.	: 31 January - 02 February 2012
DRAFT REPORT	: 13 March 2012
FINAL REPORT	: 23 March 2012

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RESPONSIBLE PERSONS

TEST FACILITY MANAGEMENT	:	Christopher Banks, DABT Managing Director Alyson Leyshon, M.Sc. Senior Director of Toxicology Operations
QUALITY ASSURANCE	:	Szabolcs Gáty, M.Sc. Head of QAU Vanda Gyimesi, M.Sc. Deputy Head of QAU Diána Czuczay, M.Sc. QA Inspector
STUDY DIRECTOR	:	Judit Hargitai, PhD. Phone: +36 88 545 273 E-mail: judit.hargitai@hu.citoxlab.com
CENTRAL DISPENSARY	:	Tamás Mészáros, PhD. (Technical Team Leader)
TECHNICAL ASSISTANTS	:	Emma Petőné Kohán Tímea Kovácsné Böröcz Ákos Klaffl Levente Jánni, B.Sc.

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1. SUMMARY

The test item Polyglyceryl-10 Decaethylhexanoate was tested for potential mutagenic activity using the Bacterial Reverse Mutation Assay.

The experiments were carried out using histidine-requiring auxotroph strains of *Salmonella typhimurium* (*Salmonella typhimurium* TA98, TA100, TA1535 and TA1537) and the tryptophan-requiring auxotroph strain of *Escherichia coli* (*Escherichia coli* WP2 *uvrA*) in the presence and absence of a post mitochondrial supernatant (S9 fraction) prepared from the livers of phenobarbital/β-naphthoflavone-induced rats.

The study included a Preliminary Solubility Test, a Preliminary Range Finding Test (Informatory Toxicity Test), an Initial Mutation Test (Plate Incorporation Method) and a Confirmatory Mutation Test (Pre-Incubation Method).

Based on the results of the Solubility Test, the test item was dissolved in DMF. Concentrations of 5000; 2500; 1000; 316; 100; 31.6 and 10 µg/plate were examined in the Range Finding Test. Based on the results of the Range Finding Test, the test item concentrations in the Initial Mutation Test and Confirmatory Mutation Test were 5000, 1581, 500, 158.1, 50, 15.81 and 5 µg/plate.

In the Initial Mutation Test and Confirmatory Mutation Test, none of the observed revertant colony numbers were above the respective biological threshold value. There were no consistent dose-related trends and no indication of any treatment effect. In all test item treated groups, the numbers of revertant colonies were below the biological relevance when compared with the solvent controls and were within the historical control range and were within the normal biological variability of the test system.

Microdrops were detected on the plates in the Initial Mutation Test in all examined strains at the highest concentration with and without metabolic activation. Slight inhibitory, cytotoxic effect of the test item was observed in the Confirmatory Mutation Test in *Salmonella typhimurium* TA98 and TA1537 strains with and without metabolic activation and in *Escherichia coli* WP2 *uvrA* strain without metabolic activation at 5000 µg/plate concentration.

The mean values of revertant colonies of the solvent control plates were within the historical control range, the reference mutagens showed the expected increase in the number of revertant colonies, the viability of the bacterial cells was checked by a plating experiment in each test. At least five analyzable concentrations were presented in all strains of the main tests. The tests were considered to be valid.

The reported data of this mutagenicity assay show (see Appendix 2 to 5) that under the experimental conditions applied the test item **did not induce gene mutations by base pair changes or frameshifts in the genome of the strains used**.

In conclusion, the test item Polyglyceryl-10 Decaethylhexanoate had no mutagenic activity in the bacterium tester strains under the test conditions used in this study.

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2. INTRODUCTION

The bacterial reverse mutation test is a microbial assay which detects point mutations induced by chemicals causing base changes or frameshift mutations in the genome of amino-acid requiring strains of *Salmonella typhimurium* and *Escherichia coli*. The auxotrophic *S. typhimurium* and *E. coli* strains are unable to grow on minimal medium - containing inorganic salts and glucose as a carbon source - except for spontaneous revertants. But in the presence of a mutagenic agent, some of them can be converted to prototrophs after a reverse mutation to the wild type. These revertants can grow and form colonies in minimal medium. An increased number of the revertant colonies indicates mutagenic activity of the test item.

The *Salmonella typhimurium* histidine (his) reversion system measures $his^- \rightarrow his^+$ reversions. The *Salmonella typhimurium* strains are constructed to differentiate between base-pair (TA1535, TA100) and frameshift (TA1537, TA98) mutations. The *Escherichia coli* WP2 *uvrA* tryptophan (trp) reversion system measures $trp^- \rightarrow trp^+$ reversions. The *Escherichia coli* WP2 *uvrA* detects mutagens that cause base-pair substitutions (AT to GC).

The bacteria used in this assay did not possess the mammalian liver enzyme system that is known to convert promutagens into mutagenic metabolites. In order to overcome this major drawback, an exogenous metabolic activation system is added in the form of a mammalian microsomal enzyme activation mixture (liver extract, S9 fraction). The activation system uses nicotinamide-adenine dinucleotide phosphate (NADP+)-cytochrome P450 dependent mixed function oxidase enzymes of the liver. The liver extract was obtained from rats, which were pre-treated with phenobarbital and β-naphthoflavone, two inducers of several drug-metabolizing enzymes.

The bacterial mutagenicity test, along with other short-term assays, is used extensively to evaluate substances for mutagenic activity. Some mutagens (e.g.: nitrosamines) are poorly detected in the standard plate incorporation assay, but they can be detected by the pre-incubation assay.

3. OBJECTIVE OF STUDY

The purpose of this study was to evaluate the mutagenic potential of Polyglyceryl-10 Decaethylhexanoate test item by measuring its ability to induce reverse mutations at selected loci of several strains of *Salmonella typhimurium* and at the tryptophan locus of *Escherichia coli* WP2 *uvr*A strain in the presence and absence of activated rat liver S9 fraction.

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4. GUIDELINES

This study followed the procedures indicated by the following internationally accepted guidelines and recommendations:

- Ninth Addendum to OECD Guidelines for Testing of Chemicals, Section 4, No. 471, "Bacterial Reverse Mutation Test", adopted 21st July, 1997
- EPA Health Effects Test Guidelines, OPPTS 870.5100 "Bacterial Reverse Mutation Test", EPA 712-C-98-247, August 1998.
- EPA Health Effects Test Guidelines, OPPTS 870.5100 "*Escherichia coli* WP2 and WP2 *uvrA* Reverse Mutation Assays", EPA 712-C-96-247, June 1996 (Public Draft)
- Commission Regulation (EC) No. 440/2008, B.13/14. "Mutagenicity: Reverse Mutation Test Using Bacteria", 30 May 2008

The method described the above mentioned guidelines conforms to the guidelines for bacterial mutagenicity testing published by the major Japanese Regulatory Authorities including METI, MHLW and MAFF.

5. MATERIALS AND METHODS

5.1. TEST ITEM

5.1.1. Name and Data of Test Item*

Name:	Polyglyceryl-10 Decaethylhexanoate
Chemical Name:	Polyglyceryl-10 Decaethylhexanoate
CAS Number:	84617-31-2
Batch/Lot number:	91015
Appearance:	Pale yellow liquid
Purity:	100 %
Manufacture date:	15 November 2009
Expiry date:	15 December 2012
Storage conditions:	Room temperature (15-25°C)
Safety precautions:	Routine safety precautions (lab coat, gloves, safety glasses, face mask) for unknown materials were applied to assure personnel health and safety.

*Note: Based on the Certificate of Analysis (dated 15 December 2011), Test Item Data Sheet (dated 15 December 2011) and Material Safety Data Sheet (dated 15 December 2011) provided by the Sponsor.

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5.1.2. Identification, Receipt

The test item of a suitable chemical purity was supplied by the Sponsor. All precautions required in the handling and disposal of the test item were outlined by the Sponsor. These documents are part of the raw data. Identification of the test item was performed in the Central Dispensary of CiToxLAB Hungary Ltd. on the basis of the information provided by the Sponsor (name, batch number, appearance and colour). Copy of the Certificate of Analysis is shown in Appendix 1.

5.1.3. Formulation

The behaviour of the test item solutions with the solution of top agar (Sections 5.4.4.) and phosphate buffer (Section 5.5.4.) was examined in a preliminary solubility test. *N,N*-Dimethylformamide was used as solvent to prepare the stock formulation of the test material. Test solutions were freshly prepared at the beginning of the experiments in the testing laboratory by diluting the stock formulation using the selected vehicle.

The respective test concentrations in the main tests are shown in Table 1.

Table 1: Formulation of the Test Item for the Main Experiments

No. of concentration	Concentration of the test item	Concentration (µg/plate)
1	100 mg/mL	5000
2	31.62mg/mL	1581
3	10 mg/mL	500
4	3.162 mg/mL	158.1
5	1 mg/mL	50
6	0.3162 mg/mL	15.81
7	0.1 mg/mL	5

Analytical determination of the test item concentration, stability and homogeneity was not performed because of the character and the short period of study.

5.2. POSITIVE AND NEGATIVE CONTROLS

Positive and negative (solvent) controls were included in the experiments. Routine safety precautions (lab coat, gloves, safety glasses and face mask) were applied to assure personnel health and safety.

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5.2.1. Positive Controls

Strain specific positive controls were included in the assay, which demonstrated the effective performance of the test. The strain specific positive control chemicals are summarized in Table 2.

Table 2: Strain-Specific Positive Control Chemicals

Metabolic activation	Strain specific positive chemical		Vehicle	Dose quantity/plate	Strain
	Chemical and Supplier	Batch number / Expiry date			
non-activation	4-nitro-1,2-phenylene-diamine (NPD) MERCK	S5061894 / 28 Feb 2013	DMSO	4 µg	*Salmonella* TA98
non-activation	Sodium azide (SAZ) SIGMA-ALDRICH	MKBF0623 / 02 Feb 2012	Distilled water	2 µg	*Salmonella* TA100 TA1535
non-activation	9-aminoacridine (9AA) MERCK	S03761 / 31 Dec 2012	DMSO	50 µg	*Salmonella* TA1537
non-activation	Methyl-methanesulfonate (MMS) SIGMA-ALDRICH	MKBG0368V / 22 Nov 2012	Distilled water	2 µL	*E coli* WP2 *uvrA*
activation	2-aminoanthracene (2AA) SIGMA-ALDRICH	STBB1901V / 06 Oct 2012	DMSO	2 µg	all *Salmonella* strains
activation	2-aminoanthracene (2AA) SIGMA-ALDRICH	STBB1901V / 06 Oct 2012	DMSO	50 µg	*E coli* WP2 *uvrA*

Abbreviation for tester strains: *Salmonella*: *Salmonella typhimurium*, *E.coli*: *Escherichia coli*

5.2.2. Negative Controls (Vehicle / Solvent Controls)

In the study three vehicle (solvent) control groups were used depending on the solubility of the test item and the solubility of strain specific positive chemicals. The following chemicals were used for vehicle (solvent) control groups:

<u>*N,N*-Dimethylformamide (DMF):</u>

Supplier:	Sigma-Aldrich Co.
Batch No.:	SZBB1030V
Expiry date:	28 March 2014
Grade:	puriss p.a., ACS reagent, reag Ph. Eur., ≥99.8% (GC)
Purity:	99.9 %

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Dimethyl sulfoxide (DMSO):

Supplier:	Sigma-Aldrich Co.
Batch No.:	BCBF1944V
Expiry date:	January 2017
Grade:	puriss, p.a., ACS reagent, ≥99.9% (GC)
Purity:	99.97%

Distilled water:

Supplier:	TEVA Hungary Co.
Batch No.:	3450611
Expiry date:	June 2014

5.3. BACTERIAL STRAINS

5.3.1. Origin

Date of arrival and origin:

Salmonella typhimurium TA98	16 November 2010, MOLTOX - Molecular Toxicology Inc., Boone, North Carolina, USA
Salmonella typhimurium TA100	16 November 2010, MOLTOX - Molecular Toxicology Inc., Boone, North Carolina, USA
Salmonella typhimurium TA1535	16 November 2010, MOLTOX - Molecular Toxicology Inc., Boone, North Carolina, USA
Salmonella typhimurium TA1537	16 November 2010, MOLTOX - Molecular Toxicology Inc., Boone, North Carolina, USA
Escherichia coli WP2 *uvrA*	16 November 2010, MOLTOX - Molecular Toxicology Inc., Boone, North Carolina, USA

The true copies of original certificates and other documents of the strains are collected and stored in the Microbiological Laboratory of the CiToxLAB Hungary Ltd.

5.3.2. Genotypes

In addition to histidine or tryptophan mutation, each strain has additional mutations, which enhances its sensitivity to mutagens. The *uvrB* (*uvrA*) strains are defective in excision repair, making them more sensitive to the mutagenic and lethal effects of a wide variety of mutagens because they cannot repair DNA damages. The presence of *rfa* mutation increases the permeability of the bacterial lipopolysaccharide wall for larger molecules. The plasmid pKM101 (TA98, TA100) carries the *muc*+ gene which participates in the error-prone "SOS" DNA repair pathway induced by DNA damage.

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This plasmid also carries an ampicillin resistance transfer factor (R-factor) which is used to identify its presence in the cell. The *Escherichia coli* strain used in this test (WP2 *uvrA*) is also defective in DNA excision repair. The genotypes of the tester strains used for mutagenicity testing are summarized in Table 3.

Table 3: Genotypes of the Strains Used for Mutagenicity Testing

Strain	Genotype		Mutations		Main DNA target	Plasmid
	trp./his mutation	type of mutation	cell wall	DNA- repair		
S.typhimurium TA98	*hisD3052*	Frameshift	*rfa*	*uvrB*	GC	pKM101
S.typhimurium TA100	*hisG46*	Base pair substitution	*rfa*	*uvrB*	GC	pKM101
S.typhimurium TA1535	*hisG46*	Base pair substitution	*rfa*	*uvrB*	GC	No
S.typhimurium, TA1537	*hisC3076*	Frameshift	*rfa*	*uvrB*	GC	No
E.coli WP2 *uvrA*	*trpE*	Base pair substitution	+	*uvrA*	AT	No

Abbreviations of tester strains: *S.typhimurium.: Salmonella typhimurium; E.coli: Escherichia coli*

5.3.3. Storage

The strains are stored at -80 ± 10°C in the Culture Collection of the Microbiological Laboratory of the CiToxLAB Hungary Ltd. Frozen permanent cultures of the tester strains were prepared from fresh, overnight cultures to which DMSO was added as a cryoprotective agent.

5.3.4. Confirmation of Phenotypes of Tester Strains

The phenotypes of the tester strains used in the bacterial reverse mutation assays with regard to membrane permeability (*rfa*), UV sensitivity (*uvrA* and *uvrB*), ampicillin resistance (*amp*), as well as spontaneous mutation frequencies are checked regularly according to Ames et al. [1] and Maron and Ames [2].

Established procedures (Standard Operating Procedures) for the preparations of each batch of frozen stock culture, raw data and reports of phenotype confirmation are stored in the Microbiological Laboratory of CiToxLAB Hungary Ltd.

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5.3.5. Spontaneous Reversion of Tester Strains

Each test strain reverts spontaneously at a frequency that is characteristic of the strain. Spontaneous reversion of the test strains to histidine (*Salmonella typhimurium* strains) or tryptophan (in *Escherichia coli* strain) independence is measured routinely in mutagenicity experiments and expressed as the number of spontaneous revertants per plate.

Historical control values for spontaneous revertants (revertants/plate) for untreated control sample without metabolic activation were in the period of 2004 to 2010 were (as guide) as follows: *Salmonella typhimurium* TA98: 8-54, TA100: 58-211, TA1535: 2-31, TA1537: 1-24, *Escherichia coli* WP2 *uvrA*: 9-86. More detailed historical control data are shown in Appendix 6.

5.3.6. Procedure for Growing Cultures

The frozen bacterial cultures were thawed at room temperature and 200 µL inoculum were used to inoculate each 50 mL of Nutrient Broth No.2 (Section 5.4.2.) for the overnight cultures in the assay. The cultures were incubated for 10-14 hours at 37°C in a Gyrotory water bath shaker.

5.3.7. Viability of the Testing Cultures

The viability of each testing culture was determined by plating 0.1 mL of the 10^5, 10^6, 10^7 and 10^8 dilutions prepared by sterile physiological saline on Nutrient Agar (Section 5.4.3.) plates. The viable cell number of the cultures was determined by manual counting after approximately 24-hour incubation at 37°C.

5.4. MEDIA

The supplier, batch number and expiry date of the used chemicals described in sections 5.4.1.-5.4.5. are summarized in Table 6 (Section 5.9. Chemicals Used in the Experiments). The composition of media refers to 1000 mL.

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5.4.1. The Typical Composition (g/1000 mL) of Minimal Glucose Agar

Glucose	20.0	g
Magnesium sulfate	0.2	g
Citric acid	2.0	g
di-Potassium hydrogenphosphate	10.0	g
Sodium ammonium hydrogenphosphate	3.5	g
Agar agar	15.0	g
Distilled water	q.s. ad[1] 1000	mL

Minimal glucose agar plates [MERCKOPLATE] (Batch number: 14706, Expiry date: 22 March 2012 used in the preliminary experiment and Initial Mutation Test; and Batch number: 14776, Expiry date: 15 May 2012 used in the Confirmatory Mutation Test) were provided by Merck. Certificates of Analysis were obtained from the Supplier.

5.4.2. Nutrient Broth No.2

Nutrient Broth No.2	25.0	g
Distilled water	q.s. ad 1000	mL

Sterilization was performed at 121°C in an autoclave.

5.4.3. Nutrient Agar

Nutrient Agar	20.0	g
Distilled water	q.s. ad 1000	mL

Sterilization was performed at 121°C in an autoclave.

5.4.4. Top Agar for *Salmonella typhimurium* Strains

Agar solution:

Agar Bacteriological	4.0	g
NaCl	5.0	g
Distilled water	q.s. ad 1000	mL

Sterilization was performed at 121°C in an autoclave.

[1] q.s. ad = quantum sufficiat ad (a sufficient quantity to make)

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Histidine – Biotin solution (0.5 mM):

D-Biotin (F.W. 244.3)	122.2	mg
L-Histidine x·HCl x H_2O (F.W. 209.63)	104.8	mg
Distilled water	q.s. ad 1000	mL

Sterilization was performed by filtration using a 0.22 µm membrane filter.

Complete Top Agar for *Salmonella typhimurium* strains:

Histidine – Biotin solution (0.5 mM)	100	mL
Agar solution	900	mL

5.4.5. Top Agar for *Escherichia coli* Strain

Tryptophan solution (2 mg/mL):

L-Tryptophan (F.W. 204.23)	2000	mg
Distilled water	q.s. ad 1000	mL

Sterilization was performed by filtration using a 0.22 µm membrane filter.

Complete Top Agar for *Escherichia coli* strain:

Nutrient Broth by 5.4.2.	50	mL
Tryptophan solution (2 mg/mL)	2.5	mL
Agar solution by 5.4.4.	947.5	mL

5.5. METABOLIC ACTIVATION SYSTEM

Test bacteria were also exposed to the test item in the presence of an appropriate metabolic activation system, which is a cofactor-supplemented post-mitochondrial S9 fraction.

The post-mitochondrial fraction (S9 fraction) was prepared by the Microbiological Laboratory of CiToxLAB Hungary Ltd. according to Ames et al. [1] and Maron and Ames [2]. The documentation of the preparation of this post-mitochondrial fraction is stored in the reagent notebook in the Microbiological Laboratory which is archived yearly.

The supplier, batch number and expiry date of the used chemicals described in sections 5.5.3. and 5.5.4. are summarized in Table 6 (Section 5.9. Chemicals Used in the Experiments). The composition of solution refers to 1000 mL.

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5.5.1. Induction of Liver Enzymes

Male Wistar rats (298-354 g, animals were 8 weeks old at the initiation) were treated with phenobarbital (PB) and β-naphthoflavone (BNF) at 80 mg/kg/day by oral gavage for three consecutive days. Rats were given drinking water and food ad libitum until 12 h before sacrifice when food was removed. Sacrifice was by ascending concentration of CO_2, confirmed by cutting through major thoracic blood vessels. Initiation of the induction of liver enzymes used for preparation S9 used in this study was 05 December 2011.

5.5.2. Preparation of Rat Liver Homogenate S9 Fraction

On Day 4, the rats were euthanized and the livers were removed aseptically using sterile surgical tools. After excision, livers were weighed and washed several times in 0.15 M KCl. The washed livers were transferred to a beaker containing 3 mL of 0.15 M KCl per g of wet liver, and homogenized. Homogenates were centrifuged for 10 min at 9000 g and the supernatant was decanted and retained. The freshly prepared S9 fraction was aliquoted into 1-3 mL portions, frozen quickly and stored at -80 ± 10°C. The date of preparation of S9 fraction for this study was 08 December 2011 (CiToxLAB code: E11250).

The sterility of the preparation was confirmed. The protein concentration of the preparation was determined by a chemical analyzer at 540 nm in the Clinical Chemistry Laboratory of CiToxLAB Hungary Ltd. The protein concentration of the S9 fraction used was determined to be 34.4 g/L.

The biological activity in the *Salmonella* assay of S9 was characterized using the two mutagens 2-Aminoanthracene and Benzo(a)pyrene, that requires metabolic activation by microsomal enzymes. The batch of S9 used in this study functioned appropriately.

5.5.3. The S9 Mix

Salt solution for S9 Mix:

NADP Na	7.66 g
D-glucose-6 phosphate Na	3.53 g
$MgCl_2 \times 6\ H_2O$	4.07 g
KCl	6.15 g
Distilled water	q.s. ad 1000 mL

Sterilization was performed by filtration through a 0.22 µm membrane filter.

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The complete S9 mix was freshly prepared containing components as follows:

Ice cold 0.2 M sodium phosphate buffer, pH 7.4	500 mL
Rat liver homogenate (S9)	100 mL
Salt solution for S9 Mix (see above)	400 mL

Prior to addition to the culture medium the S9 mix was kept in an ice bath.

5.5.4. 0.2 M Sodium Phosphate Buffer, pH 7.4

Solution A:

Na_2HPO_4	28.39	g
Distilled water	q.s. ad 1000	mL

Sterilization was performed at 121°C in an autoclave.

Solution B:

$NaH_2PO_4 \times H_2O$	27.6	g
Distilled water	q.s. ad 1000	mL

Sterilization was performed at 121°C in an autoclave.

Sodium phosphate buffer pH 7.4:

Solution A	880	mL
Solution B	120	mL

5.6. DESCRIPTION OF THE TEST PROCEDURE

The study included a Preliminary Solubility Test, a Preliminary Range Finding Test (Informatory Toxicity Test), an Initial Mutation Test and a Confirmatory Mutation Test. In the Range Finding Test as well as in the Initial Mutation Test, the plate incorporation method was used. In the Confirmatory Mutation Test, the pre-incubation method was used.

5.6.1. Concentrations

Concentrations were selected on the basis of the Preliminary Solubility Test and Preliminary Range Finding Test (Informatory Toxicity Test). In the Initial Mutation Test and Confirmatory Mutation Test the same concentrations were examined.

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5.6.1.1. Preliminary Solubility Test

Based on the available information, the test item is insoluble in Distilled water. Therefore, the solubility of the test item was examined in Dimethyl sulfoxide (DMSO) and *N,N*-Dimethylformamide (DMF). The test item was partially soluble in DMSO at 100 mg/mL concentration (opalescent formulation), but it was soluble (clear solution) using DMF at the same concentration. Due to the better solubility, DMF was selected for solvent of the study. The obtained stock solution (50 µL) with the solution of top agar (5.4.4.) and phosphate buffer (5.5.4.) was examined in a test tube without test bacterium suspension. The results of the Solubility Test are summarized in Table 4.

Table 4: The Solubility of the Test Item in DMF

Concentration of test item in DMF (mg/mL)	Solubility in DMF	Solubility in the top solution (test item solution 50 µL + phosphate buffer 500 µL + top agar 2 mL)	Test item concentration in the test tube µg/tube
100	clear solution	opalescence	5000
50	clear solution	slight opalescence	2500
20	clear solution	slight opalescence	1000
6.32	clear solution	clear solution	316

5.6.1.2. Preliminary Concentration Range Finding Test (Informatory Toxicity Test)

Based on the solubility test, 100 mg/mL stock formulation was prepared in DMF which was diluted in 6 steps by factors of 2, 2.5 and approximately √10. The revertant colony numbers and the inhibition of the background lawn of auxotrophic cells of two of the tester strains (*Salmonella typhimurium* TA98 and TA100) were determined at the concentrations of 5000, 2500, 1000, 316, 100, 31.6 and 10 µg/plate of the test item. In the Preliminary Concentration Range Finding Test the plate incorporation method (5.6.4) was used.

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5.6.1.3. Test Item Concentrations in the Mutagenicity Tests (Initial Mutation Test and Confirmatory Mutation Test)

Based on the results of the preliminary tests, 100 mg/mL stock formulation was prepared from the test item with DMF, which was diluted by serial dilution in six steps to obtain seven dosing solutions for lower doses. The maximum test concentration was 5000 µg test item/plate in the main tests.

Examined concentrations were 5000, 1581, 500; 158.1, 50; 15.81 and 5 µg test item/plate.

5.6.2. Control Groups Used in the Tests

Strain-specific positive and negative (solvent) controls, both with and without metabolic activation were included in each test. In addition, untreated control was used demonstrating that the chosen vehicle induced no deleterious or mutagenic effects. The control groups are summarized in Table 5.

Table 5: Summary of Controls

Type of control	Activation with S9 Mix	Solvent	Top Agar	Strain-Specific Positive Chemical Solutions (5.2.1.)	Phosphate Buffer
Untreated	–	–	+	–	+
Untreated	+	–	+	–	–
Solvent	–	+	+	–	+
Solvent	+	+	+	–	–
Positive Reference Control	–	–	+	+	+
Positive Reference Control	+	–	+	+	–

Note: if the solvent of the positive control substance differed from the solvent of the test item, both solvents were run in the assay.

5.6.3. Experimental Method

The experimental methods were conducted according to the methods described Ames et al. [1] and Maron and Ames [2], Kier et al. [3], Venitt and Parry [4], OECD Guideline No. 471, 1997 [5], Commission Regulation (EC) No. 440/2008, 2008 [6], EPA Guidelines, OPPTS 870.5100, 1998, 1996 [7][8], and according to the relevant SOPs of CiToxLAB Hungary Ltd.

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5.6.4. Procedure for Exposure in the Initial Mutation Test

A standard plate incorporation procedure was performed, as an Initial Mutation Test. Bacteria (cultured in Nutrient Broth No.2 as described in Section 5.3.6.) were exposed to the test item both in the presence and absence of an appropriate metabolic activation system.

Molten top agar was prepared and kept at 45°C. 2 mL of top agar was aliquoted into individual test tubes (3 tubes per control or concentration level). The equivalent number of minimal glucose agar plates was properly labelled. The test item and other components were prepared freshly and added to the overlay (45°C).

The content of the tubes:

top agar	2000 µL
solvent or test item solution (or reference controls)	50 µL
overnight culture of test strain	100 µL
phosphate buffer (pH 7.4) or S9 mix	500 µL

This solution was mixed and poured on the surface of minimal agar plates. For activation studies, instead of phosphate buffer, 0.5 mL of the S9 mix was added to each overlay tube. The entire test consisted of non-activated and activated test conditions, with the addition of untreated, negative (solvent) and positive controls. After preparation, the plates were incubated at 37°C for 48 hours.

5.6.5. Procedure for Exposure in the Confirmatory Mutation Test

A pre-incubation procedure [1][2][5][6][7] was performed as a Confirmatory Mutation Test since in the Initial Mutation Test no positive effect was observed (see details in Section 6.).

Before the overlaying, the test item solution (or solvent or reference control), the bacterial culture (Section 5.3.6.) and the S9 mix or phosphate buffer was added into appropriate tubes to provide direct contact between bacteria and the test item (in its solvent). The tubes (3 tubes per control or concentration level) were gently mixed and incubated for 20 min at 37°C in a shaking incubator.

After the incubation period, 2 mL of molten top agar was added to the tubes; the content was mixed up and poured onto minimal glucose agar plates as described for the standard plate incorporation method (Section 5.6.4.). The entire test consisted of non-activated and activated test conditions, with the addition of untreated, negative (solvent) and positive controls. After preparation, the plates were incubated at 37°C for 48 hours.

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5.7. EVALUATION OF EXPERIMENTAL DATA

The colony numbers on the untreated / negative (solvent) / positive control and test item treated plates were determined by manual counting. Visual examination of the plates was also performed; precipitation or signs of growth inhibition (if any) were recorded and reported. The mean number of revertants per plate, the standard deviation and the mutation factor* values were calculated for each concentration level of the test item and for the controls using Microsoft Excel™ software.

* Mutation factor (MF): mean number of revertants on the test item plate / mean number of revertants on the vehicle control plate.

Criteria for Validity:

The study was considered valid if:
- the number of revertant colonies of the negative (solvent) and positive controls were in the historical control range in all strains of the main tests;
- at least five analyzable concentrations were presented in all strains of the main tests.

Criteria for a Positive Response:

A test item is considered mutagenic if:
- a dose–related increase in the number of revertants occurs and/or;
- a reproducible biologically relevant positive response for at least one of the dose groups occurs in at least one strain with or without metabolic activation.

An increase is considered biologically relevant if:
- in all strains: the number of reversion more than twice higher than the reversion rate of the negative (solvent) control.

According to the guidelines [5][6][7], statistical method may be used as an aid in evaluating the test results. However, statistical significance should not be the only determining factor for a positive response.

Criteria for a Negative Response:

A test article is considered non-mutagenic if it produces neither a dose-related increase in the number of revertants nor a reproducible biologically relevant positive response at any of the dose groups, with or without metabolic activation.

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5.8. ARCHIVES

The study documents and samples:

- study plan and study plan amendment,
- all raw data,
- retained sample of the test item,
- correspondence,
- study report and any amendments

will be stored in the archives of CiToxLAB Hungary Ltd. (H-8200 Veszprém, Szabadságpuszta, Hungary) according to the Hungarian GLP regulation [9] and to test facility SOPs.

After the retention time has elapsed, all the archived materials listed above would be offered to the Sponsor or retained for a further period if agreed by a contract. No raw data or material relating to the study will be discarded without the Sponsor's prior written consent.

5.9. CHEMICALS USED IN THE EXPERIMENTS

The chemicals used in the independently performed experiments of the study are summarized in Table 6.

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Table 6: Chemicals Used in the Experiments

Chemical	Supplier	Batch Number	Expiry date
Agar Bacteriological	OXOID	1164200-02	21 March 2015
D-Biotin	SIGMA-ALDRICH	021M1530V	February 2015
Distilled water	Hungaro-Gal Ltd.	813 1011	03 April 2012
Distilled water	Hungaro-Gal Ltd.	817 1211	19 June 2012
D-Glucose 6-phosphate Na	SIGMA-ALDRICH	031M7004V	May 2014
L-Histidine HCl x H_2O	MERCK	K37522450	30 June 2012
KCl	MERCK	K42209636	30 April 2016
$MgCl_2$ x 6 H_2O	SIGMA-ALDRICH	100M0075V	October 2015
NaCl	SIGMA-ALDRICH	SZBB215BV	14 January 2015
NADP Na x H_2O	Apollo Scientific Ltd.	AS422477	28 February 2012
NaH_2PO_4 x H_2O	CARLO ERBA	0N244191A	December 2016
Na_2HPO_4	SIGMA-ALDRICH	SZBB0590V	13 February 2014
Nutrient Agar	MERCK	VM264950	18 March 2016
Nutrient Broth No.2	OXOID	1093145	30 September 2016
L-Tryptophan	SIGMA-ALDRICH	1400132	July 2012

NADP: β-Nicotinamide adenine dinucleotide phosphate

5.10. DEVIATIONS TO THE STUDY PLAN

There was no deviation to the Study Plan.

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6. RESULTS AND DISCUSSION

6.1. PRELIMINARY RANGE FINDING TEST (INFORMATORY TOXICITY TEST)

In the Preliminary Range Finding Test, the plate incorporation method was used. The preliminary test was performed using *Salmonella typhimurium* TA98 and *Salmonella typhimurium* TA100 tester strains in the presence and absence of metabolic activation system (±S9 Mix) with appropriate untreated, negative (solvent) and positive controls. In the test each samples (including the controls) were tested in triplicate.

In the Range Finding Test the concentrations examined were: 5000, 2500, 1000, 316, 100, 31.6 and 10 µg/plate.

The observed numbers of revertant colonies were mostly in the normal range. Minor differences (higher number of revertant colonies compared to the solvent control plates) were observed at some examined concentrations. However, they had no biological significance and were considered as reflecting the variability of the test system.

No precipitate or sign of cytotoxicity was observed in the Preliminary Range Finding Test.

The experimental results (revertant colony numbers per plate, mutation factors and standard deviations) are detailed in Table 7 (Appendix 2) and in Appendix 3.

6.2. INITIAL AND CONFIRMATORY MUTATION TESTS

In the Initial Mutation Test, the plate incorporation method; in the Confirmatory Mutation Test, the pre-incubation method was used. The Initial Mutation Test and Confirmatory Mutation Test were carried out using four *Salmonella typhimurium* strains (TA98, TA100, TA1535 and TA1537) and *Escherichia coli* WP2 *uvrA* strain. The Initial Mutation Test and Confirmatory Mutation Test were performed in the presence and absence of metabolic activation system (±S9 mix). Each test was performed with appropriate untreated, negative (solvent) and positive controls. In the main tests each samples (including the controls) were tested in triplicate.

The examined test item concentrations were 5000, 1581, 500, 158.1, 50, 15.81 and 5 µg/plate in both main tests.

In the Initial Mutation Test and Confirmatory Mutation Tests none of the observed revertant colony numbers were above the respective biological threshold value. There were no reproducible dose-related trends and no indication of any treatment effect.

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Using the plate incorporation method (Initial Mutation Test), the highest revertant rate was observed in *Salmonella typhimurium* TA1537 bacterial strain without metabolic activation at the concentration of 5000 µg/plate. The mutation factor value was 1.47. However, the observed mutation factor value was well below the biologically relevant threshold limit and the numbers of revertant colonies were within the historical control range.

Using the pre-incubation method (Confirmatory Mutation Test), the highest revertant rate was observed in *Salmonella typhimurium* TA98 bacterial strain with metabolic activation at 50 µg/plate concentration. The mutation factor value was 1.60. Higher numbers of revertant colonies compared to the solvent control plates were observed in the whole tested concentration range in this strain. However, no dose-dependent relationship was observed, and the observed mutation factors were below the biologically relevant threshold value. The numbers of revertant colonies were within the historical control range. Furthermore, higher number of revertant colonies compared to the DMF control was observed for untreated control (MF: 1.38) and DMSO control (MF: 1.55) also in this strain.

Higher numbers of revertant colonies compared to the solvent control were detected in the Initial Mutation Test and Confirmatory Mutation Test in other sporadic cases. However, no dose-dependence was observed and they were below the biologically relevant threshold value. The numbers of revertant colonies were within the historical control range in all cases, so they were considered as reflecting the biological variability of the test.

Sporadically, lower revertant counts compared to the solvent control were observed in the Initial Mutation Test and Confirmatory Mutation Test in some cases. However, the mean numbers of revertant colonies were in the historical control range in all cases, thus they were considered as biological variability of the test system.

Microdrops were detected on the plates in the Initial Mutation Test in all examined strains at the highest concentration (5000 µg/plate concentration) with and without metabolic activation.

Slight inhibitory, cytotoxic effect of the test item (reduced or slightly reduced background lawn development) was observed in the Confirmatory Mutation Test in *Salmonella typhimurium* TA98 and TA1537 strains at 5000 µg/plate concentration with and without metabolic activation, and in *Escherichia coli* WP2 *uvrA* strain at 5000 µg/plate concentration without metabolic activation.

The experimental results (revertant colony numbers per plate, mutation factors, and standard deviations) are summarized in Tables 8-9 (Appendix 2) and Appendices 4 and 5.

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6.3. VALIDITY OF THE TESTS

Untreated, negative (solvent) and positive controls were run concurrently. The mean values of revertant colony numbers of untreated, negative (solvent) and positive control plates were within the historical control range. At least five analyzable concentrations were presented in all strains of the main tests.

The reference mutagens showed a distinct increase of induced revertant colonies. The viability of the bacterial cells was checked by a plating experiment in each test. The tests were considered to be valid.

7. CONCLUSION

The test item Polyglyceryl-10 Decaethylhexanoate was tested for potential mutagenic activity using the Bacterial Reverse Mutation Assay.

The experiments were carried out using histidine-requiring auxotroph strains of *Salmonella typhimurium* (*Salmonella typhimurium* TA98, TA100, TA1535 and TA1537), and the tryptophan-requiring auxotroph strain of *Escherichia coli* (*Escherichia coli* WP2 *uvrA)* in the presence and absence of a metabolic activation system, which was a cofactor-supplemented post-mitochondrial S9 fraction prepared from the livers of phenobarbital/β-naphthoflavone-induced rats.

The study included a Preliminary Solubility Test, a Preliminary Range Finding Test (Informatory Toxicity Test), an Initial Mutation Test and a Confirmatory Mutation Test. In the Range Finding Test as well as in the Initial Mutation Test, the plate incorporation method was used. In the Confirmatory Mutation Test, the pre-incubation method was used.

The reported data of this mutagenicity assay show (see Appendix 2 to 5) that under the experimental conditions applied the test item **did not induce gene mutations by base pair changes or frameshifts in the genome of the strains used.**

In conclusion, the test item Polyglyceryl-10 Decaethylhexanoate had no mutagenic activity in the applied bacterium tester strains under the test conditions used in this study.

8. DISTRIBUTION OF THE FINAL REPORT

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1x copy, unbound
1x PDF file

Archive: 1x original, bound

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9. REFERENCES

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APPENDICES

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APPENDIX 1

Copy of the Certificate of Analysis

SAKAMOTO YAKUHIN KOGYO CO.,LTD.

HEAD OFFICE : 1-2-6 AWAJIMACHI,CHUO-KU,OSAKA CITY 541-0047,JAPAN
TEL.(81)6-6231-1851 FAX (81)6-6222-0631
TOKYO BRANCH : 1-8-2 MARUNOUCHI,CHIYODA-KU,TOKYO 100-0005,JAPAN
TEL.(81)3-3211-3813 FAX (081)3-3215-1789

date : Dec. 15, 2011

Product : Polyglyceryl-10 Decaethylhexanoate
Lot. No. : 91015

Certificate of analysis / Quality	Result
Description	A pale yellow viscous liquid with a faint characteristic odor
Color (Gardner)	less than 1
Acid value (mgKOH/g)	0.1
Heavy metal (ppm)	less than 20
Arsenic (ppm)	less than 2
Residue on ignition (%)	less than 0.1

Expiration date: Dec. 15, 2012

Satoshi Ohshima
Quality Control Manager

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APPENDIX 2

Summary Tables of the Results

Table 7: Summary Table of the Range Finding Test

Concentrations (µg/plate)	Mean values of revertants / Mutation factor (MF)	*Salmonella typhimurium* tester strains			
		TA98		TA100	
		-S9	+S9	-S9	+S9
Untreated control	Mean	20.7	30.0	84.0	93.3
	MF	1.11	1.18	1.04	1.01
Distilled water control	Mean	-	-	77.0	-
	MF	-	-	0.95	-
DMSO control	Mean	20.0	25.0	-	95.7
	MF	1.07	0.99	-	1.04
DMF control	Mean	18.7	25.3	80.7	92.3
	MF	1.00	1.00	1.00	1.00
5000	Mean	17.0	26.0	79.3	76.7
	MF	0.91	1.03	0.98	0.83
2500	Mean	23.3	26.3	75.3	82.3
	MF	1.25	1.04	0.93	0.89
1000	Mean	25.0	22.7	75.7	79.0
	MF	1.34	0.89	0.94	0.86
316	Mean	27.0	25.7	94.3	101.3
	MF	1.45	1.01	1.17	1.10
100	Mean	26.0	24.3	73.7	92.0
	MF	1.39	0.96	0.91	1.00
31.6	Mean	26.3	26.0	81.0	89.3
	MF	1.41	1.03	1.00	0.97
10	Mean	23.0	26.0	70.7	82.3
	MF	1.23	1.03	0.88	0.89
NPD (4µg)	Mean	296.0	-	-	-
	MF	14.80	-	-	-
2AA (2µg)	Mean	-	2490.7	-	2568.0
	MF	-	99.63	-	26.84
SAZ (2µg)	Mean	-	-	1526.0	-
	MF	-	-	19.82	-

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APPENDIX 2 (continued)

Summary Tables of the Results

Table 8: Summary Table of the Initial Mutation Test

Concentrations (µg/plate)	Mean values of revertants / Mutation factor (MF)	*Salmonella typhimurium* tester strains								*Escherichia coli*	
		TA98		TA100		TA1535		TA1537		WP2 *uvrA*	
		-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Untreated control	Mean	23.7	35.0	95.3	93.3	5.3	11.3	4.7	6.0	29.7	35.7
	MF	0.93	1.06	1.10	0.94	0.80	1.21	0.82	0.90	0.85	0.87
Distilled water control	Mean	-	-	82.7	-	6.7	-	-	-	36.0	-
	MF	-	-	0.95	-	1.00	-	-	-	1.03	-
DMSO control	Mean	24.0	35.3	-	91.3	-	10.7	4.7	5.3	-	42.3
	MF	0.95	1.07	-	0.92	-	1.14	0.82	0.80	-	1.03
DMF control	Mean	25.3	33.0	86.7	99.3	6.7	9.3	5.7	6.7	35.0	41.0
	MF	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
5000	Mean	20.7	35.3	86.0	107.0	9.3	13.3	8.3	7.0	25.7	36.0
	MF	0.82	1.07	0.99	1.08	1.40	1.43	1.47	1.05	0.73	0.88
1581	Mean	26.0	31.7	93.7	118.0	6.0	13.7	7.7	6.0	30.0	39.7
	MF	1.03	0.96	1.08	1.19	0.90	1.46	1.35	0.90	0.86	0.97
500	Mean	22.0	31.0	111.3	124.7	7.3	10.3	6.3	5.3	31.0	32.7
	MF	0.87	0.94	1.28	1.26	1.10	1.11	1.12	0.80	0.89	0.80
158.1	Mean	26.0	32.3	118.7	115.7	6.3	6.0	3.3	5.3	30.0	38.0
	MF	1.03	0.98	1.37	1.16	0.95	0.64	0.59	0.80	0.86	0.93
50	Mean	22.7	29.7	98.3	134.3	7.3	11.7	5.3	7.7	30.7	35.0
	MF	0.89	0.90	1.13	1.35	1.10	1.25	0.94	1.15	0.88	0.85
15.81	Mean	24.7	34.0	100.7	107.3	8.7	8.3	3.7	8.3	28.3	38.7
	MF	0.97	1.03	1.16	1.08	1.30	0.89	0.65	1.25	0.81	0.94
5	Mean	26.7	34.0	89.7	102.7	6.3	9.3	6.7	5.3	28.3	32.0
	MF	1.05	1.03	1.03	1.03	0.95	1.00	1.18	0.80	0.81	0.78
NPD (4µg)	Mean	334.3	-	-	-	-	-	-	-	-	-
	MF	13.93	-	-	-	-	-	-	-	-	-
2AA (2µg)	Mean	-	2468.0	-	2545.3	-	211.0	-	200.0	-	-
	MF	-	69.85	-	27.87	-	19.78	-	37.50	-	-
2AA (50µg)	Mean	-	-	-	-	-	-	-	-	-	235.3
	MF	-	-	-	-	-	-	-	-	-	5.56
SAZ (2µg)	Mean	-	-	1678.7	-	1297.3	-	-	-	-	-
	MF	-	-	20.31	-	194.60	-	-	-	-	-
9AA (50µg)	Mean	-	-	-	-	-	-	433.3	-	-	-
	MF	-	-	-	-	-	-	92.86	-	-	-
MMS (2µL)	Mean	-	-	-	-	-	-	-	-	1361.3	-
	MF	-	-	-	-	-	-	-	-	37.81	-

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APPENDIX 2 (continued)

Summary Tables of the Results

Table 9: Summary Table of the Confirmatory Mutation Test

Concentrations (µg/plate)	Mean values of revertants / Mutation factor (MF)	*Salmonella typhimurium* tester strains								*Escherichia coli*	
		TA98		TA100		TA1535		TA1537		WP2 *uvrA*	
		-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Untreated control	Mean	16.7	27.7	88.0	91.3	9.0	14.3	6.7	7.3	29.3	40.0
	MF	1.16	1.38	0.99	0.85	1.29	1.19	0.87	0.92	0.78	1.14
Distilled water control	Mean	-	-	79.7	-	9.7	-	-	-	26.7	-
	MF	-	-	0.90	-	1.38	-	-	-	0.71	-
DMSO control	Mean	18.0	31.0	-	99.0	-	13.3	6.0	6.7	-	37.3
	MF	1.26	1.55	-	0.92	-	1.11	0.78	0.83	-	1.07
DMF control	Mean	14.3	20.0	88.7	107.7	7.0	12.0	7.7	8.0	37.7	35.0
	MF	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
5000	Mean	16.0	29.3	88.0	102.3	10.7	11.0	10.3	6.0	36.3	25.7
	MF	1.12	1.47	0.99	0.95	1.52	0.92	1.35	0.75	0.96	0.73
1581	Mean	21.0	28.7	83.0	118.0	7.3	8.7	7.0	10.3	34.3	25.0
	MF	1.47	1.43	0.94	1.10	1.05	0.72	0.91	1.29	0.91	0.71
500	Mean	22.7	28.7	94.7	118.3	7.3	10.7	6.0	7.7	34.0	33.0
	MF	1.58	1.43	1.07	1.10	1.05	0.89	0.78	0.96	0.90	0.94
158.1	Mean	17.7	28.0	100.0	108.7	6.7	9.7	9.3	10.0	35.3	23.3
	MF	1.23	1.40	1.13	1.01	0.95	0.81	1.22	1.25	0.94	0.67
50	Mean	15.3	32.0	96.7	106.3	7.0	11.0	7.3	7.7	38.7	27.3
	MF	1.07	1.60	1.09	0.99	1.00	0.92	0.96	0.96	1.03	0.78
15.81	Mean	15.7	27.0	109.7	109.0	6.7	7.7	10.0	9.3	33.3	26.0
	MF	1.09	1.35	1.24	1.01	0.95	0.64	1.30	1.17	0.88	0.74
5	Mean	18.7	29.0	84.3	99.3	7.3	9.7	11.3	8.3	28.3	22.7
	MF	1.30	1.45	0.95	0.92	1.05	0.81	1.48	1.04	0.75	0.65
NPD (4µg)	Mean	346.7	-	-	-	-	-	-	-	-	-
	MF	19.26	-	-	-	-	-	-	-	-	-
2AA (2µg)	Mean	-	2348.0	-	2561.3	-	200.3	-	213.0	-	-
	MF	-	75.74	-	25.87	-	15.03	-	31.95	-	-
2AA (50µg)	Mean	-	-	-	-	-	-	-	-	-	243.0
	MF	-	-	-	-	-	-	-	-	-	6.51
SAZ (2µg)	Mean	-	-	1358.7	-	1226.7	-	-	-	-	-
	MF	-	-	17.05	-	126.90	-	-	-	-	-
9AA (50µg)	Mean	-	-	-	-	-	-	512.0	-	-	-
	MF	-	-	-	-	-	-	85.33	-	-	-
MMS (2µL)	Mean	-	-	-	-	-	-	-	-	1182.7	-
	MF	-	-	-	-	-	-	-	-	44.35	-

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APPENDIX 3

Pre-Experiment for Toxicity (Informatory Toxicity Test)

Test Item: Polyglyceryl-10 Decaethylhexanoate
Date: 13-15 January 2012
Strain: *Salmonella typhimurium* TA98
Cell count (overnight culture): 1.93×10^9 CFU/mL

Concentration: (μg/plate)		Revertant colony number -S9	Revertant colony number +S9
5000		13	29
		18	24
		20	25
	Mean	17.0	26.0
	SD	3.61	2.65
	MF	0.91	1.03
2500		25	28
		24	27
		21	24
	Mean	23.3	26.3
	SD	2.08	2.08
	MF	1.25	1.04
1000		26	19
		23	24
		26	25
	Mean	25.0	22.7
	SD	1.73	3.21
	MF	1.34	0.89
316		29	26
		27	25
		25	26
	Mean	27.0	25.7
	SD	2.00	0.58
	MF	1.45	1.01
100		26	27
		24	20
		28	26
	Mean	26.0	24.3
	SD	2.00	3.79
	MF	1.39	0.96
31.6		26	25
		29	29
		24	24
	Mean	26.3	26.0
	SD	2.52	2.65
	MF	1.41	1.03
10		20	28
		25	27
		24	23
	Mean	23.0	26.0
	SD	2.65	2.65
	MF	1.23	1.03

Concentration: (μg/plate)		Revertant colony number -S9		Revertant colony number +S9
Untreated control		21		31
		21		32
		20		27
	Mean	20.7		30.0
	SD	0.58		2.65
	MF	1.11		1.18
DMSO control*		19		28
		23		24
		18		23
	Mean	20.0		25.0
	SD	2.65		2.65
	MF	1.07		0.99
DMF control		18		27
		21		25
		17		24
	Mean	18.7		25.3
	SD	2.08		1.53
	MF	1.00		1.00
Positive control NPD (4 μg)		286	2AA (2 μg)	2498
		298		2520
		304		2454
	Mean	296.0		2490.7
	SD	9.17		33.61
	MF	14.80		99.63

MF: Mutation factor
SD: Standard deviation
+S9: with S9 mix
-S9: without S9 mix

$$\text{Mutation factor} = \frac{\text{mean revertants (test item)}}{\text{mean revertants (solvent control)}}$$

*DMSO control was used because of NPD and 2AA

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APPENDIX 3 (continued)

Pre-Experiment for Toxicity (Informatory Toxicity Test)

Test Item: Polyglyceryl-10 Decaethylhexanoate
Date: 13-15 January 2012
Strain: *Salmonella typhimurium* TA100
Cell count (overnight culture): 2.07×10^9 CFU/mL

Concentration: (µg/plate)		Revertant colony number -S9	Revertant colony number +S9
5000		76	77
		86	75
		76	78
	Mean	79.3	76.7
	SD	5.77	1.53
	MF	0.98	0.83
2500		75	85
		79	80
		72	82
	Mean	75.3	82.3
	SD	3.51	2.52
	MF	0.93	0.89
1000		75	84
		76	79
		76	74
	Mean	75.7	79.0
	SD	0.58	5.00
	MF	0.94	0.86
316		98	104
		99	102
		86	98
	Mean	94.3	101.3
	SD	7.23	3.06
	MF	1.17	1.10
100		70	84
		72	106
		79	86
	Mean	73.7	92.0
	SD	4.73	12.17
	MF	0.91	1.00
31.6		73	92
		88	92
		82	84
	Mean	81.0	89.3
	SD	7.55	4.62
	MF	1.00	0.97
10		70	82
		70	83
		72	82
	Mean	70.7	82.3
	SD	1.15	0.58
	MF	0.88	0.89

Concentration: (µg/plate)		Revertant colony number -S9		Revertant colony number +S9
Untreated control		84		97
		83		98
		85		85
	Mean	84.0		93.3
	SD	1.00		7.23
	MF	1.04		1.01
DMSO control*		78		95
		92		88
		75		104
	Mean	81.7		95.7
	SD	9.07		8.02
	MF	1.01		1.04
DMF control		79		80
		89		110
		74		87
	Mean	80.7		92.3
	SD	7.64		15.70
	MF	1.00		1.00
Distilled water** control		71		84
		82		97
		78		95
	Mean	77.0		92.0
	SD	5.57		7.00
	MF	0.95		1.00
Positive control SAZ (2 µg)		1520	2AA (2 µg)	2536
		1564		2564
		1494		2604
	Mean	1526.0		2568.0
	SD	35.38		34.18
	MF	19.82		26.84

MF: Mutation factor
SD: Standard deviation
+S9: with S9 mix
-S9: without S9 mix

$$\text{Mutation factor} = \frac{\text{mean revertants (test item)}}{\text{mean revertants (solvent control)}}$$

*DMSO control was used because of 2AA
**Distilled water control was used because of SAZ

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APPENDIX 4

Initial Mutation Test (Plate Incorporation Method)

Test Item: Polyglyceryl-10 Decaethylhexanoate
Date: 24-26 January 2012
Strain: *Salmonella typhimurium* TA98
Cell count (overnight culture): 1.58×10^9 CFU/mL

Concentration: (µg/plate)		Revertant colony number -S9	Revertant colony number +S9
5000		17 MD	37 MD
		23 MD	37 MD
		22 MD	32 MD
	Mean	20.7	35.3
	SD	3.21	2.89
	MF	0.82	1.07
1581		30	30
		22	41
		26	24
	Mean	26.0	31.7
	SD	4.00	8.62
	MF	1.03	0.96
500		30	28
		19	31
		17	34
	Mean	22.0	31.0
	SD	7.00	3.00
	MF	0.87	0.94
158.1		23	21
		38	39
		17	37
	Mean	26.0	32.3
	SD	10.82	9.87
	MF	1.03	0.98
50		19	25
		26	29
		23	35
	Mean	22.7	29.7
	SD	3.51	5.03
	MF	0.89	0.90
15.81		28	35
		23	40
		23	27
	Mean	24.7	34.0
	SD	2.89	6.56
	MF	0.97	1.03
5		27	29
		30	37
		23	36
	Mean	26.7	34.0
	SD	3.51	4.36
	MF	1.05	1.03

Concentration: (µg/plate)		Revertant colony number -S9		Revertant colony number +S9
Untreated control		30		36
		21		38
		20		31
	Mean	23.7		35.0
	SD	5.51		3.61
	MF	0.93		1.06
DMSO control*		24		26
		21		41
		27		39
	Mean	24.0		35.3
	SD	3.00		8.14
	MF	0.95		1.07
DMF control		30		32
		20		25
		26		42
	Mean	25.3		33.0
	SD	5.03		8.54
	MF	1.00		1.00
Positive control		324	2AA	2392
NPD		299	(2 µg)	2488
(4 µg)		380		2524
	Mean	334.3		2468.0
	SD	41.48		68.23
	MF	13.93		69.85

MD: Microdrops
MF: Mutation factor
SD: Standard deviation
+S9: with S9 mix
-S9: without S9 mix

$$\text{Mutation factor} = \frac{\text{mean revertants (test item)}}{\text{mean revertants (solvent control)}}$$

*DMSO control was used because of NPD and 2AA

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APPENDIX 4 (continued)

Initial Mutation Test (Plate Incorporation Method)

Test Item: Polyglyceryl-10 Decaethylhexanoate
Date: 24-26 January 2012
Strain: *Salmonella typhimurium* TA100
Cell count (overnight culture): 1.93 x 10^9 CFU/mL

Concentration: (µg/plate)		Revertant colony number -S9	Revertant colony number +S9
5000		84 MD	90 MD
		94 MD	114 MD
		80 MD	117 MD
	Mean	86.0	107.0
	SD	7.21	14.80
	MF	0.99	1.08
1581		77	124
		102	118
		102	112
	Mean	93.7	118.0
	SD	14.43	6.00
	MF	1.08	1.19
500		120	130
		117	125
		97	119
	Mean	111.3	124.7
	SD	12.50	5.51
	MF	1.28	1.26
158.1		127	111
		117	121
		112	115
	Mean	118.7	115.7
	SD	7.64	5.03
	MF	1.37	1.16
50		112	150
		96	148
		87	105
	Mean	98.3	134.3
	SD	12.66	25.42
	MF	1.13	1.35
15.81		91	104
		107	119
		104	99
	Mean	100.7	107.3
	SD	8.50	10.41
	MF	1.16	1.08
5		79	99
		89	104
		101	105
	Mean	89.7	102.7
	SD	11.02	3.21
	MF	1.03	1.03

Concentration: (µg/plate)		Revertant colony number -S9		Revertant colony number +S9
Untreated control		95		95
		100		104
		91		81
	Mean	95.3		93.3
	SD	4.51		11.59
	MF	1.10		0.94
DMSO control*		87		95
		119		96
		95		83
	Mean	100.3		91.3
	SD	16.65		7.23
	MF	1.16		0.92
Distilled water control**		96		79
		65		86
		87		90
	Mean	82.7		85.0
	SD	15.95		5.57
	MF	0.95		0.86
DMF control		84		85
		94		106
		82		107
	Mean	86.7		99.3
	SD	6.43		12.42
	MF	1.00		1.00
Positive control SAZ (2 µg)		1800	2AA (2 µg)	2536
		1720		2504
		1516		2596
	Mean	1678.7		2545.3
	SD	146.44		46.70
	MF	20.31		27.87

MD: Microdrops
MF: Mutation factor
SD: Standard deviation
+S9: with S9 mix
-S9: without S9 mix

$$\text{Mutation factor} = \frac{\text{mean revertants (test item)}}{\text{mean revertants (solvent control)}}$$

*DMSO control was used because of 2AA
**Distilled water control was used because of SAZ

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APPENDIX 4 (continued)

Initial Mutation Test (Plate Incorporation Method)

Test Item: Polyglyceryl-10 Decaethylhexanoate
Date: 24-26 January 2012
Strain: *Salmonella typhimurium* TA1535
Cell count (overnight culture): 1.89×10^9 CFU/mL

Concentration: (µg/plate)		Revertant colony number -S9	Revertant colony number +S9
5000		11 MD	13 MD
		9 MD	13 MD
		8 MD	14 MD
	Mean	9.3	13.3
	SD	1.53	0.58
	MF	1.40	1.43
1581		7	13
		5	13
		6	15
	Mean	6.0	13.7
	SD	1.00	1.15
	MF	0.90	1.46
500		9	10
		6	15
		7	6
	Mean	7.3	10.3
	SD	1.53	4.51
	MF	1.10	1.11
158.1		5	6
		8	4
		6	8
	Mean	6.3	6.0
	SD	1.53	2.00
	MF	0.95	0.64
50		4	14
		8	8
		10	13
	Mean	7.3	11.7
	SD	3.06	3.21
	MF	1.10	1.25
15.81		10	9
		7	7
		9	9
	Mean	8.7	8.3
	SD	1.53	1.15
	MF	1.30	0.89
5		8	10
		5	10
		6	8
	Mean	6.3	9.3
	SD	1.53	1.15
	MF	0.95	1.00

Concentration: (µg/plate)		Revertant colony number -S9	Revertant colony number +S9
Untreated control		6	13
		3	8
		7	13
	Mean	5.3	11.3
	SD	2.08	2.89
	MF	0.80	1.21
DMSO control*		7	10
		3	14
		7	8
	Mean	5.7	10.7
	SD	2.31	3.06
	MF	0.85	1.14
Distilled water control**		4	10
		7	5
		9	11
	Mean	6.7	8.7
	SD	2.52	3.21
	MF	1.00	0.93
DMF control		4	15
		7	6
		9	7
	Mean	6.7	9.3
	SD	2.52	4.93
	MF	1.00	1.00
Positive control SAZ (2 µg)		1300 2AA	213
		1312 (2 µg)	209
		1280	211
		1297.3	211.0
		16.17	2.00
		194.60	19.78

MD: Microdrops
MF: Mutation factor
SD: Standard deviation
+S9: with S9 mix
-S9: without S9 mix

$$\text{Mutation factor} = \frac{\text{mean revertants (test item)}}{\text{mean revertants (solvent control)}}$$

*DMSO control was used because of 2AA
**Distilled water control was used because of SAZ

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APPENDIX 4 (continued)

Initial Mutation Test (Plate Incorporation Method)

Test Item: Polyglyceryl-10 Decaethylhexanoate
Date: 24-26 January 2012
Strain: *Salmonella typhimurium* TA1537
Cell count (overnight culture): 1.93×10^9 CFU/mL

Concentration: (µg/plate)		Revertant colony number -S9	Revertant colony number +S9
5000		5 MD	6 MD
		9 MD	9 MD
		11 MD	6 MD
	Mean	8.3	7.0
	SD	3.06	1.73
	MF	1.47	1.05
1581		8	8
		7	3
		8	7
	Mean	7.7	6.0
	SD	0.58	2.65
	MF	1.35	0.90
500		5	5
		6	6
		8	5
	Mean	6.3	5.3
	SD	1.53	0.58
	MF	1.12	0.80
158.1		4	5
		2	6
		4	5
	Mean	3.3	5.3
	SD	1.15	0.58
	MF	0.59	0.80
50		4	12
		6	8
		6	3
	Mean	5.3	7.7
	SD	1.15	4.51
	MF	0.94	1.15
15.81		5	7
		3	9
		3	9
	Mean	3.7	8.3
	SD	1.15	1.15
	MF	0.65	1.25
5		4	5
		10	8
		6	3
	Mean	6.7	5.3
	SD	3.06	2.52
	MF	1.18	0.80

Concentration: (µg/plate)		Revertant colony number -S9		Revertant colony number +S9
Untreated control		3		7
		4		6
		7		5
	Mean	4.7		6.0
	SD	2.08		1.00
	MF	0.82		0.90
DMSO control*		4		7
		7		4
		3		5
	Mean	4.7		5.3
	SD	2.08		1.53
	MF	0.82		0.80
DMF control		2		8
		9		7
		6		5
	Mean	5.7		6.7
	SD	3.51		1.53
	MF	1.00		1.00
Positive control		396	2AA	208
9AA		480	(2 µg)	205
(50 µg)		424		187
	Mean	433.3		200.0
	SD	42.77		11.36
	MF	92.86		37.50

MD: Microdrops
MF: Mutation factor
SD: Standard deviation
+S9: with S9 mix
-S9: without S9 mix

$$\text{Mutation factor} = \frac{\text{mean revertants (test item)}}{\text{mean revertants (solvent control)}}$$

*DMSO control was used because of 9AA and 2AA

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APPENDIX 4 (continued)

Initial Mutation Test (Plate Incorporation Method)

Test Item: Polyglyceryl-10 Decaethylhexanoate
Date: 24-26 January 2012
Strain: *Escherichia coli* WP2 *uvrA*
Cell count (overnight culture): 3.09×10^9 CFU/mL

Concentration: (µg/plate)		Revertant colony number -S9	Revertant colony number +S9
5000		25 MD	35 MD
		34 MD	32 MD
		18 MD	41 MD
	Mean	25.7	36.0
	SD	8.02	4.58
	MF	0.73	0.88
1581		29	38
		32	42
		29	39
	Mean	30.0	39.7
	SD	1.73	2.08
	MF	0.86	0.97
500		27	28
		33	28
		33	42
	Mean	31.0	32.7
	SD	3.46	8.08
	MF	0.89	0.80
158.1		31	35
		31	43
		28	36
	Mean	30.0	38.0
	SD	1.73	4.36
	MF	0.86	0.93
50		33	35
		24	30
		35	40
	Mean	30.7	35.0
	SD	5.86	5.00
	MF	0.88	0.85
15.81		25	31
		30	43
		30	42
	Mean	28.3	38.7
	SD	2.89	6.66
	MF	0.81	0.94
5		19	33
		30	31
		36	32
	Mean	28.3	32.0
	SD	8.62	1.00
	MF	0.81	0.78

Concentration: (µg/plate)		Revertant colony number -S9		Revertant colony number +S9
Untreated control		34		38
		25		39
		30		30
	Mean	29.7		35.7
	SD	4.51		4.93
	MF	0.85		0.87
DMSO control*		28		38
		26		41
		26		48
	Mean	26.7		42.3
	SD	1.15		5.13
	MF	0.76		1.03
Distilled water control**		25		41
		44		49
		39		38
	Mean	36.0		42.7
	SD	9.85		5.69
	MF	1.03		1.04
DMF control		32		45
		27		39
		46		39
	Mean	35.0		41.0
	SD	9.85		3.46
	MF	1.00		1.00
Positive control		1344	2AA	237
MMS		1320	(50 µg)	245
(2 µL)		1420		224
		1361.3		235.3
		52.20		10.60
		37.81		5.56

MD: Microdrops
MF: Mutation factor
SD: Standard deviation
+S9: with S9 mix
-S9: without S9 mix

$$\text{Mutation factor} = \frac{\text{mean revertants (test item)}}{\text{mean revertants (solvent control)}}$$

*DMSO control was used because of 2AA
**Distilled water control was used because of MMS

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APPENDIX 5

Confirmatory Mutation Test (Pre-Incubation Method)

Test Item: Polyglyceryl-10 Decaethylhexanoate
Date: 31 January - 02 February 2012
Strain: *Salmonella typhimurium* TA98
Cell count (overnight culture): 1.90×10^9 CFU/mL

Concentration: (µg/plate)		Revertant colony number -S9	Revertant colony number +S9
5000		15 R	30 SR
		18 R	31 SR
		15 R	27 SR
	Mean	16.0	29.3
	SD	1.73	2.08
	MF	1.12	1.47
1581		22	31
		22	27
		19	28
	Mean	21.0	28.7
	SD	1.73	2.08
	MF	1.47	1.43
500		23	34
		21	24
		24	28
	Mean	22.7	28.7
	SD	1.53	5.03
	MF	1.58	1.43
158.1		17	32
		17	27
		19	25
	Mean	17.7	28.0
	SD	1.15	3.61
	MF	1.23	1.40
50		13	35
		13	31
		20	30
	Mean	15.3	32.0
	SD	4.04	2.65
	MF	1.07	1.60
15.81		19	27
		15	32
		13	22
	Mean	15.7	27.0
	SD	3.06	5.00
	MF	1.09	1.35
5		19	30
		17	33
		20	24
	Mean	18.7	29.0
	SD	1.53	4.58
	MF	1.30	1.45

Concentration: (µg/plate)		Revertant colony number -S9		Revertant colony number +S9
Untreated control		20		23
		16		33
		14		27
	Mean	16.7		27.7
	SD	3.06		5.03
	MF	1.16		1.38
DMSO control*		16		35
		21		33
		17		25
	Mean	18.0		31.0
	SD	2.65		5.29
	MF	1.26		1.55
DMF control		14		22
		16		23
		13		15
	Mean	14.3		20.0
	SD	1.53		4.36
	MF	1.00		1.00
Positive control		376	2AA	2344
NPD		320	(2 µg)	2280
(4 µg)		344		2420
	Mean	346.7		2348.0
	SD	28.10		70.09
	MF	19.26		75.74

R: Reduced background lawn
SR: Slightly reduced background lawn
MF: Mutation factor
SD: Standard deviation
+S9: with S9 mix
-S9: without S9 mix

$$\text{Mutation factor} = \frac{\text{mean revertants (test item)}}{\text{mean revertants (solvent control)}}$$

*DMSO control was used because of NPD and 2AA

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APPENDIX 5 (continued)

Confirmatory Mutation Test (Pre-Incubation Method)

Test Item: Polyglyceryl-10 Decaethylhexanoate
Date: 31 January - 02 February 2012
Strain: *Salmonella typhimurium* TA100
Cell count (overnight culture): 1.92×10^9 CFU/mL

Concentration: (µg/plate)		Revertant colony number -S9	Revertant colony number +S9
5000		75	109
		98	108
		91	90
	Mean	88.0	102.3
	SD	11.79	10.69
	MF	0.99	0.95
1581		84	126
		83	121
		82	107
	Mean	83.0	118.0
	SD	1.00	9.85
	MF	0.94	1.10
500		74	116
		104	107
		106	132
	Mean	94.7	118.3
	SD	17.93	12.66
	MF	1.07	1.10
158.1		93	104
		97	105
		110	117
	Mean	100.0	108.7
	SD	8.89	7.23
	MF	1.13	1.01
50		111	115
		76	102
		103	102
	Mean	96.7	106.3
	SD	18.34	7.51
	MF	1.09	0.99
15.81		117	99
		107	98
		105	130
	Mean	109.7	109.0
	SD	6.43	18.19
	MF	1.24	1.01
5		86	91
		85	116
		82	91
	Mean	84.3	99.3
	SD	2.08	14.43
	MF	0.95	0.92

Concentration: (µg/plate)		Revertant colony number -S9		Revertant colony number +S9
Untreated control		75		92
		101		94
		88		88
	Mean	88.0		91.3
	SD	13.00		3.06
	MF	0.99		0.85
DMSO control*		79		95
		89		111
		98		91
	Mean	88.7		99.0
	SD	9.50		10.58
	MF	1.00		0.92
Distilled water control**		84		94
		79		110
		76		90
	Mean	79.7		98.0
	SD	4.04		10.58
	MF	0.90		0.91
DMF control		80		109
		88		108
		98		106
	Mean	88.7		107.7
	SD	9.02		1.53
	MF	1.00		1.00
Positive control		1344	2AA	2496
SAZ		1292	(2 µg)	2600
(2 µg)		1440		2588
		1358.7		2561.3
		75.08		56.90
		17.05		25.87

MF: Mutation factor
SD: Standard deviation
+S9: with S9 mix
-S9: without S9 mix

$$\text{Mutation factor} = \frac{\text{mean revertants (test item)}}{\text{mean revertants (solvent control)}}$$

*DMSO control was used because of 2AA
**Distilled water control was used because of SAZ

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APPENDIX 5 (continued)

Confirmatory Mutation Test (Pre-Incubation Method)

Test Item: Polyglyceryl-10 Decaethylhexanoate
Date: 31 January - 02 February 2012
Strain: *Salmonella typhimurium* TA1535
Cell count (overnight culture): 2.52×10^9 CFU/mL

Concentration: (µg/plate)		Revertant colony number -S9	Revertant colony number +S9
5000		11	10
		10	12
		11	11
	Mean	10.7	11.0
	SD	0.58	1.00
	MF	1.52	0.92
1581		10	10
		5	8
		7	8
	Mean	7.3	8.7
	SD	2.52	1.15
	MF	1.05	0.72
500		7	10
		9	11
		6	11
	Mean	7.3	10.7
	SD	1.53	0.58
	MF	1.05	0.89
158.1		8	10
		3	6
		9	13
	Mean	6.7	9.7
	SD	3.21	3.51
	MF	0.95	0.81
50		6	13
		8	7
		7	13
	Mean	7.0	11.0
	SD	1.00	3.46
	MF	1.00	0.92
15.81		5	12
		8	6
		7	5
	Mean	6.7	7.7
	SD	1.53	3.79
	MF	0.95	0.64
5		11	11
		8	10
		3	8
	Mean	7.3	9.7
	SD	4.04	1.53
	MF	1.05	0.81

Concentration: (µg/plate)		Revertant colony number -S9		Revertant colony number +S9
Untreated control		10		14
		8		17
		9		12
	Mean	9.0		14.3
	SD	1.00		2.52
	MF	1.29		1.19
DMSO control*		13		8
		10		14
		8		18
	Mean	10.3		13.3
	SD	2.52		5.03
	MF	1.48		1.11
Distilled water control**		10		14
		12		8
		7		11
	Mean	9.7		11.0
	SD	2.52		3.00
	MF	1.38		0.92
DMF control		7		13
		7		9
		7		14
	Mean	7.0		12.0
	SD	0.00		2.65
	MF	1.00		1.00
Positive control		1200	2AA	187
SAZ		1192	(2 µg)	203
(2 µg)		1288		211
	Mean	1226.7		200.3
	SD	53.27		12.22
	MF	126.90		15.03

MF: Mutation factor
SD: Standard deviation
+S9: with S9 mix
-S9: without S9 mix

$$\text{Mutation factor} = \frac{\text{mean revertants (test item)}}{\text{mean revertants (solvent control)}}$$

*DMSO control was used because of 2AA
**Distilled water control was used because of SAZ

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APPENDIX 5 (continued)

Confirmatory Mutation Test (Pre-Incubation Method)

Test Item: Polyglyceryl-10 Decaethylhexanoate
Date: 31 January - 02 February 2012
Strain: *Salmonella typhimurium* TA1537
Cell count (overnight culture): 1.54×10^9 CFU/mL

Concentration: (µg/plate)		Revertant colony number -S9	Revertant colony number +S9
5000		12 R	5 SR
		13 R	5 SR
		6 R	8 SR
	Mean	10.3	6.0
	SD	3.79	1.73
	MF	1.35	0.75
1581		12	7
		4	11
		5	13
	Mean	7.0	10.3
	SD	4.36	3.06
	MF	0.91	1.29
500		6	6
		6	7
		6	10
	Mean	6.0	7.7
	SD	0.00	2.08
	MF	0.78	0.96
158.1		14	14
		7	11
		7	5
	Mean	9.3	10.0
	SD	4.04	4.58
	MF	1.22	1.25
50		4	12
		8	3
		10	8
	Mean	7.3	7.7
	SD	3.06	4.51
	MF	0.96	0.96
15.81		7	9
		12	10
		11	9
	Mean	10.0	9.3
	SD	2.65	0.58
	MF	1.30	1.17
5		8	6
		12	6
		14	13
	Mean	11.3	8.3
	SD	3.06	4.04
	MF	1.48	1.04

Concentration: (µg/plate)		Revertant colony number -S9		Revertant colony number +S9
Untreated control		8		6
		4		11
		8		5
	Mean	6.7		7.3
	SD	2.31		3.21
	MF	0.87		0.92
DMSO control*		6		8
		5		4
		7		8
	Mean	6.0		6.7
	SD	1.00		2.31
	MF	0.78		0.83
DMF control		5		6
		10		8
		8		10
	Mean	7.7		8.0
	SD	2.52		2.00
	MF	1.00		1.00
Positive control		564	2AA	217
9AA		520	(2 µg)	223
(50 µg)		452		199
	Mean	512.0		213.0
	SD	56.43		12.49
	MF	85.33		31.95

R: Reduced background lawn
SR: Slightly reduced background lawn
MF: Mutation factor
SD: Standard deviation
+S9: with S9 mix
-S9: without S9 mix

$$\text{Mutation factor} = \frac{\text{mean revertants (test item)}}{\text{mean revertants (solvent control)}}$$

*DMSO control was used because of 9AA and 2AA

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APPENDIX 5 (continued)

Confirmatory Mutation Test (Pre-Incubation Method)

Test Item: Polyglyceryl-10 Decaethylhexanoate
Date: 31 January - 02 February 2012
Strain: *Escherichia coli* WP2 *uvrA*
Cell count (overnight culture): 2.24×10^9 CFU/mL

Concentration: (µg/plate)		Revertant colony number -S9	Revertant colony number +S9
5000		38 SR	23
		33 SR	32
		38 SR	22
	Mean	36.3	25.7
	SD	2.89	5.51
	MF	0.96	0.73
1581		37	20
		31	27
		35	28
	Mean	34.3	25.0
	SD	3.06	4.36
	MF	0.91	0.71
500		31	35
		36	36
		35	28
	Mean	34.0	33.0
	SD	2.65	4.36
	MF	0.90	0.94
158.1		41	24
		26	24
		39	22
	Mean	35.3	23.3
	SD	8.14	1.15
	MF	0.94	0.67
50		36	27
		35	27
		45	28
	Mean	38.7	27.3
	SD	5.51	0.58
	MF	1.03	0.78
15.81		23	20
		36	30
		41	28
	Mean	33.3	26.0
	SD	9.29	5.29
	MF	0.88	0.74
5		26	22
		25	28
		34	18
	Mean	28.3	22.7
	SD	4.93	5.03
	MF	0.75	0.65

Concentration: (µg/plate)		Revertant colony number -S9		Revertant colony number +S9
Untreated control		28		41
		34		43
		26		36
	Mean	29.3		40.0
	SD	4.16		3.61
	MF	0.78		1.14
DMSO control*		29		29
		22		42
		18		41
	Mean	23.0		37.3
	SD	5.57		7.23
	MF	0.61		1.07
Distilled water control**		20		35
		35		46
		25		38
	Mean	26.7		39.7
	SD	7.64		5.69
	MF	0.71		1.13
DMF control		45		25
		33		34
		35		46
	Mean	37.7		35.0
	SD	6.43		10.54
	MF	1.00		1.00
Positive control		1188	2AA	253
MMS		1220	(50 µg)	242
(2 µL)		1140		234
		1182.7		243.0
		40.27		9.54
		44.35		6.51

SR: Slightly reduced background lawn
MF: Mutation factor
SD: Standard deviation
+S9: with S9 mix
-S9: without S9 mix

$$\text{Mutation factor} = \frac{\text{mean revertants (test item)}}{\text{mean revertants (solvent control)}}$$

*DMSO control was used because of 2AA
**Distilled water control was used because of MMS

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APPENDIX 6

Historical Control Data (Period of 2004-2010)

Untreated control data										
	without metabolic activation (-S9 Mix)					with metabolic activation (+S9 Mix)				
	TA98	TA100	TA1535	TA1537	E.coli	TA98	TA100	TA1535	TA1537	E.coli
Mean value of revertants	24.1	118.8	11.6	6.9	31.8	29.0	124.8	13.0	8.5	39.0
Minimum	8	58	2	1	9	9	62	3	1	12
Maximum	54	211	31	24	86	52	200	36	26	79

DMF control data										
	without metabolic activation (-S9 Mix)					with metabolic activation (+S9 Mix)				
	TA98	TA100	TA1535	TA1537	E.coli	TA98	TA100	TA1535	TA1537	E.coli
Mean value of revertants	22.4	117.5	10.3	7.5	34.7	27.0	126.1	11.9	9.4	40.7
Minimum	10	53	4	2	11	13	84	5	4	20
Maximum	41	195	31	20	59	39	194	21	19	64

DMSO control data										
	without metabolic activation (-S9 Mix)					with metabolic activation (+S9 Mix)				
	TA98	TA100	TA1535	TA1537	E.coli	TA98	TA100	TA1535	TA1537	E.coli
Mean value of revertants	23.5	116.8	11.5	6.4	31.1	28.2	123.0	12.8	7.7	38.7
Minimum	9	54	2	1	10	10	61	2	0	12
Maximum	54	200	35	27	69	52	199	31	27	78

TA98: *Salmonella typhimurium* TA98, TA100: *Salmonella typhimurium* TA100,
TA1535: *Salmonella typhimurium* TA1535, TA1537: *Salmonella typhimurium* TA1537,
E.coli: *Escherichia coli* WP2 *uvrA*

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APPENDIX 6 (continued)

Historical Control Data (Period of 2004-2010)

Distilled water control data										
	without metabolic activation (-S9 Mix)					with metabolic activation (+S9 Mix)				
	TA98	TA100	TA1535	TA1537	E.coli	TA98	TA100	TA1535	TA1537	E.coli
Mean value of revertants	24.6	119.4	11.8	7.2	33.5	29.9	125.6	13.1	8.7	40.1
Minimum	9	51	2	1	9	12	69	3	2	11
Maximum	46	208	34	23	65	61	223	32	23	74

Positive reference control data										
	without metabolic activation (-S9 Mix)					with metabolic activation (+S9 Mix)				
	TA98	TA100	TA1535	TA1537	E.coli	TA98	TA100	TA1535	TA1537	E.coli
Mean value of revertants	474.9	938.7	817.0	552.4	754.1	1841.7	1870.7	249.4	260.0	344.8
Minimum	102	385	167	41	146	236	649	78	66	91
Maximum	1920	2525	2625	2748	1953	4390	4288	2250	1023	2010

TA98: *Salmonella typhimurium* TA98, TA100: *Salmonella typhimurium* TA100,
TA1535: *Salmonella typhimurium* TA1535, TA1537: *Salmonella typhimurium* TA1537,
E.coli: *Escherichia coli* WP2 *uvrA*

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APPENDIX 7

Copy of the GLP Certificate

ORSZÁGOS GYÓGYSZERÉSZETI INTÉZET
National Institute of Pharmacy

FŐIGAZGATÓ
1051 Budapest, Zrínyi u. 3
tel: (1) 8869-320
fax: (1) 8869-480
e-mail: szepezdi.zsuzsanna@ogyi.hu

Ref. no: OGYI/8242-11/2010
Admin.: Urbin Magdolna Zita
Date: 16 December, 2010

GOOD LABORATORY PRACTICE (GLP) CERTIFICATE

It is hereby certified that the test facility

LAB Research Kft.

(Base facility: H-8201 Veszprém, Szabadságpuszta, Hungary)

is able to carry out

physico-chemical testing, toxicity studies, mutagenicity studies, environmental toxicity studies on aquatic or terrestrial organisms, studies on behaviour in water, soil and air;bio-accumulation , safety pharmacology testing, reproduction toxicology, inhalation toxicology, analytical chemistry and contract archiving

in compliance with the Principles of GLP (Good Laboratory Practice) and also complies with the corresponding OECD/European Community requirements.

Date of the inspection: **4-8 October, 2010.**

ORSZÁGOS GYÓGYSZERÉSZETI INTÉZET 2.

Zsuzsanna Szepezdi, Ph. D.
Director-General

The facility name until 1st September 2011 was LAB Research Ltd. From that date, the registered name has been CiToxLAB Hungary Ltd. This information has been transmitted to the GLP competent authority. The above GLP certificate is valid for this facility (now known as CiToxLAB Hungary Ltd.) until the certificate expires (16 December 2012).

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Consumer Product Testing Co.

EST. 1975

FINAL REPORT

CLIENT: Creative Strategy, Inc.
High Park Nihonbashi Bldg. 4F
2-45-4 Nihonbashi-Hamacho
Chuo-ku, Tokyo 103-0007, Japan

SPONSOR: SAKAMOTO YAKUHIN KOGYO CO., LTD.
1-20 Rinkaicho, Izumiotsu City
Osaka 595-0075, Japan

TEST: The MatTek Corporation EpiOcular™ Tissue Model *In Vitro* Toxicity Testing System

TEST ARTICLE: Polyglyceryl-10 Decaethylhexanoate (Lot. 91015)

EXPERIMENT REFERENCE NO.: V11-6351

2/2/12

Steven Nitka
Vice President
Laboratory Director

This report is submitted for the exclusive use of the person, partnership, or corporation to whom it is addressed, and neither the report nor the name of these Laboratories nor any member of its staff, may be used in connection with the advertising or sale of any product or process without written authorization.

70 New Dutch Lane • Fairfield, New Jersey 07004-2514 • (973) 808-7111 • Fax (973) 808-7234

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Consumer Product Testing Co.

EST. 1075

QUALITY ASSURANCE UNIT STATEMENT

Study No.: V11-6351

The objective of the Quality Assurance Unit (QAU) is to monitor the conduct and reporting of nonclinical laboratory studies. This study has been performed under Good Laboratory Practice principles (including government regulations to the extent applicable) and in accordance with standard operating procedures and applicable standard protocols. The QAU maintains copies of study protocols and standard operating procedures and has inspected this study on the date listed below. The findings of this inspection may have been reported to management and the Study Director.

Date of data inspection: 02/02/12

Quality Assurance:

Christine Fenbucchio 2/2/12

Signature/Date

70 New Dutch Lane • Fairfield, New Jersey 07004-2514 • (973) 808-7111 • Fax (973) 808-7234
Clinical • Toxicology • Analytical Chemistry • Microbiology

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Objective:

To evaluate the test article for irritancy potential utilizing the MatTek Corporation EpiOcular *in vitro* toxicity testing system.

Introduction:

"MatTek's patented EpiOcular corneal Model consists of normal, human-derived epidermal keratinocytes which have been cultured to form a stratified, squamous epithelium similar to that found in the cornea. The epidermal cells, which are cultured on specially prepared cell culture inserts using serum free medium, differentiate to form a multilayered structure which closely parallels the corneal epithelium . . . " This system " . . . provides a predictive, morphologically relevant *in vitro* means to assess ocular irritancy."[1]

EpiOcular, when used with the recommended cell metabolism assay, can quickly provide toxicological profiles. The procedure utilizes a water-soluble, yellow, tetrazolium salt (MTT {3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide}), which is reduced by succinate dehydrogenase in the mitochondria of viable cells to a purple, insoluble formazan derivative. Substances which damage this mitochondrial enzyme inhibit the reduction of the tetrazolium salt. The amount of MTT reduced by a culture is therefore proportional to the number of viable cells.

Test Article: Polyglyceryl-10 Decaethylhexanoate (Lot. 91015)

Experimental Interval: January 12, 2012 to January 13, 2012

Method:

The Sponsor informed this facility that the test article is not water soluble. As per MatTek's protocol, the test article was dosed as received. After the appropriate tissue preparation, 100 microliters of the test article and the negative control (distilled water) were added to the Millicells containing the EpiOcular samples. The six (6) well plates containing the dosed EpiOcular samples were then incubated at 37°C, five (5)% carbon dioxide and ≥ 90% humidity.

[1]MatTek Corporation, 200 Homer Avenue, Ashland, Massachusetts 01721

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Method (continued):

After the appropriate exposure period, each insert was individually removed from its plate and rinsed with phosphate buffered saline (PBS) to remove any residual material. Each was then rinsed a second and third time. Following the 3 rinses, each Millicell was submerged in 5 milliliters of assay media for 10 minutes, at room temperature. This final soak removed any residual, absorbed article. After the 10 minutes, excess liquid was shaken off and each EpiOcular tissue was placed into 300 microliters of MTT solution. The EpiOcular samples were then returned to the incubator.

After the three (3) hour MTT exposure, each insert was removed and gently rinsed with PBS to remove any residual MTT solution. Excess PBS was shaken from each of the inserts, which were then blotted on the bottom using paper towels. The inserts were then each placed into one (1) well of a 24 well extraction plate. Each insert was then immersed in two (2) milliliters of extraction solution, at room temperature, overnight. After the extraction procedure, the liquid within each insert was decanted back into the well from which it was taken. The remaining extractant solution was then agitated and a 200 microliter aliquot of each extract was removed for evaluation. A Dynatech MR 4000 Automatic Microplate Reader was used to determine the absorbance of each extract at 570nm. With the absorbance of the negative control (distilled water) defined as 100%, the percent absorbencies of the articles were determined. The percentages listed below directly correlate with the cell metabolism in the EpiOcular samples.

Results:

Article (% & Exposure)	System	**Percent Viability**	**Percent Inhibition**
Polyglyceryl-10 Decaethylhexanoate (Lot. 91015)			
(100% - 4 hrs.)	EpiOcular	106	-6
(100% - 1 hr.)	EpiOcular	101	-1
(100% - 20 mins.)	EpiOcular	108	-8

When possible, using a semi-log scale, the percent viabilities for the articles were plotted on the linear y axis versus the dosing time on the log x axis. By interpolation, the time at which the percent viability would be 50% was determined (ET-50). As a general guideline (provided by MatTek) the following equation can be used to estimate the rabbit Draize eye score:

$$\text{Draize} = -4.74 + 101.7/(\text{ET-50})^{0.5}$$

Based on the literature (Kay, J.H. and Calandra, J.C., "Interpretation of eye irritation tests," *J. Soc. Cosmetic Chem.*, 13, 281-289 (1962)), the ocular irritancy estimated potential has been categorized by MatTek into the following groups, based on the Draize score:

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Results (continued):

Draize Score	Irritancy Classification	Example	EpiOcular ET-50 (min)
0-15	Non-irritating, Minimal	PEG-75 Lanolin, Tween 20	>256 – 26.5
15.1 – 25	Mild	3% Sodium Dodecyl Sulfate	<26.5 – 11.7
25.1 – 50	Moderate	5% Triton X-100	<11.7 – 3.45
50.1 – 110	Severe, Extreme	5% Benzalkonium Chloride	<3.45

Discussion:

Under the conditions of this test, the Polyglyceryl-10 Decaethylhexanoate (Lot. 91015) test article, at 100%, elicited *in vitro* results which indicate that its ET-50 is greater than 256 minutes. Therefore, the test article, at 100%, has an estimated Draize ocular irritation score of approximately 0 with a "non-irritating" irritancy classification.

Conclusion:

Under the conditions of this test, the results indicate that the Polyglyceryl-10 Decaethylhexanoate (Lot. 91015) test article, at 100%, has a "non-irritating" irritancy classification.

Record Retention:

All records and documents pertaining to the conduct of this study shall be retained in the CPTC archives for a minimum of ten (10) years. At any time prior to the completion of the tenth archival year, a Sponsor may submit a written request to the CPTC QA Department to obtain custody of study records once the CPTC archive period has been completed. This transfer shall be performed at the Sponsor's expense. In the absence of a written request, study-related records shall be destroyed at the end of the CPTC archive period in a manner that renders them useless.

Professional personnel involved:

Steven Nitka, B.S.	-	Vice President Laboratory Director (Study Director)
Lillian Vazquez, B.S.	-	Laboratory Supervisor
Christine Hendricks	-	Quality Assurance Group Leader

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STUDY REPORT

Patch test on the cosmetic ingredient

Japan Hair Science Association
(Incorporated association for the public good)

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To SAKAMOTO YAKUHIN KOGYO CO.,LTD.
I hereby certify that this document is a duplicate copy of the original issued as Nichimokyo №23029-(2) on 2th November, 2011.

25th November, 2011

Keiji Kijima

Keiji Kijima ph.D
Chairman of Directors
Japan Hair Science Association
(Incorporated association for the public good)

Contract Study Report

This is the report sponsored study on the patch test of the cosmetic ingredient as you requested on 4th October, 2011. See Attachment for study results.

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Nichimokyo №23029-(2)
2th November, 2011

To SAKAMOTO YAKUHIN KOGYO CO.,LTD.

This report presents the results of patch test on the cosmetic ingredient performed by Japan Hair Science Association. It was sponsored by SAKAMOTO YAKUHIN KOGYO CO.,LTD.on 4th October, 2011. This report is an English version of the original, which was written in Japanese. The undersigned hereby declare that this version faithfully reflects the original report to the best of my knowledge.

TEST FOR IRRITATION EFFECTS OF THE COSMETIC INGREDIENT ON HUMAN SKIN

1.Purpose of the test

This patch test was performed to assess possible irritation potential of the cosmetic ingredient on healthy human skin, sponsored by SAKAMOTO YAKUHIN KOGYO CO.,LTD. on 4th October, 2011.

2.Test sample

The supplied sample is the following material

Sample : Polyglyceryl−10 Decaethylhexanoate Lot.№91015

Control : purified water

3.Test procedure

(1)Volunteers

16 males and 27 females (43 Japanese people in total), age 23 to 62, were selected as volunteers.

(2)Applied amount

Aproper quantiy of test sample was applied to each volunteer.

(3)Method of patch test

A test sheet sample was placed on FINN CHAMBER(Smart Practice®)and applied to the crooked side of upper arm of each volunteer for 24 hours in occlusive condition to for evaluation.

At the end of 24 hours exposure period, the test sample was removed. Skin reactions were evaluated 1 hour and 24 hours after removing the test sample.

(4)Criteria for evaluation

The reaction was evaluated using the following criteria. Based on the Japanese

− :	No reaction
± :	Very slight erythema
+ :	Well-defined erythema
++ :	Erythema with swelling or with papule
+++ :	Blisters in addition to the above symptom
++++ :	Large blister

The evaluation of patch test on the cosmetic ingredient performed by
Mikako Aoki, M.D., Dermatologist

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4.Term of path test

Effective October 19,2011.～October 28,2011.

5.Results

The results of this test on 43 volunteers were as shown in the Table. In all volunteers, the reaction was observed (卄)on 1 volunteer and (+)on 3 volunteers in 1 hour after removing the test sample (shown in Table as 24-hours Evaluation time). Similarly, the reaction was observed(±)on 1 volunteer in 24 hours after removing the test sample (shown in Table as 48-hours Evaluation time) in all volunteers.

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Table Results of patch test(23029-2)

No.	Volunteer	Sex	Age	Evaluationtime(hrs.)	Sample	Control	Note(anamncsis)
1	T. A.	M	33	24	−	−	Healthy
				48	−	−	
2	N. A.	M	41	24	−	−	Healthy
				48	−	−	
3	M. I.	F	38	24	++	++	Healthy
				48	±	−	
4	T. I.	M	46	24	−	−	Healthy
				48	−	−	
5	Y. I.	M	49	24	−	−	Healthy
				48	−	−	
6	Y. I.	M	36	24	−	−	Healthy
				48	−	−	
7	A. I.	F	26	24	−	−	Healthy
				48	−	−	
8	Y. U.	F	36	24	−	−	Healthy
				48	−	−	
9	H. E.	F	38	24	−	−	Healthy
				48	−	−	
10	Y. E.	M	27	24	−	−	Healthy
				48	−	−	
11	R. E.	F	30	24	−	−	Healthy
				48	−	−	
12	Y. O.	F	30	24	−	−	Healthy
				48	−	−	
13	M. O.	M	62	24	−	−	Healthy
				48	−	−	
14	K. O.	M	29	24	−	−	Healthy
				48	−	−	
15	A. O.	M	56	24	−	−	Healthy
				48	−	−	
16	M. O.	M	26	24	−	−	Healthy
				48	−	−	
17	M. O.	F	39	24	−	−	Healthy
				48	−	−	
18	K. K.	M	55	24	−	−	Healthy
				48	−	−	
19	T. K.	M	33	24	−	−	Healthy
				48	−	−	
20	Y. K.	F	35	24	−	−	Healthy
				48	−	−	
21	K. G.	F	41	24	−	−	Healthy
				48	−	−	
22	A. S.	F	42	24	+	+	Healthy
				48	−	−	
23	K. S.	M	32	24	−	−	Healthy
				48	−	−	
24	I. S.	M	52	24	−	−	Healthy
				48	−	−	
25	K. S.	F	38	24	−	−	Healthy
				48	−	−	

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Table Results of patch test(23029-2)

No.	Volunteer	Sex	Age	Evaluationtime(hrs.)	Sample	Control	Note(anamncsis)
26	S. T.	F	31	24	−	−	Healthy
				48	−	−	
27	K. T.	F	40	24	−	−	Healthy
				48	−	−	
28	T. T.	M	24	24	−	−	Healthy
				48	−	−	
29	H. T.	F	40	24	−	−	Healthy
				48	−	−	
30	T. N.	M	36	24	−	−	Healthy
				48	−	−	
31	A. N.	F	47	24	−	−	Healthy
				48	−	−	
32	H. N.	F	23	24	−	−	Healthy
				48	−	−	
33	S. H.	F	31	24	+	−	Healthy
				48	−	−	
34	M. H.	F	38	24	−	+	Healthy
				48	−	−	
35	A. H.	M	34	24	−	−	Healthy
				48	−	−	
36	Y. F.	F	44	24	−	+	Healthy
				48	−	−	
37	M. H.	F	24	24	−	−	Healthy
				48	−	−	
38	T. M.	F	40	24	+	−	Healthy
				48	−	−	
39	T. M.	F	34	24	−	+	Healthy
				48	−	−	
40	T. M.	M	42	24	−	−	Healthy
				48	−	−	
41	S. M.	F	47	24	−	−	Healthy
				48	−	−	
42	H. Y.	F	39	24	−	+	Healthy
				48	−	−	
43	M. W.	F	32	24	−	−	Healthy
				48	−	−	

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Výzkumný ústav organických syntéz a.s.
(Research Institute for Organic Syntheses Inc.)
Centre of Ecology, Toxicology and Analytics
GLP Test Facility
Rybitvi 296
533 54 Rybitvi
Czech Republic

FINAL REPORT

Polyglyceryl-10 Decaethylhexanoate

In vitro Skin Irritation Test
(EpiDerm™ Model)

Report No.:

Study No.: 4/12/4AI

Experimental part of study performed in period: 24/1-27/1/2011

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GLP Statement

Study title: Polyglyceryl-10 Decaethylhexanoate – *In vitro* Skin Irritation Test (EpiDerm™ Model)

Study No.: 4/12/4AI

The undersigned study director declares that the work described in this report has been performed under her supervision in accordance with GLP principles [OECD Principles on Good Laboratory Practice (as revised in 1997), C (97) 186 (Final)], and the report gives a correct and precise account of the results obtained.

Eva Täublová, M.Sc. Date:
Study Director

Approved by:

Viktor Mejstřík, Ph.D. Date:
Head of Test Facility

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Summary

Test substance, **Polyglyceryl-10 Decaethylhexanoate,** was assayed for the in vitro skin irritation in human epidermal model EpiDerm™. The test was performed according to Method B.46. *In vitro* skin irritation: Reconstructed human epidermis model test and Protocol for: In Vitro EpiDerm™ Skin Irritation Test For use with MatTek Corporation's Reconstructed Human Epidermal Model EpiDerm (EPI-200-SIT).

Test substance (30 µL) was placed directly atop the tissue. Length of exposition was 60 minutes. Nine tissues were used for the experiment, three per test substance (TS), three for positive control (PC) and three for negative control (NC).

After rinsing, tissues were post incubated for 42 hours due to leave of damage reparation. Three hours incubation with MTT and two hours extraction period with shaking followed then. Optical density (OD_{570}) of isopropanol extracts was measured on a spectrophotometer. Relative cell viability was calculated for each tissue as % of the mean viability of the negative control tissues.

Under the above-described experimental design, **average viability** of tissues treated by the test substance was **101.4 %** of negative control average value (i.e. viability was >50 %). According to the criteria given in Method B.46 the test substance did not fall into any of quoted categories of skin irritation.

The test substance, **Polyglyceryl-10 Decaethylhexanoate**, was **nonirritating in EpiDerm™ model.**

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1. INTRODUCTION

1.1. Nature and Purpose of Study

To assess the potential of the **Polyglyceryl-10 Decaethylhexanoate** to cause cell damage in the human epidermal model designed to the detection of skin irritation properties of test substance. The study is a part of test substance health hazard evaluation.

1.2. Sponsor

Name and Address of Sponsor
Sakamoto Yakuhin Kogyo Co., Ltd.
20, Rinkai-cho 1-chome
Izumiotsu
Osaka 595-0075
JAPAN

Study Monitor
Hidenori Tachibana
Toyo Science Corp.
Kinsan Bldg. 1-21
Nihonbashi-Muromachi, 4-chome
Chuo-ku Tokyo 103-0022
JAPAN

1.3. Test Facility

Name and Address of Test Facility
Výzkumný ústav organických syntéz a.s.
(Research Institute for Organic Syntheses, Inc.)
Centre of Ecology, Toxicology and Analytics
GLP Test Facility
Rybitvi 296
533 54 Rybitvi
Czech Republic

Study Director
Eva Täublová, M.Sc.
Výzkumný ústav organických syntéz a.s.
(Research Institute for Organic Syntheses, Inc.)
Centre of Ecology, Toxicology and Analytics
GLP Test Facility
Genetic Toxicity/In vitro Testing Group
Rybitvi 296
Czech Republic

Personnel Involved

Study performance:	Eva Täublová, M.Sc
Technical co-operation:	Martina Čapková, M.Sc., Lenka Bouškova, Lucie Cacková, Dippl. Eng.
Quality assurance:	Rostislav Čihák, Ph.D.

Address of the personnel involved in this study is the same as the address of the test facility.

1.4. Legal Items (Guidelines)

Quality Assurance
This study has been carried out in accordance with internationally valid GLP principles:
- Directive 2004/10/EC of the EP and of the Council, February 2004
- OECD Principles of Good Laboratory Practice (as revised in 1997) C (97) 186 (Final)

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Methods

The working procedure described in this final report is in accordance with the following methodical documents:

(1) Method B.46. *In vitro* skin irritation: Reconstructed human epidermis model test Commision Regulation (EC) No. 761/2009, 23th July 2009

(2) OECD TG 439, OECD Guideline for the Testing of Chemicals: *In Vitro* Skin Irritation: Reconstructed Human *Epidermis* Test Method, 22 July, 2010

(3) Protocol for: In Vitro EpiDerm™ Skin Irritation Test For use with MatTek Corporation's Reconstructed Human Epidermal Model EpiDerm, Model EPI-200-SIT, MatTek Corporation January 1st, 2010, 1-37

1.5. Study Time Schedule

Study preparation (information completion, study plan) was performed from 19/12/2011 to 6/01/2012.
Experimental part of study was performed from 24/01/2012 to 27/1/2012
Examination of results and final report elaboration occurred from 24/01/2012 to 7/02/2012

1.6. Storage and Retention of Records and Materials

The GLP Testing Section of CETA will archive all the documentation of the study at least for 15 years: contract documentation, study plan, raw data, final report with QA statement, and records of QA inspections.

The test substance sample will be archived till the end of expiry (15th December 2012).

The records of primary data from the study could be found on special data sheets archived together with final report.

2. TEST SUBSTANCE

Test substance name: **Polyglyceryl-10 Decaethylhexanoate**

a) ***Information from sponsor***

Test substance name:	**Polyglyceryl-10 Decaethylhexanoate**
Chemical name:	1,2,3-Propanetriol, homopolymer, 2-ethylhexanoate
CAS No.:	84617-31-2
Batch No.:	91015
Purity (typical):	100 % (w/w)
Appearance:	pale yellow viscous liquid
Stability/Expiration:	15th December 2012
Storage:	Store at the room temperature bellow 30°C

b) Information from test facility

Appearance:	colourless viscous liquid
Storage during the study:	The sample of test substance has been stored at room temperature during the study.

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3. STUDY PERFORMANCE

3.1. Definitions

Skin irritation Incurrence of reversible changes in skin after application of the test substance

3.2. Tissues

The reconstructed human epidermal model EpiDerm™ (EPI-200, MatTek, Ashland, USA); MTT test: EPI-200, Lot No. 16017, kit C

3.3. Chemicals and Media

Chemicals:
PBS (phosphate buffered saline) – prepared in laboratory
MTT (3-(4,5-Dimethyl thiazol-2-yl)-2,5-diphenyltetrazolium bromide), research grade, Serva, Lot No. 081153
Isopropylakohol – p.a., Lach-Ner, Lot No. PP/2007/2176/0 exp. 4/12

Positive control:
5 % SDS (sodium dodecyl sulphate), MatTek, Lot No. 062411bba

Media:
EPI-100-NMM-SIT/Maintenance Medium, MatTek, Lot No.011212TTC

3.4. Test Method

The test methods are described in the guidelines referred in chap. 1.4. Details of routine are available in Protocol for: In Vitro EpiDerm™ Skin Irritation Test For use with MatTek Corporation's Reconstructed Human Epidermal Model EpiDerm, Model EPI-200-SIT, MatTek Corporation January 1st, 2010, 1-37.

3.5. Principle of the Assay

The test consists of a topical exposure of the neat test chemical to a reconstructed human epidermis (RhE) model followed by a cell viability test. Cell viability is measured by dehydrogenase conversion of MTT [*(3-4,5-dimethyl thiazole 2-yl) 2,5-diphenyltetrazolium-bromide*], present in cell mitochondria, into a blue formazan salt that is quantitatively measured after extraction from tissues. The reduction of the viability of tissues exposed to chemicals in comparison to negative controls (treated with PBS) is used to predict the skin irritation potential.

Evaluation is determined by measuring of optical density (OD) of the formazan extracts using a spectrophotometer at 570 nm. Relative cell viability is calculated for each tissue as % of the mean OD value of the negative control tissues. Skin irritation potential of the test material is predicted if the remaining relative cell viability is below 50 %.

3.6. Test System

The reconstructed human epidermal model EpiDerm™ (EPI-212, MatTek, Ashland, USA) consists of normal human-derived epidermal keratinocytes, which have been cultured to

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form a multilayered highly differentiated model of the human epidermis. The EpiDerm™ System is manufactured according to defined quality assurance procedures.

The EpiDerm™ tissues (surface 0.63 cm^2) are cultured on specially prepared cell culture inserts and shipped world-wide as kits, containing tissues on shipping agarose together with the necessary amount of culture media.

3.7. Test Procedure

3.7.1. Direct MTT reduction - functional check in tubes

Some test substances may interfere with the MTT endpoint if it is able to directly reduce MTT. Therefore, before exposure, functional check for this possibility is performed as follows: 50 µl of the test substance is added to 1 mL MTT medium (red) and incubated in the incubator (37±1°C, 5±1 % CO_2, moistened) for 1 hour. At the end of the exposure time, the presence and intensity of the staining (if any) is observed. If the solution changes colour from red to blue, other steps to correction have to be done.

3.7.2. MTT test

a) Test substance application:

The test substance (30 µL) is placed directly atop to the previously moistened tissue. The material is spread on the tissue surface.

b) Procedure:

On the day of receipt, EpiDerm tissues are conditioned by incubation to release transport stress related compounds and debris. After pre-incubation, tissues are topically exposed to the test chemicals for 1 hour. Three tissues are used per test chemical and for the positive control (PC) and negative control (NC). Tissues are then thoroughly rinsed, blotted to remove the test substances, and transferred to fresh medium.

After 24 hour incubation period, the medium is replaced by fresh one. Tissues are incubated for another 18 hours. Afterwards, the MTT assay is performed by transferring the tissues to 24-well plates containing MTT medium (1 mg/ml). After 3 hour MTT incubation, the blue formazan salt formed by cellular mitochondria is extracted with 2.0 ml/tissue of isopropanol and the optical density of the extracted formazan is determined using a spectrophotometer at 570 nm. Detailed procedure is described in SOP M/48/3 (VUOS-CETA, 2011).

c) OD_{570} measuring:

OD_{570} was measured on a spectrophotometer Libra S22. Isopropanol served as a blank.

3.8. Assay Acceptance Criteria

1. Negative Control:

The **absolute OD** of the negative control (NC) tissues (treated with sterile PBS) in the MTT-test is an indicator of tissue viability obtained in the testing laboratory after shipping and storing procedures and under specific conditions of use. **The assay meets the acceptance criterion if the mean OD_{570} of the NC tissues is ≥ 1.0 and ≤ 2.5.**

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2. Positive Control:

A **5 % SDS** (in H_2O) solution is used as positive control (PC) and tested concurrently with the test chemicals. Concurrent means here the PC has to be tested in each assay, but not more than one PC is required per testing day. Viability of positive control should be within 95±1 % confidence interval of the historical data.

The assay meets the acceptance criterion if the mean viability of PC tissues expressed as % of the negative control tissues is ≤ 20 %.

3. Standard Deviation (SD):

Since in each test skin irritancy potential is predicted from the mean viability determined on 3 single tissues, the variability of tissue replicates should be acceptably low. **The assay meets the acceptance criterion if the SD calculated from individual % tissue viabilities of the 3 identically treated replicates is < 18 %.**

3.9. Evaluation of Results and Classification

In vitro alternatives that have been validated and accepted may also be used to help in classification decisions making (see Regulation (EC) 1272/2008, 3.2. Skin corrosion/ irritation, 3.2.2. Classification criteria for substances).

For further classification the relative cell viability is calculated for each tissue as % of the mean of the negative control tissues viability, which is set at 100 %.

The cut-off values for the prediction of irritation are given below; these values are stated in EU Method B.46., part 2.2. INTERPRETATION OF RESULTS:

The test substance is considered to be **irritant to skin** in accordance with UN GHS [1] category 2:

(i) if the tissue viability after exposure and post-treatment incubation is less than or equal (≤) to 50 %.

The test substance is considered to have **no category**:

(ii) if the tissue viability after exposure and post-treatment incubation is more than (>) 50 %.

[1] United Nations (UN) (2007). Globally Harmonized System of Classification and Labelling of Chemicals (GHS), Second revised edition, UN New York and Geneva, 2007

3.10. Deviations

At preincubation of tissues, temperature rose over 38°C (max 38,3°C).

EPI-100-NMM-SIT/Maintenance Medium expired on 01/26/2012, but we had to use it for dilution of MTT concentrate on 01/27/2012. To our enquiry we have bee informed by supplier (MatTek Corp., USA) that this anomal procedure will not influence the results.

All followed parameters of the assay were fulfilled, so these deviations **had no impact on the outcome of the study.**

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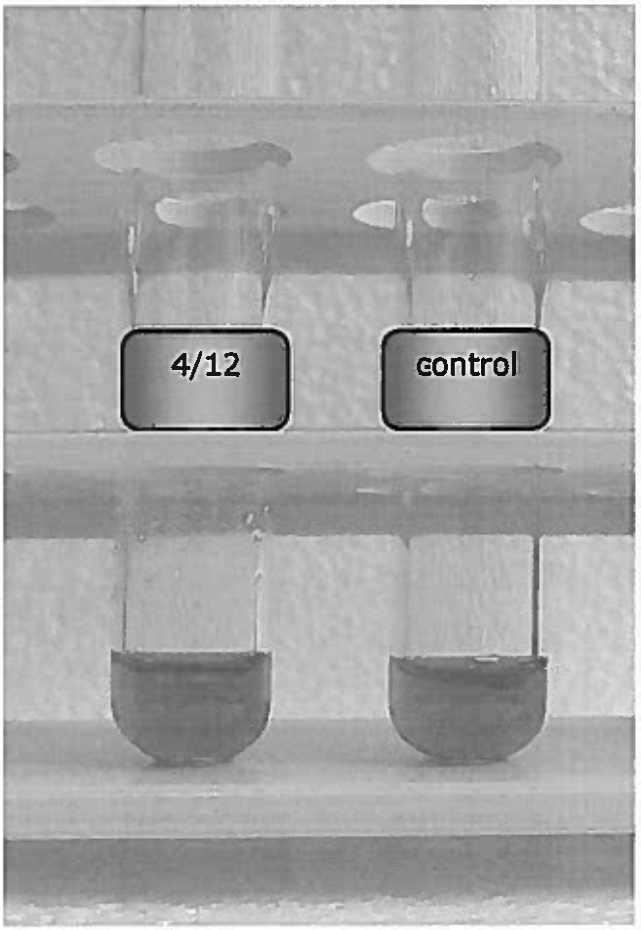
4. STUDY RESULTS

4.1. Direct MTT Reduction- Functional Check in Tubes

100 µL of the test substance was added to 1 ml of MTT medium. Suspension was incubated for 1 hour (37±1°C, 5±1 % CO_2, moistened). The test substance did not change colour (see Figure 1).

The test substance did not reduce MTT directly.

Figure 1: Direct MTT reduction (4/12 – test substance)



4.2. MTT Test

The test substance (30 µL) was placed directly atop to the previously moistened tissue. The material was spread on the tissue surface. Tissues were exposed to the test chemical for 1 hour. After exposition, tissues were thoroughly rinsed and blotted to remove the test substance. Small amount of the test substance remained in tissues. Then, tissues were let to post-incubate for 24 (medium exchange) + 18 hours (37±1°C, 5±1% CO_2, moistened).

Afterwards, the MTT assay was performed by transferring the tissues to 24-well plates containing MTT medium (1 mg/ml). After 3 hour MTT incubation, the blue formazan salt

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formed by cellular mitochondria was extracted with 2.0 ml/tissue of isopropanol and the optical density of the extracted formazan was determined using a spectrophotometer at 570 nm.

OD_{570} measuring was performed after 2-hour extraction with shaking. Results are given in the Table 1.

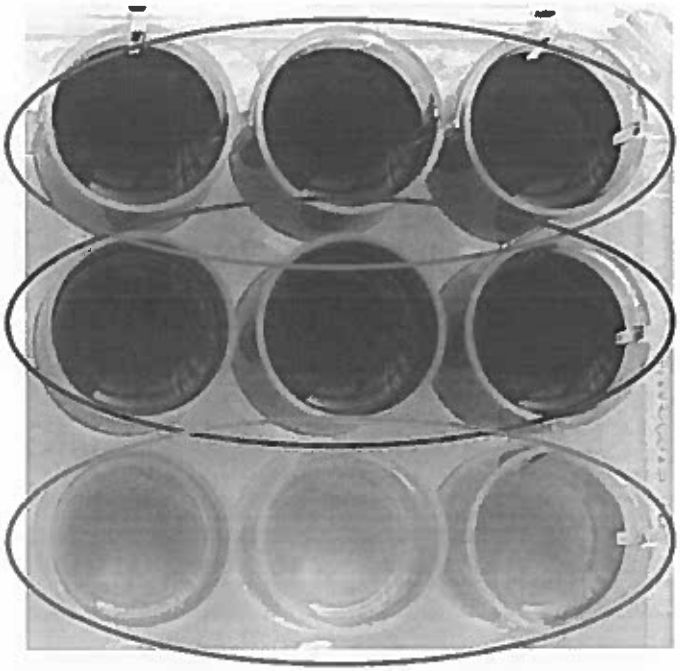
Table 1: OD_{570} values obtained at the MTT test, their averages, standard deviations (%) and relative viabilities

Treatment	Parameter	OD_{570}			Mean	SD	Average Viability (% NC)
		1	2	3			
NC (PBS)	OD_{570}	1.967	1.998	1.520	**1.828**	0.218	-
	viability (%) NC)	107.58	109.28	83.14	**100.00**	**11.945**	**100.0**
C1 Polyglyceryl-10 Decaethylhexanoate	OD_{570}	1.790	1.809	1.965	**1.855**	0.078	-
	viability (%) NC)	97.90	98.94	107.48	**101.44**	**4.288**	**101.4**
PC (5% SDS)	OD_{570}	0.216	0.098	0.248	**0.187**	0.065	-
	viability (%) NC)	11.81	5.36	13.56	**10.246**	**3.528**	**10.2**

Notes:

NC	negative control
PC	positive control
C1	test substance
mean	arithmetic mean
SD	standard deviation calculated from individual % tissue viabilities
viability (%)	viability of single tissues compared with negative control

Figure 2: Results of MTT test



- negative control (PBS)

- test substance

- positive control (SDS 5%)

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4.3. Acceptance Criteria Fulfilment

Assay acceptance criteria 1-3 given in chap. 3.8 have been met.

4.4. Evaluation of Results and Classification

Under the above-described experimental design, **average viability** of tissues treated by the test substance was **101.4 %** of negative control average value, i.e. viability was **>50 %.**

According to the classification criteria given in chap. 3.9., the test substance did not fall into any of quoted categories of skin irritation. The test substance was **nonirritating** in EpiDerm™ model.

5. CONCLUSIONS

According to study results, the test substance, **Polyglyceryl-10 Decaethylhexanoate,** was **nonirritating in EpiDerm™ model.**

6. APPENDICES

Appendix 1: Tissue Certificate

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Appendix 1: Tissue Certificate

MatTek Corporation

Certificate of Analysis

Product: EpiDerm™ Skin Tissue **Lot#** 16017

Part# : EPI-200

Description: Reconstructed skin tissue containing normal human keratinocytes.
This product is for research use only. Not for use in animals, humans or diagnostic purposes.

I. Cell source
All cells used to produce EpiDerm™ are purchased or derived from tissue obtained by MatTek Corporation from accredited institutions. In all cases, consent was obtained by these institutions from the donor or the donor's legal next of kin, for use of the cells or derivatives of the tissue for research purposes.

Keratinocyte Strain: 4F1188

II. Analysis for potential biological contaminants:
The cells used to produce EpiDerm™ tissue are screened for potential biological contaminants. Tests performed for each of the potential biological contaminant listed in the analysis that follows, were performed according to the test method given. The product resulted in "no detection" for the following potential biological contaminants determined by the stated test method:

Keratinocytes:	
HIV-1 virus – Oligonucleotide-directed amplification	Not detected
Hepatitis B virus – Oligonucleotide- directed amplification	Not detected
Hepatitis C virus – Oligonucleotide- directed amplification	Not detected
Bacteria, yeast, and other fungi – long term antibiotic, antimycotic free culture	Not detected

III. Analysis for tissue functionality:
Qualitative observation of MTT reduction: 1% Triton X-100, 5hr Exposure

Appearance: Pass

Initials: SL
Date: 27/1/2012

Paul Kearney
Paul Kearney
Quality Assurance Manager

Jan 27, 2012
Date

CAUTION: Whereas all information above is believed to be accurate and correct, no absolute guarantee that human derived material is non-infectious can be made or is implied by this certificate of analysis. All tissues should be treated as potential pathogens. The use of protective clothing and eyeware and appropriate disposal procedures are strongly recommended.

200 Homer Avenue
Ashland MA 01721 *Web Site:* www.mattek.com *Email:* information@mattek.com
(508) 881-6771
Fax (508) 879-1532

QC-10-012-0005

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ASSESSMENT OF THE SENSITIZING POTENTIAL OF A COSMETIC PRODUCT: FINAL CLINICAL SAFETY TEST UNDER DERMATOLOGICAL CONTROL

STUDY REF.	**DA11071-2**
PRODUCTS	**Polyglyceryl-10 Decaethylhexanoate (Batch No. : 91015)**
SPONSOR	**Sakamoto Yakuhin Kogyo Co., Ltd. 20, Rinkai-cho, 1-chome, Izumiotsu, Osaka 595-0075 JAPAN**
MONITOR	**Dermscan Asia**
INVESTIGATOR	**Dr Sujittra Napathorn, Dermatologist**

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SUMMARY

<table>
<tr><td colspan="3">Sponsor: Sakamoto Yakuhin Kogyo Co., Ltd.

Address:
20, Rinkai-cho, 1-chome,
Izumiotsu, Osaka 595-0075
JAPAN</td><td colspan="2">Investigator : Dermscan Asia Co., Ltd

Address:
3300/46-47 29th Flr., Tower A
Elephant Tower, Phaholyothin Rd., Chomphon,
Chatujak,Bangkok,10900
THAILAND</td></tr>
<tr><td>Study title</td><td colspan="4">ASSESSMENT OF THE SENSITIZING POTENTIAL OF A COSMETIC PRODUCT: FINAL CLINICAL SAFETY TEST UNDER DERMATOLOGICAL CONTROL</td></tr>
<tr><td rowspan="2">Product</td><td colspan="4">Reference:
Polyglyceryl-10 Decaethylhexanoate (Batch No. : 91015)</td></tr>
<tr><td colspan="2">Galenic form:
Colorless viscous liquid</td><td colspan="2">Concentration:
Pure</td></tr>
<tr><td>Patch type:</td><td colspan="4">Occlusive</td></tr>
<tr><td>Study dates</td><td colspan="4">From : January 9 to February 17, 2012.</td></tr>
<tr><td>Objective</td><td colspan="4">To confirm that repeated applications of the product under maximized conditions, in healthy subjects, does not induce delayed contact allergy reactions.</td></tr>
<tr><td>Study design</td><td colspan="4">Monocentric and simple blind study.</td></tr>
<tr><td>Assessment criteria</td><td colspan="2">• Assessment of erythema and edema
• Irritation: MCII calculation
• Sensitization: ICDRG scoring</td><td>Zone</td><td>Scapular part of the back</td></tr>
<tr><td></td><td></td><td>Kinetics</td><td>Induction : 3 weeks
Rest : 2 weeks
Challenge : 1 week</td></tr>
<tr><td rowspan="3">Study population</td><td colspan="4">Number of analyzed subjects: 50.</td></tr>
<tr><td colspan="4">Average age : 30 ± 1 years old (between 21 and 40)</td></tr>
<tr><td colspan="4"><u>Main inclusion criteria</u>
• Asian Female or male with normal skin.
• Age ≥ 18 years old
• Phototype I to IV.</td></tr>
<tr><td>Results</td><td colspan="4">Irritating potential - Induction phase: Non irritating

Sensitizing potential - Challenge phase: Non-sensitizing

<u>CONCLUSION</u>

Under these study conditions, the product Polyglyceryl-10 Decaethylhexanoate (Batch No. : 91015) can be considered "Hypoallergenic product".</td></tr>
</table>

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1. PROTOCOL

1.1. Aim of the study

The main objective of this study is to confirm that repeated applications of a cosmetic product under maximized conditions, in healthy subjects, does not induce delayed contact allergy reactions.

Its secondary objective is to determine the irritating potential of the product first after a single application and second after repeated applications.

1.2. Study design

This was an open study, without randomisation of the application site.

1.3. Study subjects

1.3.1. Inclusion criteria

- Healthy subjects.
- Asian female or male subjects with normal skin
- Age ≥ 18 years old
- Phototype I to IV.
- Subjects without scars, tattoos, any pigmentary marks, excessive pilosity and uneven skin tone or active dermal lesions, on the concerned areas of the back.
- Subjects having given their informed, written consent.
- Cooperative subjects, aware of the necessity and duration of controls so that perfect adhesion to the protocol established by DERMSCAN could be expected.

1.3.2. Non-Inclusion criteria

- Pregnant or nursing women or women without any medically efficient contraceptive method (for women of childbearing potential).
- Sun or UV exposure during the 15 days before or during the study.
- Subject having taken part to a repeated patch tests study or having received photopatch-tests from less than 4 months (with a maximum of three studies of this kind each year).
- Hyper irritable skin.
- Known allergy or sensitivity to adhesive plaster and/or cosmetic products.
- History of abnormal response to sunlight or presence of active dermal lesions, scars, beauty spots, freckles or any abnormality, on the back.
- Serious or progressive disease (mainly skin cancer background).
- Subjects undergoing a topical or systemic treatment:
  - anti-inflammatories and/or anti-histamines during the previous week and during the study,
  - photo-allergic and/or phototoxic substances from less than one month and during the study,

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- immuno-suppressors and/or corticoids during the four previous weeks and during the study,
- retinoids during the six previous months and during the study

- Any condition considered, by the investigator, as incompatible with the study protocol

1.3.3. Restriction

- No exposure to sun or U.V rays during the whole study.
- Do not apply any cosmetic products nor topic drugs to the application zone. No swimming pool, sauna... during the whole study.
- Do not start any medical treatment without informing the investigator.

1.4. Study product

The product supplied by Sponsor, had the following characteristics:

Product name	Product aspect	Product code
Polyglyceryl-10 Decaethylhexanoate (Batch No. : 91015)	Colorless viscous liquid	P1D

The product was received on 30/12/2011.

1.5. Methodology

1.5.1. Instruments, dose

The studied product was applied to a cleansed (with water) and dry skin under the following conditions:

Areas:	Scapular zones: homolateral (induction zone) and controlateral (challenge zone)
Patch tests type:	Finn Chamber® 8mm (50mm^2) - occlusive
Dose* :	25 µl
Application conditions:	Pure
Application frequency:	Induction phase: 3 times a week during 48 hours. Challenge phase: once during 48 hours.
Contact time:	Induction phase: 3 weeks. Rest phase: 2 weeks. Challenge phase: 1 week
Control:	patch without product

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1.5.2. Clinical assessment

1.5.2.1. Clinical criteria regarding the irritating potential (induction phase)

After each application, the patch is removed and the clinical examination is performed by the investigator 30 minutes later in order to eliminate the pressure and the occlusion effects.

The result of examination is negative if the skin looks normal. The clinical examination is made on the back using the following criteria and scale (Quotation 0 to 3)

Score	Quotation	CRITERIA: description :	
		ERYTHEMA «E»	OEDEMA «O»
0	Absent	no erythema	no oedema
0.5	Very slight	barely perceptible: pinkish coloration of one part of the tested area	palpable, barely visible
1	Slight	pinkish coloration of the complete tested area or rather visible on one part of the tested area	palpable, visible
2	Obvious	obvious erythema covering the whole tested area	obvious oedema (thickness < 1 mm) with or without papule(s) or vesicle(s)
3	Important	severe erythema covering all the tested area or obvious erythema diffusing outside the tested area	severe oedema (thickness ≥ 1 mm or diffusing outside the tested area) with or without papule(s) or vesicle(s)

1.5.2.2. Clinical criteria regarding the sensitizing potential (challenge phase)

The occurrence of allergic reactions was assessed according to the following scale:

Criterion	ICDRG (*) Quotation	Numeric score Quotation
No reaction	0	0
Doubtful reaction	?	?
Erythema and edema	+	1
Erythema, edema and vesicles	++	2
Severe reaction with blisters or post-blisters ulcerations	+++	3

* (International Contact Dermatitis Research Group)

A score ≥ 2 represents a contact allergy reaction.

1.5.2.3. Behaviour in case of specific reactions

In case of reaction to the support (patch), this one is moved and applied next to the initial site. If a reaction is observed again, applications are definitely stopped.

During the induction phase, if the investigator observes, at the level of the product application site, as soon as the second application, a reaction like :

- erythema ≥ 2 or
- erythema ≥ 1 with edema or discreet infiltration with a few papules,

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applications continue on an adjacent site with readings on both sites until the end of induction.
If a new reaction occurs, applications are definitely stopped.

In case of allergy suspicion, a new product application is proposed to confirm the reaction et possibly identify the concerned substance(s). This new exposure takes place at least three weeks after disappearance of the previous reaction. The new exposure conditions have to be discussed between the investigator and the sponsor. The subject is followed until total disappearance of the signs.

1.5.3. Premature study termination

The subjects had the right to leave the study at any time whatever the reason.

The premature study termination could be due to multiple reasons:
- non-compliance with the visits schedule,
- adverse events (including intercurrent diseases),
- protocol non-adherence/departures from protocol,
- withdrawal of subject consent.

1.5.4. Study schedule

1.5.4.1. Induction phase

W1:

Day of the week	Mo	Tu	We	Th	Fr	Sa	Su
Study day	D1	D2	D3	D4	D5	D6	D7
Product application	↓		↓		↓		
Removal/Assessment			↓		↓		

W2:

Day of the week	Mo	Tu	We	Th	Fr	Sa	Su
Study day	D8	D9	D10	D11	D12	D13	D14
Product application	↓		↓		↓		
Removal/Assessment	↓		↓		↓		

W3:

Day of the week	Mo	Tu	We	Th	Fr	Sa	Su	Mo
Study day	D15	D16	D17	D18	D19	D20	D21	D22
Product application	↓		↓		↓			
Removal/Assessment	↓		↓		↓			↓

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1.5.4.2. Rest phase

W4 and W5: No application and no assessment.

1.5.4.3. Challenge phase

W6:

Day of the week	Mo	Tu	We	Th	Fr
Study day	D36	D37	D38	D39	D40
Product application	2 zones ↓				
Removal/Assessment			↓		↓

1.5.5. Results interpretation

1.5.5.1. Induction phase

At the end of the 9 readings of the induction phase, the average score of each subject (Cumulative Irritation Index (C.I.I.)) was calculated by adding the scores obtained for each of the readings and by dividing this sum by the actual number of readings (a reading was not taken into account if there was reaction on the control or a global irritation).

$$C.I.I. = \frac{\sum \text{of the grade (erythema + edema)}}{\text{Number of readings}}$$

The irritating potential of the product was estimated by calculating the mean of the reactions observed during the inductionh phase in order to obtain the Mean Cumulative Irritation Index (M.C.I.I.):

MCII = CII / nb of subjects

The obtained index allow to arbitrarily classify the studied product according to the following scale:

M.C.I.I.	Class
M.C.I.I. < 0.25	Non irritating (NI)
0.25 ≤ M.C.I.I. < 0.50	Very slightly irritating (VSI)
0.5 ≤ M.C.I.I. < 1	Slightly irritating (SI)
1 ≤ M.C.I.I. < 2	Moderately irritating (MI)
M.C.I.I. ≥ 2	Irritating (I)

Individual values and the product class were taken into account to write a suitable conclusion under the study conditions.

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1.5.5.2. Challenge phase

A possible allergic reaction, during the Induction or Challenge Phase, was rated from 0 to 3 according to ICDRG (International Contact Dermatitis Research Group) – see the table paragraph 1.5.2.2.
During the Challenge Phase, the reading took place 30 minutes after patch-tests removal and 48 hours later.

The sensitizing potential of the product was assessed by the readings on D38 and D40 (Challenge Phase) according to the following criteria: reaction ++ (2) or +++ (3) in the absence of added irritation phenomenon.

The presence of only one case of active sensitizing **(upper or equal score in ++ (2))** on controlateral side leads to the conclusion "Potentially sensitive product".

2. STUDY FOLLOW-UP

2.1. Studied population

2.1.1. Inclusion

55 healthy subjects were selected according to the inclusion and the non-inclusion criteria, and 50 subjects completed study.
The table below presents the information concerning all the included subjects.

	Non included	Included	Drop out	Untraceable
Number of subjects	0	55	5*	0

Remark: * withdrawal of subject consent

2.1.2. Subjects characteristics

The summary table below presents a synthesis of the observations concerning exclusively the subjects taken into account for data analysis.

Number of subjects	Sex	Age (mean±SEM)	Phototype	Medical or surgical events and medical treatments	
				Before the study	During the study
50	38: F 12: M	30 ± 1	III: 11 IV: 39	see Tables in the APPENDIX II	

2.2. Protocol amendment and/or non-adherence

There was no protocol amendment and no non-adherence to the protocol was observed.

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3. RESULTS

3.1. Irritating potential: induction phase

The TABLE OF READINGS regarding the Induction Phase is presented in the APPENDIX III.

The readings done 30 minutes after having removed the patch-tests showed the following results:

Product	Score	D3		D5		D8		D10		D12		D15		D17		D19		D22		Global result
		n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	
Polyglyceryl-10 Decaethylhexanoate (Batch No. : 91015)	T+	0	0	7	14	0	0	0	0	7	14	0	0	1	2	0	0	1	2	Non-irritating (0.007)
	0:	50	100	41	82	49	98	50	100	43	86	49	98	48	96	50	100	48	96	
	0.5:	0	0	2	4	1	2	0	0	0	0	1	2	1	2	0	0	1	2	
	1:	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	2:	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	3:	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Patch move to adjacent site		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Not patch		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	

n = number of subjects
T+ = positive control

% = % of subjects
*see score in Appendix III

Under these study conditions, product Polyglyceryl-10 Decaethylhexanoate (Batch No. : 91015) showed a score, so it can be considered as non-irritating.

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3.2. Sensitizing potential: challenge phase

The TABLE OF READINGS regarding the Challenge Phase is presented in APPENDIX IV. These reading made 30 minutes and 48 hours after having removed the patch-tests showed the following results:

Product	Zones	score	Day of the reading				Global result
			D38		D40		
			n	%	n	%	
Polyglyceryl-10 Decaethylhexanoate (Batch No. : 91015)	Homolateral	T+	0	0	0	0	Non-sensitizing
		0 :	48	96	50	100	
		? :	2	4	0	0	
		+ :	0	0	0	0	
		++ :	0	0	0	0	
		+++ :	0	0	0	0	
	Controlateral	T+	0	0	0	0	
		0 :	49	98	50	100	
		? :	1	2	0	0	
		+ :	0	0	0	0	
		++ :	0	0	0	0	
		+++ :	0	0	0	0	

n = number of subjects % = % of subjects
T+ = positive control

Under these study conditions no reaction ++ (2) nor +++ (3) were observed, so the product Polyglyceryl-10 Decaethylhexanoate (Batch No. : 91015) can be considered non-sensitizing.

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4. CONCLUSION

Under these study conditions, the product Polyglyceryl-10 Decaethylhexanoate (Batch No. : 91015) can be considered non-irritating and non-sensitizing.

According to these elements, this product can claim the label "HYPOALLERGENIC".

Bangkok
March 23, 2012

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5. BIBLIOGRAPHY

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- AFSSAPS : "TEST CLINIQUE FINAL DE SECURITE D'UN PRODUIT COSMETIQUE EN VUE DE CONFIRMER SON ABSENCE DE POTENTIEL SENSIBILISANT CUTANE RETARDE : RECOMMANDATIONS AUX PROMOTEURS DE RECHERCHE ET AUX PRESTATAIRES DE SERVICE" - Recommandations TCFS – version finale de décembre 2008.

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6. APPENDIX

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APPENDIX I

AUTHENTIFICATION SHEET

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AUTHENTIFICATION PAGE

I am aware that the study N° DA11071-2,

has been conducted according to the STUDY PROTOCOL.

Dr Sujittra NAPATHORN Date 23 MAR 2012 Signature

Dermatologist

Arisa SURINPAO Date 23 MAR 2012 Signature Arisa Surinpao

Technician

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APPENDIX II

SUBJHECTS CHARACTERISTICS

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SUBJECTS CHARACTERISTICS

Subject	Name (the 3 first letters)	First name (the 2 first letters)	Age	Sex	Phototype	Skin type	Medical or surgical events and medical treatment	
							before the study	during the study
(1)*	(KON)*	(JA)*	(40)*	(F)*	(III)*	(N)*	(NO)*	(NO)*
2	TIA	KA	35	F	IV	N	NO	NO
3	TAB	UB	30	F	IV	N	NO	NO
4	SUP	SA	37	F	III	N	NO	NO
(5)*	(SRI)*	(KA)*	(23)*	(F)*	(IV)*	(N)*	(NO)*	(NO)*
6	PRA	PR	22	M	III	N	NO	NO
7	TON	SU	22	F	IV	N	NO	NO
8	KHU	SU	22	F	IV	N	NO	NO
9	MAN	RU	38	F	IV	N	NO	NO
10	KEE	NH	37	F	IV	N	NO	NO
11	SAK	AR	40	F	IV	N	NO	NO
12	RUN	NI	31	F	III	N	NO	NO
13	BUN	AP	30	F	III	N	NO	NO
14	BUR	SU	39	F	IV	N	NO	NO
(15)*	(PON)*	(TA)*	(30)*	(F)*	(IV)*	(N)*	(NO)*	(NO)*
16	YOY	SU	23	F	IV	N	NO	NO
17	MAN	WA	22	M	IV	N	NO	NO
18	SAS	SU	22	F	IV	N	NO	NO
19	JAI	NA	21	F	IV	N	NO	NO
20	CHU	SU	22	F	IV	N	NO	NO
21	RAH	JU	34	F	IV	N	NO	NO
22	UEA	KI	36	M	IV	N	NO	NO
23	SAD	DU	31	F	IV	N	NO	NO
24	TAB	BU	36	F	IV	N	NO	NO
25	MEE	PR	33	M	IV	N	NO	NO
26	PAN	TA	26	F	III	N	NO	NO
27	PEN	KA	26	F	III	N	NO	NO
28	POE	KA	38	F	IV	N	NO	NO
29	JAM	WI	37	M	IV	N	NO	NO
30	JAI	NO	32	M	IV	N	NO	NO
31	SAN	PI	23	M	IV	N	NO	NO
32	SAR	YU	33	F	IV	N	NO	NO
33	MEE	DA	31	M	IV	N	NO	NO
34	TAN	JA	22	F	III	N	NO	NO
35	SOI	KO	26	M	IV	N	NO	NO
(36)*	(JAM)*	(WA)*	(36)*	(F)*	(III)*	(N)*	(NO)*	(NO)*
(37)*	(PEE)*	(RA)*	(38)*	(F)*	(IV)*	(N)*	(NO)*	(NO)*
38	KAW	TO	31	F	III	N	NO	NO
39	KUH	KA	27	F	IV	N	NO	NO
40	KAM	UD	31	F	IV	N	NO	NO
41	MEE	NI	34	F	IV	N	NO	NO
42	WAN	KO	34	F	IV	N	NO	NO
43	RAJ	PO	25	M	IV	N	NO	NO
44	KID	RA	21	F	IV	N	NO	NO
45	CHA	SU	36	F	IV	N	NO	NO
46	LUE	HA	39	F	IV	N	NO	NO
47	BUN	RA	34	F	IV	N	NO	NO
48	TOE	KA	35	F	III	N	NO	NO
49	RUN	SI	39	F	IV	N	NO	NO
50	MAO	KI	30	M	IV	N	NO	NO
51	WOR	MI	23	F	IV	N	NO	NO
52	ROJ	AT	25	F	III	N	NO	NO
53	MAN	CH	23	M	IV	N	NO	NO
54	SUR	AR	24	F	III	N	NO	NO
55	KAE	WA	39	F	IV	N	NO	NO

Mean	30	38	F	0	I
Median	31	12	M	0	II
Minimum	21			11	III
Maximum	40			39	IV
SEM	1			0	V

()*: values not included in data analysis
F = Female
M = Male

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APPENDIX III

TABLES OF THE READINGS – INDUCTION PHASE

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TABLES OF THE READINGS – INDUCTION PHASE

No.of subject	D3		D5		D8		D10		D12		D15		D17		D19		D22	
	induction site		induction site		induction site		induction site		induction site		induction site		induction site		induction site		induction site	
	P1D	C	P1D	C	P1D	C	P1D	C	P1D	C	P1D	C	P1D	C	P1D	C	P1D	C
(1)*	(0)*	(0)*	Drop Out															
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	(0)*	(0.5)*	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
(5)*	(0)*	(0)*	Drop Out															
6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	(0)*	(1)*	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
(15)*	(0)*	(0)*	(0)*	(0)*	(0)*	(0)*	Drop Out											
16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0	(0)*	(0.5)*	0.5	0	0	0	0	0	0	0
18	0	0	0	0	0.5	0	0	0	0	0	0	0	0	0	0	0	0	0
19	0	0	0.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
22	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
23	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
26	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
27	0	0	(0)*	(0.5)*	0	0	0	0	0	0	0	0	0	0	0	0	0	0
28	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
29	0	0	0.5	0	0	0	0	0	(0)*	(0.5)*	0	0	(0)*	(0.5)*	0	0	0	0
30	0	0	(0.5)*	(0.5)*	0	0	0	0	(0.5)*	(1)*	0	0	0	0	0	0	0.5	0
31	0	0	(0)*	(0.5)*	0	0	0	0	0	0	0	0	0	0	0	0	0	0
32	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
33	0	0	0	0	0	0	0	0	(0.5)*	(0.5)*	0	0	0	0	0	0	0	0
34	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
35	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
(36)*	Drop Out																	
(37)*	Drop Out																	
38	0	0	(0)*	(0.5)*	0	0	0	0	(0)*	(0.5)*	0	0	0	0	0	0	0	0
39	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
40	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
41	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
42	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
43	0	0	(0)*	(0.5)*	0	0	0	0	(0)*	(0.5)*	0	0	0	0	0	0	(0)*	(0.5)*
44	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
45	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
46	0	0	(0)*	(0.5)*	0	0	0	0	0	0	0	0	0	0	0	0	0	0
47	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
48	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
49	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
50	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
51	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
52	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
53	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
54	0	0	0	0	0	0	0	0	0	0	0	0	0.5	0	0	0	0	0
55	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

()*: values not included in data analysis
P1D = Tested product
C = control

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APPENDIX IV

TABLES OF THE READINGS – CHALLENGE PHASE

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TABLES OF THE READINGS – CHALLENGE PHASE

No.of subject	D38				D40			
	induction site		challenge site		induction site		challenge site	
	RBM	C	RBM	C	RBM	C	RBM	C
(1)*	Drop Out							
2	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0
4	+	0	?	0	?	0	?	0
(5)*	Drop Out							
6	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0
8	?	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0
13	0	0	?	0	0	0	0	0
14	0	0	0	0	0	0	0	0
(15)*	Drop Out							
16	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0
20	?	0	0	0	0	0	0	0
21	0	0	0	0	0	0	0	0
22	0	0	0	0	0	0	0	0
23	0	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0	0
25	0	0	0	0	0	0	0	0
26	0	0	0	0	0	0	0	0
27	0	0	0	0	0	0	0	0
28	0	0	0	0	0	0	0	0
29	?	0	0	0	0	0	0	0
30	0	0	0	0	0	0	0	0
31	0	0	0	0	0	0	0	0
32	?	0	0	0	0	0	0	0
33	0	0	0	0	0	0	0	0
34	0	0	0	0	0	0	0	0
35	0	0	0	0	0	0	0	0
(36)*	Drop Out							
(37)*	Drop Out							
38	+	0	+	0	0	0	0	0
39	0	0	0	0	0	0	0	0
40	0	0	0	0	0	0	0	0
41	0	0	0	0	0	0	0	0
42	+	0	?	0	0	0	0	0
43	0	0	0	0	0	0	0	0
44	+	0	+	0	?	0	+	0
45	0	0	0	0	0	0	0	0
46	0	0	0	0	0	0	0	0
47	0	0	0	0	0	0	0	0
48	++	0	+	0	?	0	?	0
49	0	0	0	0	0	0	0	0
50	0	0	0	0	0	0	0	0
51	0	0	0	0	0	0	0	0
52	0	0	0	0	0	0	0	0
53	0	0	0	0	0	0	0	0
54	?	0	?	0	?	0	?	0
55	0	0	0	0	0	0	0	0

()*: values not included in data analysis
P1D = Tested product
C = control

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Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: Lillian Gill, D.P.A.
Director - COSMETIC INGREDIENT REVIEW (CIR)

FROM: Beth A. Lange, Ph.D.
Industry Liaison to the CIR Expert Panel

DATE: March 25, 2016

SUBJECT: Information on Polyglyceryl-10 Pentaisostearate (SLR posted January 28, 2016)

CiTox LAB Hungary Ltd. 2011. The testing of Polyglyceryl-10 Pentaisostearate with bacterial reverse mutation assay.

Consumer Product Testing Co. 2011. The MatTek Corporation EpiOcular™ tissue model *in vitro* toxicity testing system (Polyglyceryl-10 Pentaisostearate).

Japan Hair Science Association. 2011. Patch test on the cosmetic ingredient (Polyglyceryl-10 Pentaisostearate).

Research Institute for Organic Syntheses Inc. 2011. In vitro skin irritation test (EpiDerm™ Model) (Polyglyceryl-10 Pentaisostearate).

Dermscan Asia. 2011. Assessment of the sensitizing potential of a cosmetic product: final clinical safety test under dermatological control (Polyglyceryl-10 Pentaisostearate).

1620 L Street, N.W., Suite 1200 | Washington, D.C. 20036 | 202.331.1770 | 202.331.1969 (fax) | www.personalcarecouncil.org

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CiToxLAB Hungary Ltd.
Veszprém - Szabadságpuszta

FINAL REPORT

The Testing of
S Face IS-1005P
with
Bacterial Reverse Mutation Assay

Polyglyceryl-10 Pentaisostearate

Study code: 11/193-007M

Study Director: Judit Hargitai, PhD.

03 November 2011

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STATEMENT OF THE STUDY DIRECTOR

This study has been performed in accordance with the study plan agreed upon by the Sponsor, the OECD Guidelines for Testing of Chemicals (No. 471, 21 July 1997), Commission Regulation (EC) No.440/2008, B.13/14., EPA Health Effects Test Guidelines, OPPTS 870.5100 (EPA 712-C-98-247, 1998 and EPA 712-C-96-247, 1996) and the Principles of Good Laboratory Practice (Hungarian GLP Regulations: 9/2001 (III.30.) EüM-FVM joint decree of the Minister of Health and the Minister of Agriculture and Regional Development, which corresponds to the OECD GLP, ENV/MC/CHEM(98)17).

I, the undersigned, declare that this report constitutes a true record of the actions undertaken and the results obtained in this study. By virtue of my dated signature I accept the responsibility for the validity of the data and the following conclusion drawn from them:

In conclusion, the test item S Face IS-1005P (Batch Number: 10301) had no mutagenic activity on the growth of the applied bacterium tester strains under the test conditions used in this study.

Signature: _____ Date: 03 November 2011

Judit Hargitai, PhD.
Study Director

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STATEMENT OF THE MANAGEMENT

According to the conditions of the research and development agreement between SAKAMOTO YAKUHIN KOGYO CO., LTD. (as Sponsor) and CiToxLAB Hungary [formerly: LAB Research Ltd.] (as Testing Facility) the study titled "The Testing of S Face IS-1005P with Bacterial Reverse Mutation Assay" has been performed in compliance with the study plan and the Principles of Good Laboratory Practice.

Signature: _____ Date: 03 Nov 2011

Christopher Banks, DABT
Managing Director

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QUALITY ASSURANCE STATEMENT

Study Code: 11/193-007M

Study Title: The Testing of S Face IS-1005P with Bacterial Reverse Mutation Assay

Test Item: S Face IS-1005P

In order to enable the results of this study to meet legal requirements including regulatory submission and/or safety assessment , this study has been inspected and this report audited by the Quality Assurance Unit in compliance with the Principles of Good Laboratory Practice. As far as it can be reasonably established the methods described and the results incorporated in this report accurately reflect the raw data produced during this study.

All inspections, data reviews and the report audit were reported in written form to the study director and to management. The dates of such inspections and of the report audit are given below:

Date	Phase(s) Inspected/Audited	Date of report to	
		Management	Study Director
10 August 2011	Study Plan	10 August 2011	10 August 2011
06 September 2011	Test Item formulation	06 September 2011	06 September 2011
14 October 2011	Draft Report	14 October 2011	14 October 2011
03 November 2011	Final Report	03 November 2011	03 November 2011

Signature: Fábiánné Makovi Éva Date: 03 November 2011

Éva Fábiánné Makovi, B.Sc.
On behalf of QA

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GENERAL INFORMATION

STUDY TITLE	:	The Testing of S Face IS-1005P with Bacterial Reverse Mutation Assay
TEST ITEM	:	S Face IS-1005P
SPONSOR	:	SAKAMOTO YAKUHIN KOGYO CO., LTD. Address: 1-20, Rinkaicho, Izumiotsu-City, Osaka, 595-0075, Japan Phone: +81 725 33 6981 Fax: +81 725 23 1198
TEST FACILITY	:	CiToxLAB Hungary Ltd. * (Formerly known as LAB Research Ltd. before 1st September 2011) Address: H-8200 Veszprém, Szabadságpuszta, Hungary Phone: +36 88 545 300 Fax: +36 88 545 301

*: *After the Study Plan was issued the legal name of the company was changed to CiToxLAB Hungary Ltd.*

BASIS OF STUDY	:	OECD Guidelines for Testing of Chemicals, No.471 EPA Health Effects Test Guidelines, OPPTS 870.5100 Commission Regulation (EC) No. 440/2008, B.13/14.

STUDY PLAN	:	11 August 2011
AMENDMENT 1 TO THE STUDY PLAN	:	30 August 2011
RANGE FINDING TEST	:	17-19 August 2011
EXPERIMENTAL PERIOD I.	:	30 August – 01 September 2011
EXPERIMENTAL PERIOD II.	:	06-08 September 2011
DRAFT REPORT	:	19 October 2011
FINAL REPORT	:	03 November 2011

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RESPONSIBLE PERSONS:

TEST FACILITY MANAGEMENT	:	Christopher Banks, DABT Managing Director Alyson Leyshon, M.Sc. Senior Director of Toxicology Operations
QUALITY ASSURANCE	:	Szabolcs Gáty, M.Sc. Head of QAU Vanda Gyimesi, M.Sc. Deputy Head of QAU Éva Fábiánné Makovi, B.Sc. QA Inspector
STUDY DIRECTOR	:	Judit Hargitai, PhD. Phone: +36 88 545 273 E-mail: judit.hargitai@hu.citoxlab.com
SCIENTIFIC ASSISTANT	:	Béla Oláh, PhD.
CENTRAL DISPENSARY	:	Tamás Mészáros, PhD. (Technical Team Leader)
TECHNICAL ASSISTANTS	:	Levente Jánni, B.Sc. Tímea Kovácsné Böröcz Emma Petőné Kohán Krisztina Pátkainé Fejes Ákos Klaffl Kata Gönczöl Zoltánné Marcsik

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1. SUMMARY

The test item S Face IS-1005P was tested for potential mutagenic activity using the Bacterial Reverse Mutation Assay.

The experiments were carried out using histidine-requiring auxotroph strains of *Salmonella typhimurium* (*Salmonella typhimurium* TA98, TA100, TA1535 and TA1537) and the tryptophan-requiring auxotroph strain of *Escherichia coli* (*Escherichia coli* WP2 *uvrA*) in the presence and absence of a post mitochondrial supernatant (S9 fraction) prepared from the livers of phenobarbital/β-naphthoflavone-induced rats.

The study included a Preliminary Solubility Test, a Preliminary Range Finding Test (Informatory Toxicity Test), an Initial Mutation Test (Plate Incorporation Method) and a Confirmatory Mutation Test (Pre-Incubation Method).

Based on the results of the Solubility Test and available information, the test item was dissolved in Acetone. Concentrations of 5000; 2500; 1000; 316; 100; 31.6 and 10 µg/plate were examined in the Range Finding Test. Based on the results of the Range Finding Test, the test item concentrations in the two independently performed main experiments were: 5000; 1581; 500; 158.1; 50; 15.81 and 5 µg/plate.

In the Initial Mutation Test and Confirmatory Mutation Test, none of the observed revertant colony numbers were above the respective biological threshold value. There were no consistent dose-related trends and no indication of any treatment effect. In all test item treated groups, the numbers of revertant colonies were below the biological relevance when compared with the solvent controls and were within the historical control range and were within the normal biological variability of the test system.

Oily patches were observed in the Confirmatory Mutation Test at 5000 µg/plate concentration with and without metabolic activation in all strains.

The mean values of revertant colonies of the solvent control plates were within the historical control data range, the reference mutagens showed the expected increase in the number of revertant colonies, the viability of the bacterial cells was checked by a plating experiment in each test. The tests were considered to be valid.

The reported data of this mutagenicity assay show (see Appendix 2 to 5) that under the experimental conditions applied the test item **did not induce gene mutations by base pair changes or frameshifts in the genome of the strains used.**

In conclusion, the test item S Face IS-1005P had no mutagenic activity on the growth of the bacterium tester strains under the test conditions used in this study.

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2. INTRODUCTION

The bacterial reverse mutation test is a microbial assay which detects point mutations induced by chemicals causing base changes or frameshift mutations in the genome of amino-acid requiring strains of *Salmonella typhimurium* and *Escherichia coli*. The auxotrophic *S. typhimurium* and *E. coli* strains are unable to grow on minimal medium – containing inorganic salts and glucose as a carbon source – except for spontaneous revertants. But in the presence of a mutagenic agent, some of them can be converted to prototrophs after a reverse mutation to the wild type. These revertants can grow and form colonies in minimal medium. An increased number of the revertant colonies indicates mutagenic activity of the test item.

The *Salmonella typhimurium* histidine (his) reversion system measures $his^- \rightarrow his^+$ reversions. The *Salmonella typhimurium* strains are constructed to differentiate between base-pair (TA1535, TA100) and frameshift (TA1537, TA98) mutations. The *Escherichia coli* WP2 *uvrA* tryptophan (trp) reversion system measures $trp^- \rightarrow trp^+$ reversions. The *Escherichia coli* WP2 *uvrA* detects mutagens that cause base-pair substitutions (AT to GC).

The bacteria used in this assay did not possess the mammalian enzyme system that is known to convert promutagens into mutagenic metabolites. In order to overcome this major drawback, an exogenous metabolic activation system is added in the form of a mammalian microsomal enzyme activation mixture (liver extract, S9 fraction). The activation system uses nicotinamide-adenine dinucleotide phosphate (NADP+)-cytochrome P450 dependent mixed function oxidase enzymes of the liver. The liver extract was obtained from rats, which were pre-treated with □henobarbital and β-naphthoflavone, two inducers of several drug-metabolizing enzymes.

The bacterial mutagenicity test, along with other short-term assays, is used extensively to evaluate substances for mutagenic activity. Some mutagens (e.g.: nitrosamines) are poorly detected in the standard plate incorporation assay, but they can be detected by the pre-incubation assay.

3. OBJECTIVE OF STUDY

The purpose of this study was to evaluate the mutagenic potential of S Face IS-1005P test item by measuring its ability to induce reverse mutations at selected loci of several strains of *Salmonella typhimurium* and at the tryptophan locus of *Escherichia coli* WP2 *uvr*A strain in the presence and absence of activated rat liver S9 fraction.

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4. GUIDELINES

This study followed the procedures indicated by the following internationally accepted guidelines and recommendations:

- Ninth Addendum to OECD Guidelines for Testing of Chemicals, Section 4, No. 471, "Bacterial Reverse Mutation Test", 21 July 1997
- EPA Health Effects Test Guidelines, OPPTS 870.5100 "Bacterial Reverse Mutation Test", EPA 712-C-98-247, August 1998
- EPA Health Effects Test Guidelines, OPPTS 870.5100 "*Escherichia coli* WP2 and WP2 *uvrA* Reverse Mutation Assays", EPA 712-C-96-247, June 1996 (Public Draft)
- Commission Regulation (EC) No. 440/2008, B.13/14. "Mutagenicity: Reverse Mutation Test Using Bacteria", 30 May 2008

The method described the above mentioned guidelines conforms to the guidelines for bacterial mutagenicity testing published by the major Japanese Regulatory Authorities including METI, MHLW and MAFF.

5. MATERIALS AND METHODS

5.1. TEST ITEM

5.1.1. Name and Data of Test Item*

Name:	S Face IS-1005P
Chemical Name:	Polyglyceryl-10 Pentaisostearate
CAS number:	83138-62-9, 126449-40-9
Batch/Lot number:	10301
Molecular weight:	ca. 2350
Appearance:	Pale yellow liquid with faint characteristic odour
Specific gravity:	0.989
Purity:	100%
Manufacture date:	01 March 2011
Expiry date:	01 August 2012
Storage conditions:	Room temperature (15-25°C)
Safety precautions:	Routine safety precautions (lab coat, gloves, safety glasses, face mask) for unknown materials were applied to assure personnel health and safety.

*Based on the Certificate of Analysis (dated 01 August 2011), Test Item Data Sheet (dated 01 August 2011) and Material Safety Data Sheet (dated 10 March 2011) provided by the Sponsor.

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5.1.2. Identification, Receipt

The test item of a suitable chemical purity was supplied by the Sponsor. All precautions required in the handling and disposal of the test item were outlined by the Sponsor. These documents are part of the raw data. Identification of the test item was performed in the Central Dispensary of CiToxLAB Hungary Ltd. On the basis of the information provided by the Sponsor. The copy of the Certificate of Analysis is shown in Appendix 1.

5.1.3. Formulation

The behaviour of the test item solutions with the solution of top agar (Sections 5.4.4.) and phosphate buffer (Section 5.5.4.) was examined in a preliminary solubility test. Acetone was used as solvent to prepare the stock formulation of the test material. Test solutions were freshly prepared at the beginning of the experiments in the testing laboratory by diluting the stock formulation using the selected vehicle.

Analytical determination of the test item concentration, stability and homogeneity was not performed because of the character and the short period of study.

Table 1: Formulation of the Test Item for the Main Experiments

No. of concentration	Concentration of the test item	Concentration (µg/plate)
1	100 mg/mL	5000
2	31.62 mg/mL	1581
3	10 mg/mL	500
4	3.162 mg/mL	158.1
5	1 mg/mL	50
6	0.3162 mg/mL	15.81
7	0.1 mg/mL	5

5.2. POSITIVE AND NEGATIVE CONTROLS

Positive and negative (solvent) controls were included in the experiments. Routine safety precautions (lab coat, gloves, safety glasses and face mask) were applied to assure personnel health and safety.

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5.2.1. Positive Controls

Strain specific positive controls were included in the assay, which demonstrated the effective performance of the test. The strain specific positive control chemicals are summarized in Table 2.

Table 2: Strain-Specific Positive Control Chemicals

Metabolic activation	Strain specific positive chemical		Vehicle	Dose quantity/plate	Strain
	Chemical and Supplier	Batch number / Expiry date			
non-activation	4-nitro-1,2-phenylene-diamine (NPD) MERCK	S5061894 / 28 Feb 2013	DMSO	4 µg	*Salmonella* TA98
non-activation	Sodium azide (SAZ) SIGMA-ALDRICH	MKBF0623 / 02 Feb 2012	Distilled water	2 µg	*Salmonella* TA100 and TA1535
non-activation	9-aminoacridine (9AA) MERCK	S03761 / 31 Dec 2012	DMSO	50 µg	*Salmonella* TA1537
non-activation	Methyl-methanesulfonate (MMS) SIGMA-ALDRICH	MKBD8856V / 30 Dec 2011	Distilled water	2 µL	*E.coli* WP2 *uvrA*
activation	2-aminoanthracene (2AA) SIGMA-ALDRICH	STBB1901 / 31 Oct 2011	DMSO	2 µg	all *Salmonella* strains
activation	2-aminoanthracene (2AA) SIGMA-ALDRICH	STBB1901 / 31 Oct 2011	DMSO	50 µg	*E.coli* WP2 *uvrA*

Abbreviation for tester strains: *Salmonella*: *Salmonella typhimurium; E.coli*: *Escherichia coli*

5.2.2. Negative Controls (Solvent Controls)

In the study three vehicle control groups were used depending on the solubility of the test item and the solubility of strain specific positive chemicals. The following chemicals were used for vehicle (solvent) control groups:

<u>Acetone:</u>

Supplier: REANAL
Batch No.: KBM62741
Expiry date: February 2016
Grade: PhEur.6
Purity: >99.9%

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Dimethyl sulfoxide (DMSO):

Supplier:	Sigma-Aldrich Co.
Batch No.:	BCBD8784V
Expiry date:	December 2016
Grade:	puriss, p.a., ACS reagent, ≥99.9% (GC)
Purity:	99.96%

Distilled water:

Supplier:	TEVA Hungary Co.
Batch No.:	0110111 / 2170511
Expiry date:	January 2014 / May 2014

Note: Lot 0110111 was used in the preliminary experiment; lot 2170511 was used in the main tests.

5.3. BACTERIAL STRAINS

5.3.1. Origin

Date of arrival and origin:

Salmonella typhimurium TA98	16 November 2010, MOLTOX – Molecular Toxicology Inc., Boone, North Carolina, USA
Salmonella typhimurium TA100	04 December 2008, BioReliance Corporation, Rockville, Maryland, USA
Salmonella typhimurium TA1535	16 November 2010, MOLTOX – Molecular Toxicology Inc., Boone, North Carolina, USA
Salmonella typhimurium TA1537	16 November 2010, MOLTOX – Molecular Toxicology Inc., Boone, North Carolina, USA
Escherichia coli WP2 *uvrA*	16 November 2010, MOLTOX – Molecular Toxicology Inc., Boone, North Carolina, USA

The true copies of original certificates and other documents of the strains are collected and stored in the Microbiological Laboratory of the CiToxLAB Hungary Ltd.

5.3.2. Genotypes

In addition to histidine or tryptophan mutation, each strain has additional mutations, which enhances its sensitivity to mutagens. The *uvrB* (*uvrA*) strains are defective in excision repair, making them more sensitive to the mutagenic and lethal effects of a wide variety of mutagens because they cannot repair DNA damages. The presence of *rfa* mutation increases the permeability of the bacterial lipopolysaccharide wall for larger molecules.

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The plasmid pKM101 (TA98, TA100) carries the *muc+* gene which participates in the error-prone "SOS" DNA repair pathway induced by DNA damage. This plasmid also carries an ampicillin resistance transfer factor (R-factor) which is used to identify its presence in the cell. The *Escherichia coli* strain used in this test (WP2 *uvrA*) is also defective in DNA excision repair. The genotypes of the tester strains used for mutagenicity testing are summarized in Table 3.

Table 3: Genotypes of the Strains Used for Mutagenicity Testing

Strain	Genotype		Mutations		Main DNA target	Plasmid
	trp./his mutation	type of mutation	cell wall	DNA- repair		
S.typhimurium TA98	*hisD3052*	Frameshift	*rfa*	*uvrB*	GC	pKM101
S.typhimurium TA100	*hisG46*	Base pair substitution	*rfa*	*uvrB*	GC	pKM101
S.typhimurium TA1535	*hisG46*	Base pair substitution	*rfa*	*uvrB*	GC	No
S.typhimurium TA1537	*hisC3076*	Frameshift	*rfa*	*uvrB*	GC	No
E.coli WP2 *uvrA*	*trpE*	Base pair substitution	+	*uvrA*	AT	No

Abbreviations of tester strains: *S.typhimurium.: Salmonella typhimurium; E.coli: Escherichia coli*

5.3.3. Storage

The strains are stored at -80 ± 10°C in the Culture Collection of the Microbiological Laboratory of the CiToxLAB Hungary Ltd. Frozen permanent cultures of the tester strains were prepared from fresh, overnight cultures to which DMSO was added as a cryoprotective agent.

5.3.4. Confirmation of Phenotypes of Tester Strains

The phenotypes of the tester strains used in the bacterial reverse mutation assays with regard to membrane permeability (*rfa*), UV sensitivity (*uvrA* and *uvrB*), ampicillin resistance (*amp*), as well as spontaneous mutation frequencies are checked regularly according to Ames et al. [1] and Maron and Ames [2].

Established procedures (Standard Operating Procedures) for the preparations of each batch of frozen stock culture, raw data and reports of phenotype confirmation are stored in the Microbiological Laboratory of CiToxLAB Hungary Ltd.

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5.3.5. Spontaneous Reversion of Tester Strains

Each test strain reverts spontaneously at a frequency that is characteristic of the strain. Spontaneous reversion of the test strains to histidine (*Salmonella typhimurium* strains) or tryptophan (in *Escherichia coli* strain) independence is measured routinely in mutagenicity experiments and expressed as the number of spontaneous revertants per plate. Historical control values for spontaneous revertants (revertants/plate) for untreated control sample without metabolic activation were in the period of 2004 to 2010 were (as guide) as follows: *Salmonella typhimurium* TA98: 8-54, TA100: 58-211, TA1535: 2-31, TA1537: 1-24, *Escherichia coli* WP2 *uvrA*: 9-86. More detailed historical control data are shown in Appendix 6.

5.3.6. Procedure for Growing Cultures

The frozen bacterial cultures were thawed at room temperature and 200 µL inoculum were used to inoculate each 50 mL of Nutrient Broth No.2 (Section 5.4.2.) for the overnight cultures in the assay. The cultures were incubated for 10-14 hours at 37°C in a Gyrotory water bath shaker.

5.3.7. Viability of the Testing Cultures

The viability of each testing culture was determined by plating 0.1 mL of the10^5, 10^6, 10^7 and 10^8 dilutions of cultures on Nutrient Agar (Section 5.4.3.) plates. The viable cell number of the cultures was determined by manual counting.

5.4. MEDIA

The supplier, batch number and expiry date of the used chemicals described in sections 5.4.1.-5.4.5. are summarized in Table 6 (Section 5.9. Chemicals Used in the Experiments). The composition of media refers to 1000 mL.

5.4.1. The Typical Composition (g/1000 mL) of Minimal Glucose Agar

Glucose	20.0	g
Magnesium sulfate	0.2	g
Citric acid	2.0	g
di-Potassium hydrogenphosphate	10.0	g
Sodium ammonium hydrogenphosphate	3.5	g
Agar agar	15.0	g
Distilled water	q.s. ad[1] 1000	mL

[1] q.s. ad = quantum sufficiat ad (a sufficient quantity to make)

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Minimal glucose agar plates [MERCKOPLATE] (Batch number: 14584, Expiry date: 16 November 2011, used in the preliminary experiment; and Batch number: 14588, Expiry date: 19 November 2011, used in the main tests) were provided by Merck. Certificates of Analysis were obtained from the Supplier.

5.4.2. Nutrient Broth No.2

Nutrient Broth No.2.	25.0	g
Distilled water	q.s. ad 1000	mL

Sterilization was performed at 121°C in an autoclave.

5.4.3. Nutrient Agar

Nutrient Agar	20.0	g
Distilled water	q.s. ad 1000	mL

Sterilization was performed at 121°C in an autoclave.

5.4.4. Top Agar for *Salmonella typhimurium* Strains

Agar solution:

Agar Bacteriological	4.0	g
NaCl	5.0	g
Distilled water	q.s. ad 1000	mL

Sterilization was performed at 121°C in an autoclave.

Histidine – Biotin solution (0.5 mM):

D-Biotin (F.W. 244.3)	122.2	mg
L-Histidine x·HCl x H_2O (F.W. 209.63)	104.8	mg
Distilled water	q.s. ad 1000	mL

Sterilization was performed by filtration using a 0.22 µm membrane filter.

Complete Top Agar for *Salmonella typhimurium* strains:

Histidine – Biotin solution (0.5 mM)	100	mL
Agar solution	900	mL

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5.4.5. Top Agar for Escherichia coli Strain

Tryptophan solution (2 mg/mL):

L-Tryptophan (F.W. 204.23)	2000	mg
Distilled water	q.s. ad 1000	mL

Sterilization was performed by filtration using a 0.22 µm membrane filter.

Complete Top Agar for *Escherichia coli* strain:

Nutrient Broth by 5.4.2.	50	mL
Tryptophan solution (2 mg/mL)	2.5	mL
Agar solution by 5.4.4.	947.5	mL

5.5. METABOLIC ACTIVATION SYSTEM

Test bacteria were also exposed to the test item in the presence of an appropriate metabolic activation system, which is a cofactor-supplemented post-mitochondrial S9 fraction.

The post-mitochondrial fraction (S9 fraction) was prepared by the Microbiological Laboratory of CiToxLAB Hungary Ltd. According to Ames et al. [1] and Maron and Ames [2]. The documentation of the preparation of this post-mitochondrial fraction is stored in the reagent notebook in the Microbiological Laboratory which is archived yearly.

The supplier, batch number and expiry date of the used chemicals described in sections 5.5.3. and 5.5.4. are summarized in Table 6 (Section 5.9. Chemicals Used in the Experiments). The composition of solution refers to 1000 mL.

5.5.1. Induction of Liver Enzymes

Male Wistar rats (257-305 g, animals were 7-8 weeks old at the initiation) were treated with □henobarbital (PB) and β-naphthoflavone (BNF) at 80 mg/kg/day by oral gavage for three consecutive days. Rats were given drinking water and food ad libitum until 12 h before sacrifice when food was removed. Sacrifice was by ascending concentration of CO_2, confirmed by cutting through major thoracic blood vessels. Initiation of the induction of liver enzymes used for preparation S9 used in this study was 07 March 2011.

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5.5.2. Preparation of Rat Liver Homogenate S9 Fraction

On Day 4, the rats were euthanized and the livers were removed aseptically using sterile surgical tools. After excision, livers were weighed and washed several times in 0.15 M KCl. The washed livers were transferred to a beaker containing 3 mL of 0.15 M KCl per g of wet liver, and homogenized. Homogenates were centrifuged for 10 min at 9000 g and the supernatant was decanted and retained. The freshly prepared S9 fraction was aliquoted into 1-3 mL portions, frozen quickly and stored at -80 ± 10°C.

The sterility of the preparation was confirmed.

The protein concentration of the preparation was determined by a chemical analyzer at 540 nm in the Clinical Chemistry Laboratory of CiToxLAB Hungary Ltd. The protein concentration of the S9 fraction used was determined to be 35.3 g/L. The date of preparation of S9 fraction for this study was 10 March 2011 (CiToxLAB code: E11041).

The biological activity in the *Salmonella* assay of S9 was characterized using the two mutagens 2-Aminoanthracene and Benzo(a)pyrene, that requires metabolic activation by microsomal enzymes. The batch of S9 used in this study functioned appropriately.

5.5.3. The S9 Mix

Salt solution for S9 Mix:

NADP Na	7.66 g
D-glucose-6 phosphate Na	3.53 g
$MgCl_2 \times 6\ H_2O$	4.07 g
KCl	6.15 g
Distilled water	q.s. ad 1000 mL

Sterilization was performed by filtration through a 0.22 µm membrane filter.

The complete S9 mix was freshly prepared containing components as follows:

Ice cold 0.2 M sodium phosphate-buffer, pH 7.4	500 mL
Rat liver homogenate (S9)	100 mL
Salt solution for S9 Mix (see above)	400 mL

Prior to addition to the culture medium the S9 mix was kept in an ice bath.

5.5.4. 0.2 M Sodium Phosphate Buffer, pH 7.4

Solution A:

$Na_2HPO_4 \times 12H_2O$	71.63 g
Distilled water	q.s. ad 1000 mL

Sterilization was performed at 121°C in an autoclave.

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Solution B:

NaH_2PO_4	24.0	g
Distilled water	q.s. ad 1000	mL

Sterilization was performed at 121°C in an autoclave.

Sodium phosphate buffer pH 7.4:

Solution A	880	mL
Solution B	120	mL

5.6. DESCRIPTION OF THE TEST PROCEDURE

The study included a Preliminary Solubility Test, a Preliminary Range Finding Test (Informatory Toxicity Test), an Initial Mutation Test and a Confirmatory Mutation Test. In the Range Finding Test as well as in the Initial Mutation Test, the plate incorporation method was used. In the Confirmatory Mutation Test, the pre-incubation method was used.

5.6.1. Concentrations

Concentrations were selected on the basis of the Preliminary Solubility Test and Preliminary Range Finding Test (Informatory Toxicity Test). In the Initial Mutation Test and Confirmatory Mutation Test the same concentrations were used.

5.6.2. Preliminary Solubility Test

According to the available information provided by the Sponsor, the test item is non-soluble in water, but soluble in Acetone. Therefore, the solubility of the test item was examined in Acetone and was found to be soluble in this solvent at 100 mg/mL concentration. As Acetone is compatible with the test system, it was chosen for solvent of the study. The obtained formulations (50 µL) with the solution of top agar (5.4.4.) and phosphate buffer (5.5.4.) were examined in a test tube without test bacterium suspension. The results of the Solubility Test are summarized in Table 4.

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Table 4: The Solubility of the Test Item in Acetone

Concentration of test item in Acetone (mg/mL)	Solubility in Acetone	Solubility in the top solution (test item solution 50 µL + phosphate buffer 500 µL + top agar 2 mL)	Test item concentration in the test tube µg/tube
100	clear solution	strong opalescence	5000
50	clear solution	strong opalescence	2500
20	clear solution	opalescence	1000
6.32	clear solution	slight opalescence	316
2	clear solution	clear solution	100

5.6.2.1.Concentration Range Finding Test (Informatory Toxicity Test)

Based on the solubility test, 100 mg/mL stock formulation was prepared in Acetone, which was diluted in 6 steps by factors of 2, 2.5 and approximately √10. The revertant colony numbers and the inhibition of the background lawn of auxotrophic cells of two of the tester strains (*Salmonella typhimurium* TA98 and TA100) were determined at the concentrations of 5000, 2500, 1000, 316, 100, 31.6 and 10 µg/plate of the test item.

5.6.2.2.Test Item Concentrations in the Mutagenicity Tests (Initial Mutation Test and Confirmatory Mutation Test)

Based on the results of the preliminary tests, 100 mg/mL stock formulation was prepared from the test item with Acetone, which was diluted by serial dilutions in six steps to obtain seven dosing solutions for lower doses. The maximum test concentration was 5000 µg test item/plate.

Examined concentrations in the Initial Mutation Test and Confirmatory Mutation Test were: 5000; 1581; 500; 158.1; 50; 15.81 and 5 µg/plate.

5.6.3. Control Groups Used in the Tests

Strain-specific positive and negative (solvent) controls, both with and without metabolic activation were included in each test. In addition, untreated control was used demonstrating that the chosen vehicle induced no deleterious or mutagenic effects. The control groups are summarized in Table 5.

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Table 5: Summary of Controls

Type of control	Activation with S9 Mix	Solvent	Top Agar	Strain-Specific Positive Chemical Solutions (5.2.1.)	Phosphate Buffer
Untreated	–	–	+	–	+
Untreated	+	–	+	–	–
Solvent	–	+	+	–	+
Solvent	+	+	+	–	–
Positive Reference Control	–	–	+	+	+
Positive Reference Control	+	–	+	+	–

Note: if the solvent of the positive control substance differed from the solvent of the test item, both solvents were run in the assay.

5.6.4. Experimental Method

The experimental methods were conducted according to the methods described Ames et al. [1] and Maron and Ames [2], Kier et al. [3], Venitt and Parry [4], OECD Guideline No. 471, 1997 [5], Commission Regulation (EC) No. 440/2008, 2008 [6] and EPA Guidelines, OPPTS 870.5100, 1998, 1996 [7][8].

5.6.5. Procedure for Exposure in the Initial Mutation Test

A standard plate incorporation procedure was performed, as an Initial Mutation Test. Bacteria (cultured in Nutrient Broth No.2. as described in Section 5.3.6.) were exposed to the test item both in the presence and absence of an appropriate metabolic activation system.

Molten top agar was prepared and kept at 45°C. 2 mL of top agar was aliquoted into individual test tubes (3 tubes per control or concentration level). The equivalent number of minimal glucose agar plates was properly labelled. The test item and other components were prepared freshly and added to the overlay (45°C).

The content of the tubes:

top agar	2000 µL
solvent or test item solution (or reference controls)	50 µL
overnight culture of test strain	100 µL
phosphate buffer (pH 7.4) or S9 mix	500 µL

This solution was mixed and poured on the surface of minimal agar plates. For activation studies, instead of phosphate buffer, 0.5 mL of the S9 mix was added to each overlay tube. The entire test consisted of non-activated and activated test conditions, with the addition of untreated, negative and positive controls. After preparation, the plates were incubated at 37°C for 48 hours.

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5.6.6. Procedure for Exposure in the Confirmatory Mutation Test

A pre-incubation procedure [1][2][5][6][7] was performed as a Confirmatory Mutation Test since in the Initial Mutation Test no positive effect was observed (see details in Section 6.).

Before the overlaying, the test item solution (or solvent or reference control), the bacterial culture (Section 5.3.6.) and the S9 mix or phosphate buffer was added into appropriate tubes (3 tubes per control or concentration level) to provide direct contact between bacteria and the test item (in its solvent). The tubes were gently mixed and incubated for 20 min at 37°C in a shaking incubator.

After the incubation period, 2 mL of molten top agar was added to the tubes; the content was mixed up and poured onto minimal glucose agar plates as described for the standard plate incorporation method (Section 5.6.4.). The entire test consisted of non-activated and activated test conditions, with the addition of untreated, negative and positive controls. After preparation, the plates were incubated at 37°C for 48 hours.

5.7. EVALUATION OF EXPERIMENTAL DATA

The colony numbers on the untreated / negative / positive control and test item treated plates were determined by manual counting. The mean number of revertants per plate, the standard deviation and the mutation factor* values were calculated for each concentration level of the test item and for the controls using Microsoft Excel™ software.

* Mutation factor (MF): mean number of revertants on the test item plate / mean number of revertants on the vehicle control plate.

Criteria for a Positive Response:

A test item was considered mutagenic if:

- a dose–related increase in the number of revertants occurred and/or;
- a reproducible biologically relevant positive response for at least one of the dose groups occurred in at least one strain with or without metabolic activation.

An increase was considered biologically relevant if:

- in all strains: the number of reversion more than twice higher than the reversion rate of the solvent control

According to the guidelines [5][6][7], statistical method may be used as an aid in evaluating the test results. However, statistical significance should not be the only determining factor for a positive response.

Criteria for a Negative Response:

A test article was considered non-mutagenic if it produced neither a dose-related increase in the number of revertants nor a reproducible biologically relevant positive response at any of the dose groups, with or without metabolic activation.

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5.8. ARCHIVES

The study documents and samples:

- study plan and study plan amendment,
- all raw data,
- retained sample of the test item,
- correspondence,
- study report and any amendments

will be stored in the archives of CiToxLAB Hungary Ltd. (H-8200 Veszprém, Szabadságpuszta, Hungary) according to the Hungarian GLP regulation [9] and to test facility SOPs.

After the retention time has elapsed, all the archived materials listed above would be offered to the Sponsor or retained for a further period if agreed by a contract. No raw data or material relating to the study will be discarded without the Sponsor's prior written consent.

5.9. CHEMICALS USED IN THE EXPERIMENTS

The chemicals used in the independently performed experiments of the study are summarized in Table 6.

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Table 6: Chemicals Used in the Experiments

Chemical	Supplier	Batch Number	Expiry date
Agar Bacteriological	OXOID	1164200-02	21 March 2015
D-Biotin	SIGMA-ALDRICH	036K1504V	October 2014
D-Biotin	SIGMA-ALDRICH	021M1154V	February 2015
Distilled water	HungaroPharma	805 0411	05 October 2011
Distilled water	HungaroPharma	808 0611	06 December 2011
Distilled water	HungaroPharma	810 0811	01 February 2012
D-Glucose 6-phosphate Na	SIGMA-ALDRICH	079K3788	August 2012
D-Glucose 6-phosphate Na	SIGMA-ALDRICH	031M7004V	May 2014
L-Histidine HCl x H_2O	MERCK	K37522450	30 June 2012
KCl	MERCK	K41042236	31 May 2015
$MgCl_2$ x 6 H_2O	SIGMA-ALDRICH	100M0075V	October 2015
NaCl	Lachner	PP/2010/10084	31 May 2013
NADP Na x H_2O	SIGMA-ALDRICH	020M7009V	March 2013
NaH_2PO_4 x H_2O	CARLO ERBA	0N244191A	December 2016
Na_2HPO_4	SIGMA-ALDRICH	SZBB0590V	13 February 2014
Nutrient Agar	MERCK	VM100650	06 October 2014
Nutrient Broth No.2	OXOID	987078	31 January 2016
Nutrient Broth No.2	OXOID	1025421	30 April 2016
L-Tryptophan	SIGMA-ALDRICH	1400132	July 2012

NADP: β-Nicotinamide adenine dinucleotide phosphate

Note: For Nutrient Broth No.2, lot #987078 was used in the preliminary experiment and lot #1025421 was used in the main tests.

5.10. DEVIATIONS TO THE STUDY PLAN

There was no deviation to the Study Plan.

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6. RESULTS AND DISCUSSION

6.1. PRELIMINARY RANGE FINDING TEST (INFORMATORY TOXICITY TEST)

In the Preliminary Range Finding Test, the plate incorporation method was used. The preliminary test was performed using *Salmonella typhimurium* TA98 and *Salmonella typhimurium* TA100 tester strains in the presence and absence of metabolic activation system (±S9 Mix) with appropriate untreated, negative (solvent) and positive controls. In the test each samples (including the controls) were tested in triplicate.

In the Range Finding Test the concentrations examined were: 5000, 2500, 1000, 316, 100, 31.6 and 10 µg/plate.

The observed numbers of revertant colonies were mostly in the normal range. Minor differences (slightly higher or slightly lower colony counts compared to the solvent control) were observed in some sporadic cases; however, they were without biological significance and in the historical control range in all cases. Therefore, they were considered to reflect the biological variability of the test system.

The experimental results (revertant colony numbers per plate, mutation factors and standard deviations) are detailed in Table 7 (Appendix 2) and in Appendix 3.

6.2. INITIAL AND CONFIRMATORY MUTATION TESTS

In the Initial Mutation Test, the plate incorporation method; in the Confirmatory Mutation Test, the pre-incubation method was used. The Initial Mutation Test and Confirmatory Mutation Test were carried out using four *Salmonella typhimurium* strains (TA98, TA100, TA1535, TA1537) and *Escherichia coli* WP2 *uvrA* strain. The Initial Mutation Test and Confirmatory Mutation Test were performed in the presence and absence of metabolic activation system (±S9 mix). Each test was performed with appropriate untreated, negative (solvent) and positive controls. In the main tests each sample (including the controls) were tested in triplicate.

The examined test item concentrations in the Initial Mutation Test and Confirmatory Mutation Test were: 5000; 1581; 500; 158.1; 50; 15.81 and 5 µg/plate.

In the Initial Mutation Test and Confirmatory Mutation Tests none of the observed revertant colony numbers were above the respective biological threshold value. There were no reproducible dose-related trends and no indication of any treatment effect.

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Using the plate incorporation method (Initial Mutation Test), the highest revertant rate was observed in *Salmonella typhimurium* TA1537 bacterial strain without metabolic activation at the concentrations of 500 µg/plate. The mutation factor value was 1.76. Higher revertant counts were detected at some other tested concentrations in this experiment. However, no dose-dependence was observed, the observed mutation factor values were below the biologically relevant threshold limit and the numbers of revertant colonies were within the historical control range in each case.

Using the pre-incubation method (Confirmatory Mutation Test), the highest revertant rate was observed in *Salmonella typhimurium* TA1537 bacterial strain with metabolic activation at 15.81 µg/plate concentration. The observed mutation factor value was 1.94. Higher revertant counts were detected at some other tested concentrations in this experiment. However, no dose-dependent relationship was observed, and the observed mutation factor values did not reach the biologically relevant threshold value. The numbers of revertant colonies were within the historical control range in all cases. Furthermore, higher numbers of revertant colonies compared to the Acetone solvent control plates were observed for untreated control (MF: 1.38) also in this experiment.

Higher numbers of revertant colonies compared to the solvent control were detected in the Initial Mutation Test in *Salmonella typhimurium* TA1537 bacterial strain with metabolic activation; in the Confirmatory Mutation Test in *Salmonella typhimurium* TA1535 strain without metabolic activation; furthermore, in other sporadic cases in the two main tests. However, no dose-dependence was observed and they were below the biologically relevant threshold value. The numbers of revertant colonies were within the historical control range in all cases, so they were considered as reflecting the biological variability of the test.

Sporadically, lower revertant counts compared to the solvent control were observed in the Initial Mutation Test and Confirmatory Mutation Test in some cases. However, the mean numbers of revertant colonies were in the historical control range in all cases, thus they were considered as biological variability of the test system.

Oily patches were observed in the Confirmatory Mutation Test at 5000 µg/plate concentration with and without metabolic activation in all strains.

The experimental results (revertant colony numbers per plate, mutation factors, and standard deviations) are summarized in Tables 8-9 (Appendix 2) and Appendices 4 and 5.

6.3. VALIDITY OF THE TESTS

Untreated, negative (solvent) and positive controls were run concurrently. The mean values of revertant colony numbers of untreated and solvent control plates were within the historical control data range. The reference mutagens showed a distinct increase of induced revertant colonies. The viability of the bacterial cells was checked by a plating experiment in each test. The tests were considered to be valid.

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7. CONCLUSION

The test item S Face IS-1005P was tested for potential mutagenic activity using the Bacterial Reverse Mutation Assay.

The experiments were carried out using histidine-requiring auxotroph strains of *Salmonella typhimurium* (*Salmonella typhimurium* TA98, TA100, TA1535 and TA1537), and the tryptophan-requiring auxotroph strain of *Escherichia coli* (*Escherichia coli* WP2 *uvrA)* in the presence and absence of a metabolic activation system, which was a cofactor-supplemented post-mitochondrial S9 fraction prepared from the livers of phenobarbital/ β-naphthoflavone-induced rats.

The study included a Preliminary Solubility Test, a Preliminary Range Finding Test (Informatory Toxicity Test), an Initial Mutation Test and a Confirmatory Mutation Test. In the Range Finding Test as well as in the Initial Mutation Test, the plate incorporation method was used. In the Confirmatory Mutation Test, the pre-incubation method was used.

The reported data of this mutagenicity assay show (see Appendix 2 to 5) that under the experimental conditions applied the test item **did not induce gene mutations by base pair changes or frameshifts in the genome of the strains used.**

In conclusion, the test item S Face IS-1005P series had no mutagenic activity on the growth of the applied bacterium tester strains under the test conditions used in this study.

8. DISTRIBUTION OF THE FINAL REPORT

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1x copy, unbound
1x PDF file

Archive: 1x original, bound

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9. REFERENCES

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9. Hungarian Good Laboratory Practice Regulations: 9/2001 (III. 30.) EüM-FVM joint decree of the Minister of Health and the Minister of the Agriculture and Regional Development (in Hungarian) which corresponds to the OECD GLP, ENV/MC/CHEM(98)17

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APPENDICES

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APPENDIX 1

Copy of the Certificate of Analysis

SAKAMOTO YAKUHIN KOGYO CO.,LTD.

HEAD OFFICE : 1-2-6 AWAJIMACHI,CHUO-KU,OSAKA CITY 541-0047,JAPAN
TEL (81)6-6231-1851 FAX (81)6-6222-0631
TOKYO BRANCH : 1-6-2 MARUNOUCHI,CHIYODA-KU,TOKYO 100-0005,JAPAN
TEL (81)3-3211-3613 FAX (81)3-3215-1789

date : AUG-1-2011

Product : S Face IS-1005P
Lot. No. : 10301

Certificate of analysis / Quality

	Result	Specification
Description	pass	A pale yellow liquid with a faint characteristic odor
Identification (1)	Pass	JSQI 2006 (Polyglyceryl-10 Pentaisostearate)
(2)	Pass	JSQI 2006 (Polyglyceryl-10 Pentaisostearate)
Acid value (mgKOH/g)	0.07	1.5 max
Saponification value (mgKOH/g)	143.1	137～151
Heavy metal (ppm)	Pass	less than 20
Arsenic (ppm)	Pass	less than 2
Residue on ignition (%)	Pass	less than 1.0

Expiration date : AUG-1-2012

Satoshi Ohshima
Quality Control Manager

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APPENDIX 2

Summary Tables of the Results

Table 7: Summary Table of the Range Finding Test

Concentrations (µg/plate)	Mean values of revertants / Mutation factor (MF)	*Salmonella typhimurium* tester strains			
		TA98		TA100	
		-S9	+S9	-S9	+S9
Untreated control	Mean	29.0	20.0	77.7	98.0
	MF	1.14	0.74	1.00	0.96
DMSO control	Mean	21.3	35.7	-	77.3
	MF	0.84	1.32	-	0.76
Distilled water control	Mean	-	-	49.7	-
	MF	-	-	0.64	-
Acetone control	Mean	25.3	27.0	78.0	102.3
	MF	1.00	1.00	1.00	1.00
5000	Mean	27.3	33.7	82.3	93.3
	MF	1.08	1.25	1.06	0.91
2500	Mean	22.0	23.3	77.3	102.0
	MF	0.87	0.86	0.99	1.00
1000	Mean	23.3	24.0	79.7	112.3
	MF	0.92	0.89	1.02	1.10
316	Mean	19.0	28.3	80.3	113.0
	MF	0.75	1.05	1.03	1.10
100	Mean	17.0	27.7	85.0	106.7
	MF	0.67	1.02	1.09	1.04
31.6	Mean	25.3	31.3	71.7	118.7
	MF	1.00	1.16	0.92	1.16
10	Mean	20.7	28.3	82.0	123.3
	MF	0.82	1.05	1.05	1.21
NPD (4µg)	Mean	323.7	-	-	-
	MF	15.17	-	-	-
2AA (2µg)	Mean	-	2696.0	-	2037.3
	MF	-	75.59	-	26.34
SAZ (2µg)	Mean	-	-	1092.0	-
	MF	-	-	21.99	-

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APPENDIX 2 (continued)

Summary Tables of the Results

Table 8: Summary Table of the Initial Mutation Test

Concentrations (µg/plate)	Mean values of revertants / Mutation factor (MF)	*Salmonella typhimurium* tester strains								*Escherichia coli*	
		TA98		TA100		TA1535		TA1537		WP2 *uvrA*	
		-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Untreated control	Mean	26.3	37.7	92.0	96.3	8.7	13.7	5.0	6.7	31.7	43.0
	MF	0.93	0.99	1.12	0.94	0.79	1.28	0.88	1.05	1.09	1.01
DMSO control	Mean	27.7	37.0	-	91.7	-	14.7	6.7	9.0	-	31.7
	MF	0.98	0.97	-	0.89	-	1.38	1.18	1.42	-	0.74
Distilled water control	Mean	-	-	80.0	-	11.3	-	-	-	35.3	-
	MF	-	-	0.98	-	1.03	-	-	-	1.22	-
Acetone control	Mean	28.3	38.0	82.0	103.0	11.0	10.7	5.7	6.3	29.0	42.7
	MF	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
5000	Mean	29.0	32.7	99.3	98.7	11.7	15.0	8.3	9.3	24.3	45.7
	MF	1.02	0.86	1.21	0.96	1.06	1.41	1.47	1.47	0.84	1.07
1581	Mean	26.7	31.7	91.0	82.0	9.7	11.7	7.0	9.7	33.3	41.7
	MF	0.94	0.83	1.11	0.80	0.88	1.09	1.24	1.53	1.15	0.98
500	Mean	31.7	37.3	92.3	90.3	12.7	13.7	10.0	7.7	29.0	35.7
	MF	1.12	0.98	1.13	0.88	1.15	1.28	1.76	1.21	1.00	0.84
158.1	Mean	29.0	38.0	92.0	101.0	11.0	11.3	8.3	8.3	29.3	38.7
	MF	1.02	1.00	1.12	0.98	1.00	1.06	1.47	1.32	1.01	0.91
50	Mean	28.7	32.3	90.0	112.3	9.3	14.0	6.3	8.0	31.7	40.0
	MF	1.01	0.85	1.10	1.09	0.85	1.31	1.12	1.26	1.09	0.94
15.81	Mean	30.0	33.0	91.0	106.0	9.3	12.3	6.7	8.7	31.7	33.0
	MF	1.06	0.87	1.11	1.03	0.85	1.16	1.18	1.37	1.09	0.77
5	Mean	28.7	32.0	80.3	107.0	11.7	11.3	7.7	9.7	27.7	37.3
	MF	1.01	0.84	0.98	1.04	1.06	1.06	1.35	1.53	0.95	0.88
NPD (4µg)	Mean	303.7	-	-	-	-	-	-	-	-	-
	MF	10.98	-	-	-	-	-	-	-	-	-
2AA (2µg)	Mean	-	2773.3	-	2382.7	-	198.3	-	200.7	-	-
	MF	-	74.95	-	25.99	-	13.52	-	22.30	-	-
2AA (50µg)	Mean	-	-	-	-	-	-	-	-	-	296.7
	MF	-	-	-	-	-	-	-	-	-	9.37
SAZ (2µg)	Mean	-	-	1966.7	-	1565.3	-	-	-	-	-
	MF	-	-	24.58	-	138.12	-	-	-	-	-
9AA (50µg)	Mean	-	-	-	-	-	-	426.7	-	-	-
	MF	-	-	-	-	-	-	64.00	-	-	-
MMS (2µL)	Mean	-	-	-	-	-	-	-	-	890.7	-
	MF	-	-	-	-	-	-	-	-	25.21	-

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APPENDIX 2 (continued)

Summary Tables of the Results

Table 9: Summary Table of the Confirmatory Mutation Test

Concentrations (µg/plate)	Mean values of revertants / Mutation factor (MF)	*Salmonella typhimurium* tester strains								*Escherichia coli*	
		TA98		TA100		TA1535		TA1537		WP2 *uvrA*	
		-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Untreated control	Mean	21.0	29.3	80.7	111.0	6.7	8.0	6.3	7.3	28.3	35.7
	MF	0.80	0.84	0.92	1.02	0.95	1.04	1.27	1.38	1.09	0.97
DMSO control	Mean	22.7	36.3	-	117.7	-	9.3	4.3	4.0	-	32.7
	MF	0.86	1.04	-	1.08	-	1.22	0.87	0.75	-	0.89
Distilled water control	Mean	-	-	91.3	-	7.7	-	-	-	20.7	-
	MF	-	-	1.04	-	1.10	-	-	-	0.79	-
Acetone control	Mean	26.3	35.0	87.7	108.7	7.0	7.7	5.0	5.3	26.0	36.7
	MF	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
5000	Mean	24.3	34.7	77.7	122.0	7.0	12.3	5.3	8.0	20.0	36.3
	MF	0.92	0.99	0.89	1.12	1.00	1.61	1.07	1.50	0.77	0.99
1581	Mean	18.3	33.0	82.0	88.0	6.7	11.3	4.0	9.3	22.7	33.0
	MF	0.70	0.94	0.94	0.81	0.95	1.48	0.80	1.75	0.87	0.90
500	Mean	20.3	36.7	70.0	98.7	6.7	11.0	6.7	5.3	22.3	38.7
	MF	0.77	1.05	0.80	0.91	0.95	1.43	1.33	1.00	0.86	1.05
158.1	Mean	22.3	34.7	66.7	100.7	6.3	11.0	6.7	8.3	22.0	34.0
	MF	0.85	0.99	0.76	0.93	0.90	1.43	1.33	1.56	0.85	0.93
50	Mean	23.7	33.3	80.7	108.3	7.0	8.7	5.3	4.7	22.3	37.7
	MF	0.90	0.95	0.92	1.00	1.00	1.13	1.07	0.88	0.86	1.03
15.81	Mean	19.3	32.3	77.3	98.3	8.0	8.3	4.0	10.3	25.3	31.3
	MF	0.73	0.92	0.88	0.90	1.14	1.09	0.80	1.94	0.97	0.85
5	Mean	28.0	27.7	77.3	92.7	7.0	11.7	7.0	8.7	21.3	38.3
	MF	1.06	0.79	0.88	0.85	1.00	1.52	1.40	1.63	0.82	1.05
NPD (4µg)	Mean	386.7	-	-	-	-	-	-	-	-	-
	MF	17.06	-	-	-	-	-	-	-	-	-
2AA (2µg)	Mean	-	2446.7	-	2348.0	-	236.0	-	213.7	-	-
	MF	-	67.34	-	19.95	-	25.29	-	53.42	-	-
2AA (50µg)	Mean	-	-	-	-	-	-	-	-	-	213.0
	MF	-	-	-	-	-	-	-	-	-	6.52
SAZ (2µg)	Mean	-	-	1426.7	-	1458.7	-	-	-	-	-
	MF	-	-	15.62	-	190.26	-	-	-	-	-
9AA (50µg)	Mean	-	-	-	-	-	-	414.7	-	-	-
	MF	-	-	-	-	-	-	95.69	-	-	-
MMS (2µL)	Mean	-	-	-	-	-	-	-	-	1134.7	-
	MF	-	-	-	-	-	-	-	-	54.90	-

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APPENDIX 3

Preliminary Range Finding Test (Informatory Toxicity Test)

Test Item: S Face lS-1005P
Date: 17-19 August 2011
Strain: *Salmonella typhimurium* TA98
Cell count (overnight culture): 2.44 x 10^9 CFU/mL

Concentration: (µg/plate)		Revertant colony number -S9	Revertant colony number +S9
5000		27	19
		33	42
		22	40
	Mean	27.3	33.7
	SD	5.51	12.74
	MF	1.08	1.25
2500		16	23
		23	29
		27	18
	Mean	22.0	23.3
	SD	5.57	5.51
	MF	0.87	0.86
1000		36	18
		17	23
		17	31
	Mean	23.3	24.0
	SD	10.97	6.56
	MF	0.92	0.89
316		20	28
		19	32
		18	25
	Mean	19.0	28.3
	SD	1.00	3.51
	MF	0.75	1.05
100		12	26
		19	26
		20	31
	Mean	17.0	27.7
	SD	4.36	2.89
	MF	0.67	1.02
31.6		20	41
		27	22
		29	31
	Mean	25.3	31.3
	SD	4.73	9.50
	MF	1.00	1.16
10		26	28
		17	30
		19	27
	Mean	20.7	28.3
	SD	4.73	1.53
	MF	0.82	1.05

Concentration: (µg/plate)		Revertant colony number -S9		Revertant colony number +S9
Untreated control		33		18
		25		20
		29		22
	Mean	29.0		20.0
	SD	4.00		2.00
	MF	1.14		0.74
DMSO control*		21		38
		27		30
		16		39
	Mean	21.3		35.7
	SD	5.51		4.93
	MF	0.84		1.32
Acetone control		19		28
		33		25
		24		28
	Mean	25.3		27.0
	SD	7.09		1.73
	MF	1.00		1.00
Positive control NPD (4 µg)		332	2AA (2 µg)	2696
		341		2640
		298		2752
	Mean	323.7		2696.0
	SD	22.68		56.00
	MF	15.17		75.59

MF: Mutation factor
SD: Standard deviation
+S9: with S9 mix
-S9: without S9 mix

$$\text{Mutation factor} = \frac{\text{mean revertants (test item)}}{\text{mean revertants (solvent control)}}$$

*DMSO control was used because of NPD and 2AA

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APPENDIX 3 (continued)

Preliminary Range Finding Test (Informatory Toxicity Test)

Test Item: S Face IS-1005P
Date: 17-19 August 2011
Strain: *Salmonella typhimurium* TA100
Cell count (overnight culture): 2.01 x 10^9 CFU/mL

Concentration: (µg/plate)		Revertant colony number -S9	Revertant colony number +S9
5000		70	104
		87	77
		90	99
	Mean	82.3	93.3
	SD	10.79	14.36
	MF	1.06	0.91
2500		70	105
		83	104
		79	97
	Mean	77.3	102.0
	SD	6.66	4.36
	MF	0.99	1.00
1000		80	116
		64	99
		95	122
	Mean	79.7	112.3
	SD	15.50	11.93
	MF	1.02	1.10
316		82	114
		70	116
		89	109
	Mean	80.3	113.0
	SD	9.61	3.61
	MF	1.03	1.10
100		82	105
		95	113
		78	102
	Mean	85.0	106.7
	SD	8.89	5.69
	MF	1.09	1.04
31.6		76	126
		66	120
		73	110
	Mean	71.7	118.7
	SD	5.13	8.08
	MF	0.92	1.16
10		90	126
		93	121
		63	123
	Mean	82.0	123.3
	SD	16.52	2.52
	MF	1.05	1.21

Concentration: (µg/plate)		Revertant colony number -S9		Revertant colony number +S9
Untreated control		77		94
		72		102
		84		98
	Mean	77.7		98.0
	SD	6.03		4.00
	MF	1.00		0.96
DMSO control*		70		83
		40		73
		59		76
	Mean	56.3		77.3
	SD	15.18		5.13
	MF	0.72		0.76
Acetone control		72		108
		83		100
		79		99
	Mean	78.0		102.3
	SD	5.57		4.93
	MF	1.00		1.00
Distilled water** control		58		104
		46		93
		45		97
	Mean	49.7		98.0
	SD	7.23		5.57
	MF	0.64		0.96
Positive control SAZ (2 µg)		1080	2AA (2 µg)	2100
		1100		1988
		1096		2024
	Mean	1092.0		2037.3
	SD	10.58		57.18
	MF	21.99		26.34

MF: Mutation factor
SD: Standard deviation
+S9: with S9 mix
-S9: without S9 mix

$$\text{Mutation factor} = \frac{\text{mean revertants (test item)}}{\text{mean revertants (solvent control)}}$$

*DMSO control was used because of 2AA
**Distilled water control was used because of SAZ

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APPENDIX 4

Initial Mutation Test (Plate Incorporation Method)

Test Item: S Face IS-1005P
Date: 30 August - 01 September 2011
Strain: *Salmonella typhimurium* TA98
Cell count (overnight culture): 1.40 x10^9 CFU/mL

Concentration: (µg/plate)		Revertant colony number r -S9	+S9
5000		22	32
		34	33
		31	33
	Mean	29.0	32.7
	SD	6.24	0.58
	MF	1.02	0.86
1581		22	39
		24	34
		34	22
	Mean	26.7	31.7
	SD	6.43	8.74
	MF	0.94	0.83
500		30	40
		41	33
		24	39
	Mean	31.7	37.3
	SD	8.62	3.79
	MF	1.12	0.98
158.1		31	34
		22	36
		34	44
	Mean	29.0	38.0
	SD	6.24	5.29
	MF	1.02	1.00
50		35	22
		25	38
		26	37
	Mean	28.7	32.3
	SD	5.51	8.96
	MF	1.01	0.85
15.81		32	38
		35	24
		23	37
	Mean	30.0	33.0
	SD	6.24	7.81
	MF	1.06	0.87
5		32	44
		29	26
		25	26
	Mean	28.7	32.0
	SD	3.51	10.39
	MF	1.01	0.84

Concentration: (µg/plate)		Revertant colony number -S9		+S9
Untreated control		27		33
		26		39
		26		41
	Mean	26.3		37.7
	SD	0.58		4.16
	MF	0.93		0.99
DMSO control*		32		35
		28		39
		23		37
	Mean	27.7		37.0
	SD	4.51		2.00
	MF	0.98		0.97
Acetone control		26		45
		30		40
		29		29
	Mean	28.3		38.0
	SD	2.08		8.19
	MF	1.00		1.00
Positive control		308	2AA	2680
NPD		312	(2 µg)	2792
(4 µg)		291		2848
	Mean	303.7		2773.3
	SD	11.15		85.54
	MF	10.98		74.95

MF: Mutation factor
SD: Standard deviation
+S9: with S9 mix
-S9: without S9 mix

$$\text{Mutation factor} = \frac{\text{mean revertants (test item)}}{\text{mean revertants (solvent control)}}$$

*DMSO control was used because of NPD and 2AA

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APPENDIX 4 (continued)

Initial Mutation Test (Plate Incorporation Method)

Test Item: S Face IS-1005P
Date: 30 August - 01 September 2011
Strain: *Salmonella typhimurium* TA100
Cell count (overnight culture): 2.69×10^9 CFU/mL

Concentration: (µg/plate)		Revertant colony number -S9	Revertant colony number +S9
5000		101	105
		90	91
		107	100
	Mean	99.3	98.7
	SD	8.62	7.09
	MF	1.21	0.96
1581		92	75
		87	86
		94	85
	Mean	91.0	82.0
	SD	3.61	6.08
	MF	1.11	0.80
500		108	99
		89	92
		80	80
	Mean	92.3	90.3
	SD	14.29	9.61
	MF	1.13	0.88
158.1		97	97
		89	97
		90	109
	Mean	92.0	101.0
	SD	4.36	6.93
	MF	1.12	0.98
50		92	108
		96	119
		82	110
	Mean	90.0	112.3
	SD	7.21	5.86
	MF	1.10	1.09
15.81		98	91
		88	117
		87	110
	Mean	91.0	106.0
	SD	6.08	13.45
	MF	1.11	1.03
5		72	100
		74	118
		95	103
	Mean	80.3	107.0
	SD	12.74	9.64
	MF	0.98	1.04

Concentration: (µg/plate)		Revertant colony number -S9		Revertant colony number +S9
Untreated control		93		94
		82		87
		101		108
	Mean	92.0		96.3
	SD	9.54		10.69
	MF	1.12		0.94
DMSO control*		81		93
		98		79
		83		103
	Mean	87.3		91.7
	SD	9.29		12.06
	MF	1.07		0.89
Distilled water control**		75		92
		77		93
		88		97
	Mean	80.0		94.0
	SD	7.00		2.65
	MF	0.98		0.91
Acetone control		72		104
		73		110
		101		95
	Mean	82.0		103.0
	SD	16.46		7.55
	MF	1.00		1.00
Positive control SAZ (2 µg)		2100	2AA (2 µg)	2356
		1920		2552
		1880		2240
	Mean	1966.7		2382.7
	SD	117.19		157.70
	MF	24.58		25.99

MF: Mutation factor
SD: Standard deviation
+S9: with S9 mix
-S9: without S9 mix

$$\text{Mutation factor} = \frac{\text{mean revertants (test item)}}{\text{mean revertants (solvent control)}}$$

*DMSO control was used because of 2AA
**Distilled water control was used because of SAZ

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APPENDIX 4 (continued)

Initial Mutation Test (Plate Incorporation Method)

Test Item: S Face IS-1005P
Date: 30 August - 01 September 2011
Strain: *Salmonella typhimurium* TA1535
Cell count (overnight culture): 2.67×10^9 CFU/mL

Concentration: (µg/plate)		Revertant colony number -S9	Revertant colony number +S9
5000		15	17
		6	12
		14	16
	Mean	11.7	15.0
	SD	4.93	2.65
	MF	1.06	1.41
1581		9	11
		11	10
		9	14
	Mean	9.7	11.7
	SD	1.15	2.08
	MF	0.88	1.09
500		15	14
		13	16
		10	11
	Mean	12.7	13.7
	SD	2.52	2.52
	MF	1.15	1.28
158.1		8	12
		12	9
		13	13
	Mean	11.0	11.3
	SD	2.65	2.08
	MF	1.00	1.06
50		9	15
		9	16
		10	11
	Mean	9.3	14.0
	SD	0.58	2.65
	MF	0.85	1.31
15.81		7	16
		12	12
		9	9
	Mean	9.3	12.3
	SD	2.52	3.51
	MF	0.85	1.16
5		10	11
		14	11
		11	12
	Mean	11.7	11.3
	SD	2.08	0.58
	MF	1.06	1.06

Concentration: (µg/plate)		Revertant colony number -S9		Revertant colony number +S9
Untreated control		11		17
		8		13
		7		11
	Mean	8.7		13.7
	SD	2.08		3.06
	MF	0.79		1.28
DMSO control*		5		15
		13		14
		10		15
	Mean	9.3		14.7
	SD	4.04		0.58
	MF	0.85		1.38
Distilled water control**		13		17
		9		11
		12		14
	Mean	11.3		14.0
	SD	2.08		3.00
	MF	1.03		1.31
Acetone control		15		15
		7		7
		11		10
	Mean	11.0		10.7
	SD	4.00		4.04
	MF	1.00		1.00
Positive control		1612	2AA	189
SAZ		1488	(2 µg)	190
(2 µg)		1596		216
	Mean	1565.3		198.3
	SD	67.45		15.31
	MF	138.12		13.52

MF: Mutation factor
SD: Standard deviation
+S9: with S9 mix
-S9: without S9 mix

$$\text{Mutation factor} = \frac{\text{mean revertants (test item)}}{\text{mean revertants (solvent control)}}$$

*DMSO control was used because of 2AA
**Distilled water control was used because of SAZ

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APPENDIX 4 (continued)

Initial Mutation Test (Plate Incorporation Method)

Test Item: S Face IS-1005P
Date: 30 August - 01 September 2011
Strain: *Salmonella typhimurium* TA1537
Cell count (overnight culture): 2.63×10^9 CFU/mL

Concentration: (µg/plate)		Revertant colony number -S9	Revertant colony number +S9
5000		8	12
		8	9
		9	7
	Mean	8.3	9.3
	SD	0.58	2.52
	MF	1.47	1.47
1581		4	7
		9	10
		8	12
	Mean	7.0	9.7
	SD	2.65	2.52
	MF	1.24	1.53
500		11	10
		7	7
		12	6
	Mean	10.0	7.7
	SD	2.65	2.08
	MF	1.76	1.21
158.1		12	6
		9	10
		4	9
	Mean	8.3	8.3
	SD	4.04	2.08
	MF	1.47	1.32
50		7	5
		8	12
		4	7
	Mean	6.3	8.0
	SD	2.08	3.61
	MF	1.12	1.26
15.81		7	5
		4	11
		9	10
	Mean	6.7	8.7
	SD	2.52	3.21
	MF	1.18	1.37
5		5	10
		11	9
		7	10
	Mean	7.7	9.7
	SD	3.06	0.58
	MF	1,35	1,53

Concentration: (µg/plate)		Revertant colony number -S9		Revertant colony number +S9
Untreated control		6		7
		4		5
		5		8
	Mean	5.0		6.7
	SD	1.00		1.53
	MF	0.88		1.05
DMSO control*		9		7
		7		12
		4		8
	Mean	6.7		9.0
	SD	2.52		2.65
	MF	1.18		1.42
Acetone control		5		9
		8		4
		4		6
	Mean	5.7		6.3
	SD	2.08		2.52
	MF	1.00		1.00
Positive control 9AA (50 µg)		388	2AA (2 µg)	198
		452		193
		440		211
	Mean	426.7		200.7
	SD	34.02		9.29
	MF	64.00		22.30

MF: Mutation factor
SD: Standard deviation
+S9: with S9 mix
-S9: without S9 mix

$$\text{Mutation factor} = \frac{\text{mean revertants (test item)}}{\text{mean revertants (solvent control)}}$$

*DMSO control was used because of 9AA and 2AA

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APPENDIX 4 (continued)

Initial Mutation Test (Plate Incorporation Method)

Test Item: S Face IS-1005P
Date: 30 August - 01 September 2011
Strain: *Escherichia coli* WP2 *uvrA*
Cell count (overnight culture): 3.61×10^9 CFU/mL

Concentration: (µg/plate)		Revertant colony number -S9	Revertant colony number +S9
5000		23	48
		20	47
		30	42
	Mean	24.3	45.7
	SD	5.13	3.21
	MF	0.84	1.07
1581		28	48
		38	40
		34	37
	Mean	33.3	41.7
	SD	5.03	5.69
	MF	1.15	0.98
500		28	45
		27	33
		32	29
	Mean	29.0	35.7
	SD	2.65	8.33
	MF	1.00	0.84
158.1		27	26
		29	45
		32	45
	Mean	29.3	38.7
	SD	2.52	10.97
	MF	1.01	0.91
50		36	45
		23	34
		36	41
	Mean	31.7	40.0
	SD	7.51	5.57
	MF	1.09	0.94
15.81		34	33
		30	32
		31	34
	Mean	31.7	33.0
	SD	2.08	1.00
	MF	1.09	0.77
5		31	43
		28	31
		24	38
	Mean	27.7	37.3
	SD	3.51	6.03
	MF	0.95	0.88

Concentration: (µg/plate)		Revertant colony number -S9		Revertant colony number +S9
Untreated control		24		42
		38		40
		33		47
	Mean	31.7		43.0
	SD	7.09		3.61
	MF	1.09		1.01
DMSO control*		31		33
		33		32
		27		30
	Mean	30.3		31.7
	SD	3.06		1.53
	MF	1.05		0.74
Distilled water control**		38		45
		31		38
		37		37
	Mean	35.3		40.0
	SD	3.79		4.36
	MF	1.22		0.94
Acetone control		22		45
		30		36
		35		47
	Mean	29.0		42.7
	SD	6.56		5.86
	MF	1.00		1.00
Positive control		1012	2AA	288
MMS		780	(50 µg)	312
(2 µL)		880		290
	Mean	890.7		296.7
	SD	116.37		13.32
	MF	25.21		9.37

MF: Mutation factor
SD: Standard deviation
+S9: with S9 mix
-S9: without S9 mix

$$\text{Mutation factor} = \frac{\text{mean revertants (test item)}}{\text{mean revertants (solvent control)}}$$

*DMSO control was used because of 2AA
**Distilled water control was used because of MMS

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APPENDIX 5

Confirmatory Mutation Test (Pre-Incubation Method)

Test Item: S Face IS-1005P
Date: 06-08 September 2011
Strain: *Salmonella typhimurium* TA98
Cell count (ovemight culture): 1.24×10^9 CFU/mL

Concentration: (µg/plate)		Revertant colony number -S9	Revertant colony number +S9
5000		24	33
		24	33
		25	38
	Mean	24.3	34.7
	SD	0.58	2.89
	MF	0.92	0.99
1581		17	35
		20	36
		18	28
	Mean	18.3	33.0
	SD	1.53	4.36
	MF	0.70	0.94
500		17	38
		20	35
		24	37
	Mean	20.3	36.7
	SD	3.51	1.53
	MF	0.77	1.05
158.1		17	30
		26	34
		24	40
	Mean	22.3	34.7
	SD	4.73	5.03
	MF	0.85	0.99
50		26	26
		24	38
		21	36
	Mean	23.7	33.3
	SD	2.52	6.43
	MF	0.90	0.95
15.81		19	24
		18	39
		21	34
	Mean	19.3	32.3
	SD	1.53	7.64
	MF	0.73	0.92
5		29	24
		28	31
		27	28
	Mean	28.0	27.7
	SD	1.00	3.51
	MF	1.06	0.79

Concentration: (µg/plate)		Revertant colony number -S9		Revertant colony number +S9
Untreated control		15		32
		25		31
		23		25
	Mean	21.0		29.3
	SD	5.29		3.79
	MF	0.80		0.84
DMSO control*		15		38
		32		34
		21		37
	Mean	22.7		36.3
	SD	8.62		2.08
	MF	0.86		1.04
Acetone control		25		36
		22		33
		32		36
	Mean	26.3		35.0
	SD	5.13		1.73
	MF	1.00		1.00
Positive control		396	2AA	2388
NPD		412	(2 µg)	2400
(4 µg)		352		2552
	Mean	386.7		2446.7
	SD	31.07		91.42
	MF	17.06		67.34

MF: Mutation factor
SD: Standard deviation
+S9: with S9 mix
-S9: without S9 mix

$$\text{Mutation factor} = \frac{\text{mean revertants (test item)}}{\text{mean revertants (solvent control)}}$$

*DMSO control was used because of NPD and 2AA

Oily patches were observed at 5000 µg/plate with and without metabolic activation.

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APPENDIX 5 (continued)

Confirmatory Mutation Test (Pre-Incubation Method)

Test Item: S Face lS-1005P
Date: 06-08 September 2011
Strain: *Salmonella typhimurium* TA100
Cell count (overnight culture): 1.70×10^9 CFU/mL

Concentration: (µg/plate)		Revertant colony number -S9	Revertant colony number +S9
5000		64	127
		75	122
		94	117
	Mean	77.7	122.0
	SD	15.18	5.00
	MF	0.89	1.12
1581		91	81
		68	88
		87	95
	Mean	82.0	88.0
	SD	12.29	7.00
	MF	0.94	0.81
500		57	95
		71	107
		82	94
	Mean	70.0	98.7
	SD	12.53	7.23
	MF	0.80	0.91
158.1		58	96
		66	91
		76	115
	Mean	66.7	100.7
	SD	9.02	12.66
	MF	0.76	0.93
50		81	105
		89	110
		72	110
	Mean	80.7	108.3
	SD	8.50	2.89
	MF	0.92	1.00
15.81		74	91
		74	118
		84	86
	Mean	77.3	98.3
	SD	5.77	17.21
	MF	0.88	0.90
5		72	91
		72	98
		88	89
	Mean	77.3	92.7
	SD	9.24	4.73
	MF	0.88	0.85

Concentration: (µg/plate)		Revertant colony number -S9		Revertant colony number +S9
Untreated control		85		104
		74		119
		83		110
	Mean	80.7		111.0
	SD	5.86		7.55
	MF	0.92		1.02
DMSO control*		65		129
		66		114
		82		110
	Mean	71.0		117.7
	SD	9.54		10.02
	MF	0.81		1.08
Distilled water control**		91		117
		95		117
		88		115
	Mean	91.3		116.3
	SD	3.51		1.15
	MF	1.04		1.07
Acetone control		83		105
		97		113
		83		108
	Mean	87.7		108.7
	SD	8.08		4.04
	MF	1.00		1.00
Positive control SAZ (2 µg)		1488	2AA (2 µg)	2244
		1400		2380
		1392		2420
	Mean	1426.7		2348.0
	SD	53.27		92.26
	MF	15.62		19.95

MF: Mutation factor
SD: Standard deviation
+S9: with S9 mix
-S9: without S9 mix

$$\text{Mutation factor} = \frac{\text{mean revertants (test item)}}{\text{mean revertants (solvent control)}}$$

*DMSO control was used because of 2AA
**Distilled water control was used because of SAZ

Oily patches were observed at 5000 µg/plate with and without metabolic activation

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APPENDIX 5 (continued)

Confirmatory Mutation Test (Pre-Incubation Method)

Test Item: S Face IS-1005P
Date: 06-08 September 2011
Strain: *Salmonella typhimurium* TA1535
Cell count (overnight culture): 1.35×10^9 CFU/mL

Concentration: (µg/plate)		Revertant colony number -S9	Revertant colony number +S9
5000		5	11
		9	15
		7	11
	Mean	7.0	12.3
	SD	2.00	2.31
	MF	1.00	1.61
1581		7	12
		8	8
		5	14
	Mean	6.7	11.3
	SD	1.53	3.06
	MF	0.95	1.48
500		2	18
		9	7
		9	8
	Mean	6.7	11.0
	SD	4.04	6.08
	MF	0.95	1.43
158.1		10	12
		5	8
		4	13
	Mean	6.3	11.0
	SD	3.21	2.65
	MF	0.90	1.43
50		3	5
		10	11
		8	10
	Mean	7.0	8.7
	SD	3.61	3.21
	MF	1.00	1.13
15.81		6	6
		6	7
		12	12
	Mean	8.0	8.3
	SD	3.46	3.21
	MF	1.14	1.09
5		6	13
		6	7
		9	15
	Mean	7.0	11.7
	SD	1.73	4.16
	MF	1.00	1.52

Concentration: (µg/plate)		Revertant colony number -S9		Revertant colony number +S9
Untreated control		6		10
		6		9
		8		5
	Mean	6.7		8.0
	SD	1.15		2.65
	MF	0.95		1.04
DMSO control*		3		6
		4		9
		10		13
	Mean	5.7		9.3
	SD	3.79		3.51
	MF	0.81		1.22
Distilled water control**		8		12
		8		12
		7		6
	Mean	7.7		10.0
	SD	0.58		3.46
	MF	1.10		1.30
Acetone control		4		8
		10		11
		7		4
	Mean	7.0		7.7
	SD	3.00		3.51
	MF	1.00		1.00
Positive control		1396	2AA	218
SAZ		1440	(2 µg)	210
(2 µg)		1540		280
	Mean	1458.7		236.0
	SD	73.79		38.31
	MF	190.26		25.29

MF: Mutation factor
SD: Standard deviation
+S9: with S9 mix
-S9: without S9 mix

$$\text{Mutation factor} = \frac{\text{mean revertants (test item)}}{\text{mean revertants (solvent control)}}$$

*DMSO control was used because of 2AA
**Distilled water control was used because of SAZ

Oily patches were observed at 5000 µg/plate with and without metabolic activation

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APPENDIX 5 (continued)

Confirmatory Mutation Test (Pre-Incubation Method)

Test Item: S Face IS-1005P
Date: 06-08 September 2011
Strain: *Salmonella typhimurium* TA1537
Cell count (overnight culture): 1.65×10^9 CFU/mL

Concentration: (µg/plate)		Revertant colony number -S9	Revertant colony number +S9
5000		4	8
		6	9
		6	7
	Mean	5.3	8.0
	SD	1.15	1.00
	MF	1.07	1.50
1581		1	9
		4	10
		7	9
	Mean	4.0	9.3
	SD	3.00	0.58
	MF	0.80	1.75
500		6	6
		6	5
		8	5
	Mean	6.7	5.3
	SD	1.15	0.58
	MF	1.33	1.00
158.1		5	4
		4	13
		11	8
	Mean	6.7	8.3
	SD	3.79	4.51
	MF	1.33	1.56
50		5	9
		5	1
		6	4
	Mean	5.3	4.7
	SD	0.58	4.04
	MF	1.07	0.88
15.81		6	9
		2	11
		4	11
	Mean	4.0	10.3
	SD	2.00	1.15
	MF	0.80	1.94
5		5	8
		8	12
		8	6
	Mean	7.0	8.7
	SD	1.73	3.06
	MF	1.40	1.63

Concentration: (µg/plate)		Revertant colony number -S9		Revertant colony number +S9
Untreated control		6		11
		7		4
		6		7
	Mean	6.3		7.3
	SD	0.58		3.51
	MF	1.27		1.38
DMSO control*		5		4
		3		4
		5		4
	Mean	4.3		4.0
	SD	1.15		0.00
	MF	0.87		0.75
Acetone control		7		10
		4		3
		4		3
	Mean	5.0		5.3
	SD	1.73		4.04
	MF	1.00		1.00
Positive control 9AA (50 µg)		440	2AA (2 µg)	198
		412		231
		392		212
	Mean	414.7		213.7
	SD	24.11		16.56
	MF	95.69		53.42

MF: Mutation factor
SD: Standard deviation
+S9: with S9 mix
-S9: without S9 mix

$$\text{Mutation factor} = \frac{\text{mean revertants (test item)}}{\text{mean revertants (solvent control)}}$$

*DMSO control was used because of 9AA and 2AA

Oily patches were observed at 5000 µg/plate with and without metabolic activation

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APPENDIX 5 (continued)

Confirmatory Mutation Test (Pre-Incubation Method)

Test Item: S Face IS-1005P
Date: 06-08 September 2011
Strain: *Escherichia coli* WP2 *uvrA*
Cell count (overnight culture): 2.88 x 10^9 CFU/mL

Concentration: (µg/plate)		Revertant colony number -S9	Revertant colony number +S9
5000		17	42
		18	36
		25	31
	Mean	20.0	36.3
	SD	4.36	5.51
	MF	0.77	0.99
1581		18	37
		21	28
		29	34
	Mean	22.7	33.0
	SD	5.69	4.58
	MF	0.87	0.90
500		26	38
		18	40
		23	38
	Mean	22.3	38.7
	SD	4.04	1.15
	MF	0.86	1.05
158.1		18	28
		24	31
		24	43
	Mean	22.0	34.0
	SD	3.46	7.94
	MF	0.85	0.93
50		20	45
		27	35
		20	33
	Mean	22.3	37.7
	SD	4.04	6.43
	MF	0.86	1.03
15.81		28	38
		22	25
		26	31
	Mean	25.3	31.3
	SD	3.06	6.51
	MF	0.97	0.85
5.0		16	42
		27	35
		21	38
	Mean	21.3	38.3
	SD	5.51	3.51
	MF	0.82	1.05

Concentration: (µg/plate)		Revertant colony number -S9		Revertant colony number +S9
Untreated control		30		46
		31		29
		24		32
	Mean	28.3		35.7
	SD	3.79		9.07
	MF	1.09		0.97
DMSO control*		22		21
		33		42
		20		35
	Mean	25.0		32.7
	SD	7.00		10.69
	MF	0.96		0.89
Distilled water control**		24		37
		21		42
		17		34
	Mean	20.7		37.7
	SD	3.51		4.04
	MF	0.79		1.03
Aceton		25		39
		24		37
		29		34
	Mean	26.0		36.7
	SD	2.65		2.52
	MF	1.00		1.00
Positive control MMS (2 µL)		1080	2AA (50 µg)	226
		1224		199
		1100		214
	Mean	1134.7		213.0
	SD	78.01		13.53
	MF	54.90		6.52

MF: Mutation factor
SD: Standard deviation
+S9: with S9 mix
-S9: without S9 mix

$$\text{Mutation factor} = \frac{\text{mean revertants (test item)}}{\text{mean revertants (solvent control)}}$$

*DMSO control was used because of 2AA
**Distilled water control was used because of MMS

Oily patches were observed at 5000 µg/plate with and without metabolic activation

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APPENDIX 6

Historical Control Data
(Period of 2004-2010)

Untreated control data										
	without metabolic activation (-S9 Mix)					with metabolic activation (+S9 Mix)				
	TA98	TA100	TA1535	TA1537	E.coli	TA98	TA100	TA1535	TA1537	E.coli
Mean value of revertants	24.1	120.8	11.6	6.9	31.8	29.0	124.8	13.0	8.5	39.0
Minimum	8	58	2	1	9	9	62	3	1	12
Maximum	54	211	31	24	86	52	200	36	26	79

Acetone control data										
	without metabolic activation (-S9 Mix)					with metabolic activation (+S9 Mix)				
	TA98	TA100	TA1535	TA1537	E.coli	TA98	TA100	TA1535	TA1537	E.coli
Mean value of revertants	23.5	109.0	10.7	6.2	30.6	29.5	119.8	11.1	8.0	37.7
Minimum	9	77	5	2	13	14	79	4	2	23
Maximum	43	184	20	14	56	47	188	20	22	60

DMSO control data										
	without metabolic activation (-S9 Mix)					with metabolic activation (+S9 Mix)				
	TA98	TA100	TA1535	TA1537	E.coli	TA98	TA100	TA1535	TA1537	E.coli
Mean value of revertants	23.5	116.8	11.5	6.4	31.1	28.2	123.0	12.8	7.7	38.7
Minimum	9	54	2	1	10	10	61	2	0	12
Maximum	54	200	35	27	69	52	199	31	27	78

TA98: *Salmonella typhimurium* TA98, TA100: *Salmonella typhimurium* TA100,
TA1535: *Salmonella typhimurium* TA1535, TA1537: *Salmonella typhimurium* TA1537,
E.coli: *Escherichia coli* WP2 *uvrA*

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APPENDIX 6 (continued)

Historical Control Data
(Period of 2004-2010)

Distilled water control data										
	without metabolic activation (-S9 Mix)					with metabolic activation (+S9 Mix)				
	TA98	TA100	TA1535	TA1537	E.coli	TA98	TA100	TA1535	TA1537	E.coli
Mean value of revertants	24.6	119.4	11.8	7.2	33.5	29.9	125.6	13.1	8.7	40.1
Minimum	9	51	2	1	9	12	69	3	2	11
Maximum	46	208	34	23	65	61	223	32	23	74

Positive reference control data										
	without metabolic activation (-S9 Mix)					with metabolic activation (+S9 Mix)				
	TA98	TA100	TA1535	TA1537	E.coli	TA98	TA100	TA1535	TA1537	E.coli
Mean value of revertants	474.9	938.7	817.0	552.4	754.1	1841.7	1870.7	249.4	260.0	344.8
Minimum	102	385	167	41	146	236	649	78	66	91
Maximum	1920	2525	2625	2748	1953	4390	4288	2250	1023	2010

TA98: *Salmonella typhimurium* TA98, TA100: *Salmonella typhimurium* TA100,
TA1535: *Salmonella typhimurium* TA1535, TA1537: *Salmonella typhimurium* TA1537,
E.coli: *Escherichia coli* WP2 *uvrA*

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APPENDIX 7

Copy of the GLP Certificate

ORSZÁGOS GYÓGYSZERÉSZETI INTÉZET
National Institute of Pharmacy

FŐIGAZGATÓ
1051 Budapest, Zrínyi u. 3.
tel: (1) 8869-320
fax: (1) 8869-480
e-mail: szepezdi.zsuzsanna@ogyi.hu

Ref. no: OGYI/8242-11/2010
Admin.: Urbin Magdolna Zita
Date: 16 December, 2010

GOOD LABORATORY PRACTICE (GLP) CERTIFICATE

It is hereby certified that the test facility

LAB Research Kft.

(Base facility: H-8201 Veszprém, Szabadságpuszta, Hungary)

is able to carry out

physico-chemical testing, toxicity studies, mutagenicity studies, environmental toxicity studies on aquatic or terrestrial organisms, studies on behaviour in water, soil and air;bio-accumulation , safety pharmacology testing, reproduction toxicology, inhalation toxicology, analytical chemistry and contract archiving

in compliance with the Principles of GLP (Good Laboratory Practice) and also complies with the corresponding OECD/European Community requirements.

Date of the inspection: **4-8 October, 2010.**

ORSZÁGOS GYÓGYSZERÉSZETI INTÉZET 2

Zsuzsanna Szepezdi, Ph. D.
Director-General

The facility name until 1st September 2011 was LAB Research Ltd. From that date, the registered name has been CiToxLAB Hungary Ltd., this information has been transmitted to the GLP competent authority. The above GLP certificate is valid for this facility (now known as CiToxLAB) until the certificate expires (16 December 2012).

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Consumer Product Testing Co.

EST. 1975

FINAL REPORT

CLIENT:

Creative Strategy, Inc.
High Park Nihonbashi Bldg. 4F
2-45-4 Nihonbashi-Hamacho
Chuo-ku, Tokyo 103-0007, Japan

SPONSOR:

SAKAMOTO YAKUHIN KOGYO CO., LTD.
1-20 Rinkaicho, Izumiotsu City
Osaka 595-0075, Japan

TEST:

The MatTek Corporation EpiOcular™ Tissue Model *In Vitro* Toxicity Testing System

TEST ARTICLE:

S Face IS-1005P (Lot.10301)

Polyglyceryl-10 Pentaisostearate

EXPERIMENT REFERENCE NO.:

V11-3646-1

9/20/04

Steven Nitka
Vice President
Laboratory Director

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70 New Dutch Lane • Fairfield, New Jersey 07004-2514 • (973) 808-7111 • Fax (973) 808-7234

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Consumer Product Testing Co.
FSL 1975

QUALITY ASSURANCE UNIT STATEMENT

Study No.: V11-3646-1

The objective of the Quality Assurance Unit (QAU) is to monitor the conduct and reporting of nonclinical laboratory studies. This study has been performed under Good Laboratory Practice principles (including government regulations to the extent applicable) and in accordance with standard operating procedures and applicable standard protocols. The QAU maintains copies of study protocols and standard operating procedures and has inspected this study on the date listed below. The findings of this inspection may have been reported to management and the Study Director.

Date of data inspection: 9/17/11

Quality Assurance:

Christine Hendricks 9/20/11
Signature/Date

70 New Dutch Lane • Fairfield, New Jersey 07004-2514 • (973) 808-7111 • Fax (973) 808-7234
Clinical • Toxicology • Analytical Chemistry • Microbiology

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Objective:

To evaluate the test article for irritancy potential utilizing the MatTek Corporation EpiOcular *in vitro* toxicity testing system.

Introduction:

"MatTek's patented EpiOcular corneal Model consists of normal, human-derived epidermal keratinocytes which have been cultured to form a stratified, squamous epithelium similar to that found in the cornea. The epidermal cells, which are cultured on specially prepared cell culture inserts using serum free medium, differentiate to form a multilayered structure which closely parallels the corneal epithelium . . . " This system " . . . provides a predictive, morphologically relevant *in vitro* means to assess ocular irritancy."[1]

EpiOcular, when used with the recommended cell metabolism assay, can quickly provide toxicological profiles. The procedure utilizes a water-soluble, yellow, tetrazolium salt (MTT {3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide}), which is reduced by succinate dehydrogenase in the mitochondria of viable cells to a purple, insoluble formazan derivative. Substances which damage this mitochondrial enzyme inhibit the reduction of the tetrazolium salt. The amount of MTT reduced by a culture is therefore proportional to the number of viable cells.

Test Article: S Face IS-1005P (Lot.10301)

Experimental Interval: August 17, 2011 – August 18, 2011

Method:

The Sponsor stated that the test article is not water soluble. As per MatTek's protocol, the test article was dosed as received. After the appropriate tissue preparation, 100 microliters of the test article and the negative control (distilled water) were added to the Millicells containing the EpiOcular samples. The six (6) well plates containing the dosed EpiOcular samples were then incubated at 37°C, five (5)% carbon dioxide and ≥ 90% humidity.

[1]MatTek Corporation, 200 Homer Avenue, Ashland, Massachusetts 01721

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Method (continued):

After the appropriate exposure period, each insert was individually removed from its plate and rinsed with phosphate buffered saline (PBS) to remove any residual material. Each was then rinsed a second and third time. Following the 3 rinses, each Millicell was submerged in 5 milliliters of assay media for 10 minutes, at room temperature. This final soak removed any residual, absorbed article. After the 10 minutes, excess liquid was shaken off and each EpiOcular tissue was placed into 300 microliters of MTT solution. The EpiOcular samples were then returned to the incubator.

After the three (3) hour MTT exposure, each insert was removed and gently rinsed with PBS to remove any residual MTT solution. Excess PBS was shaken from each of the inserts, which were then blotted on the bottom using paper towels. The inserts were then each placed into one (1) well of a 24 well extraction plate. Each insert was then immersed in two (2) milliliters of extraction solution, at room temperature, overnight. After the extraction procedure, the liquid within each insert was decanted back into the well from which it was taken. The remaining extractant solution was then agitated and a 200 microliter aliquot of each extract was removed for evaluation. A Dynatech MR 4000 Automatic Microplate Reader was used to determine the absorbance of each extract at 570nm. With the absorbance of the negative control (distilled water) defined as 100%, the percent absorbencies of the articles were determined. The percentages listed below directly correlate with the cell metabolism in the EpiOcular samples.

Results:

Article (% & Exposure)	System	Percent Viability	Percent Inhibition
S Face IS-1005P (Lot.10301)			
(100% - 4 hrs.)	EpiOcular	107	-7
(100% - 1 hr.)	EpiOcular	102	-2
(100% - 20 mins.)	EpiOcular	106	-6

When possible, using a semi-log scale, the percent viabilities for the articles were plotted on the linear y axis versus the dosing time on the log x axis. By interpolation, the time at which the percent viability would be 50% was determined (ET-50). As a general guideline (provided by MatTek) the following equation can be used to estimate the rabbit Draize eye score:

$$\text{Draize} = -4.74 + 101.7/(\text{ET-50})^{0.5}$$

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Based on the literature (Kay, J.H. and Calandra, J.C., "Interpretation of eye irritation tests," *J. Soc. Cosmetic Chem.*, 13, 281-289 (1962)), the ocular irritancy estimated potential has been categorized by MatTek into the following groups, based on the Draize score:

Draize Score	Irritancy Classification	Example	EpiOcular ET-50 (min)
0-15	Non-irritating, Minimal	PEG-75 Lanolin, Tween 20	>256 – 26.5
15.1 – 25	Mild	3% Sodium Dodecyl Sulfate	<26.5 – 11.7
25.1 – 50	Moderate	5% Triton X-100	<11.7 – 3.45
50.1 – 110	Severe, Extreme	5% Benzalkonium Chloride	<3.45

Discussion:

Under the conditions of this test, the S Face IS-1005P (Lot.10301) test article, at 100%, elicited *in vitro* results which indicate that its ET-50 is greater than 256 minutes. Therefore, the test article, at 100%, has an estimated Draize ocular irritation score of approximately 0 with a "non-irritating" irritancy classification.

Conclusion:

Under the conditions of this test, the results indicate that the S Face IS-1005P (Lot.10301) test article, at 100%, has a "non-irritating" irritancy classification.

Record Retention:

All records and documents pertaining to the conduct of this study shall be retained in the CPTC archives for a minimum of ten (10) years. At any time prior to the completion of the tenth archival year, a Sponsor may submit a written request to the CPTC QA Department to obtain custody of study records once the CPTC archive period has been completed. This transfer shall be performed at the Sponsor's expense. In the absence of a written request, study-related records shall be destroyed at the end of the CPTC archive period in a manner that renders them useless.

Professional personnel involved:

Steven Nitka, B.S.	-	Vice President Laboratory Director (Study Director)
Lillian Vazquez, B.S.	-	Laboratory Supervisor
Christine Hendricks	-	Quality Assurance Group Leader

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STUDY REPORT

Patch test on the cosmetic ingredient

Japan Hair Science Association
(Incorporated association for the public good)

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To SAKAMOTO YAKUHIN KOGYO CO.,LTD.
I hereby certify that this document is a duplicate copy of the original issued as Nichimokyo №23029-(1) on 2th November, 2011.

25th November, 2011

Keiji Kijima

Keiji Kijima ph.D
Chairman of Directors
Japan Hair Science Association
(Incorporated association for the public good)

Contract Study Report

This is the report sponsored study on the patch test of the cosmetic ingredient as you requested on 4th October, 2011. See Attachment for study results.

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Nichimokyo №23029-(1)
2th November, 2011

To SAKAMOTO YAKUHIN KOGYO CO.,LTD.

This report presents the results of patch test on the cosmetic ingredient performed by Japan Hair Science Association. It was sponsored by SAKAMOTO YAKUHIN KOGYO CO.,LTD.on 4th October, 2011. This report is an English version of the original, which was written in Japanese. The undersigned hereby declare that this version faithfully reflects the original report to the best of my knowledge.

TEST FOR IRRITATION EFFECTS OF THE COSMETIC INGREDIENT ON HUMAN SKIN

1.Purpose of the test

This patch test was performed to assess possible irritation potential of the the cosmetic ingredient on healthy human skin, sponsored by SAKAMOTO YAKUHIN KOGYO CO.,LTD. on 4th October, 2011.

2.Test sample

The supplied sample is the following material

Sample : S face IS−1005P Lot.№10301 Polyglyceryl-10 Pentaisostearate

Control : purified water

3.Test procedure

(1)Volunteers

16 males and 27 females (43 Japanese people in total), age 23 to 62, were selected as volunteers.

(2)Applied amount

Aproper quantiy of test sample was applied to each volunteer.

(3)Method of patch test

A test sheet sample was placed on FINN CHAMBER(Smart Practice®)and applied to the crooked side of upper arm of each volunteer for 24 hours in occlusive condition to for evaluation.

At the end of 24 hours exposure period, the test sample was removed. Skin reactions were evaluated 1 hour and 24 hours after removing the test sample.

(4)Criteria for evaluation

The reaction was evaluated using the following criteria. Based on the Japanese

− :	No reaction
± :	Very slight erythema
+ :	Well-defined erythema
++ :	Erythema with swelling or with papule
+++ :	Blisters in addition to the above symptom
++++ :	Large blister

The evaluation of patch test on the cosmetic ingredient performed by
Mikako Aoki, M.D., Dermatologist

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4.Term of path test

Effective October 19,2011.~October 28,2011.

5.Results

The results of this test on 43 volunteers were as shown in the Table. In all volunteers, the reaction was observed (+)on 1 volunteer in 1 hour after removing the test sample (shown in Table as 24-hours Evaluation time). Similarly, no reaction was observed in 24 hours after removing the test sample (shown in Table as 48-hours Evaluation time) in all volunteers.

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Table Results of patch test(23029-1)

No.	Volunteer	Sex	Age	Evaluationtime(hrs.)	Sample	Control	Note(anamnesis)
1	T. A.	M	33	24	−	−	Healthy
				48	−	−	
2	N. A.	M	41	24	−	−	Healthy
				48	−	−	
3	M. I.	F	38	24	+	++	Healthy
				48	−	−	
4	T. I.	M	46	24	−	−	Healthy
				48	−	−	
5	Y. I.	M	49	24	−	−	Healthy
				48	−	−	
6	Y. I.	M	36	24	−	−	Healthy
				48	−	−	
7	A. I.	F	26	24	−	−	Healthy
				48	−	−	
8	Y. U.	F	36	24	−	−	Healthy
				48	−	−	
9	H. E.	F	38	24	−	−	Healthy
				48	−	−	
10	Y. E.	M	27	24	−	−	Healthy
				48	−	−	
11	R. E.	F	30	24	−	−	Healthy
				48	−	−	
12	Y. O.	F	30	24	−	−	Healthy
				48	−	−	
13	M. O.	M	62	24	−	−	Healthy
				48	−	−	
14	K. O.	M	29	24	−	−	Healthy
				48	−	−	
15	A. O.	M	56	24	−	−	Healthy
				48	−	−	
16	M. O.	M	26	24	−	−	Healthy
				48	−	−	
17	M. O.	F	39	24	−	−	Healthy
				48	−	−	
18	K. K.	M	55	24	−	−	Healthy
				48	−	−	
19	T. K.	M	33	24	−	−	Healthy
				48	−	−	
20	Y. K.	F	35	24	−	−	Healthy
				48	−	−	
21	K. G.	F	41	24	−	−	Healthy
				48	−	−	
22	A. S.	F	42	24	−	+	Healthy
				48	−	−	
23	K. S.	M	32	24	−	−	Healthy
				48	−	−	
24	I. S.	M	52	24	−	−	Healthy
				48	−	−	
25	K. S.	F	38	24	−	−	Healthy
				48	−	−	

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Table Results of patch test(23029-1)

No.	Volunteer	Sex	Age	Evaluationtime(hrs.)	Sample	Control	Note(anamncsis)
26	S. T.	F	31	24	−	−	Healthy
				48	−	−	
27	K. T.	F	40	24	−	−	Healthy
				48	−	−	
28	T. T.	M	24	24	−	−	Healthy
				48	−	−	
29	H. T.	F	40	24	−	−	Healthy
				48	−	−	
30	T. N.	M	36	24	−	−	Healthy
				48	−	−	
31	A. N.	F	47	24	−	−	Healthy
				48	−	−	
32	H. N.	F	23	24	−	−	Healthy
				48	−	−	
33	S. H.	F	31	24	−	−	Healthy
				48	−	−	
34	M. H.	F	38	24	−	+	Healthy
				48	−	−	
35	A. H.	M	34	24	−	−	Healthy
				48	−	−	
36	Y. F.	F	44	24	−	+	Healthy
				48	−	−	
37	M. H.	F	24	24	−	−	Healthy
				48	−	−	
38	T. M.	F	40	24	−	−	Healthy
				48	−	−	
39	T. M.	F	34	24	−	+	Healthy
				48	−	−	
40	T. M.	M	42	24	−	−	Healthy
				48	−	−	
41	S. M.	F	47	24	−	−	Healthy
				48	−	−	
42	H. Y.	F	39	24	−	+	Healthy
				48	−	−	
43	M. W.	F	32	24	−	−	Healthy
				48	−	−	

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Výzkumný ústav organických syntéz a.s.
(Research Institute for Organic Syntheses Inc.)
Centre of Ecology, Toxicology and Analytics
GLP Test Facility
Rybitvi 296
533 54 Rybitvi
Czech Republic

FINAL REPORT

S Face IS-1005P
In vitro Skin Irritation Test
(EpiDerm™ Model)

Polyglyceryl-10 Pentaisostearate

Report No.: 11-299

Study No.: 174/11/4AI

Experimental part of study performed in period: 15/09/2011-24/09/2011

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JAPAN

Rybitvi, 15 -11- 2011

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GLP Statement

Study title: S Face IS-1005P – *In vitro* Skin Irritation Test (EpiDerm™ Model)

Study No.: 174/11/4AI

The undersigned study director declares that the work described in this report has been performed under her supervision in accordance with GLP principles [OECD Principles on Good Laboratory Practice (as revised in 1997), C (97) 186 (Final)], and the report gives a correct and precise account of the results obtained.

Martina Čapková, M.Sc.
Study Director

Date: 15 -11- 2011

Approved by:

Viktor Mejstřík, Ph.D.
Head of Test Facility

Date: 15 -11- 2011

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Quality Assurance Statement

Study title: S Face IS-1005P – In vitro Skin Irritation Test

Study No: 174/11/4AI

Inspection (revision)	**Date**
Study plan	14. 9. 2011
Test procedure (*194/11/4AI*)	22. 10. 2011
Study documentation	14. 10. 2011
Draft final report	14. 10. 2011
Final report	4. 11. 2011

The performance of this type of study is not always inspected individually in every phase. Critical steps of test procedure could be audited in one of studies (see brackets) performed in similar time period.

Final report has been audited by QA personnel and is considered to describe closely the methods and procedures used in the study. The reported results accurately reflect the original data of the study.

It is the practice of Quality Assurance Unit to report the results of each inspection/revision to both the Study Director and test facility management immediately.

.....

R.Čihák, Ph.D.
Head of Quality Assurance Unit

Date : 10.11.11

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Summary

Test substance **S Face IS-1005P** was assayed for the in vitro skin irritation in human epidermal model EpiDerm™. The test was performed according to Method B.46. *In vitro* skin irritation: Reconstructed human epidermis model test and Protocol for: In Vitro EpiDerm™ Skin Irritation Test For use with MatTek Corporation's Reconstructed Human Epidermal Model EpiDerm (see par. 1.4, (1), (3)).

Test substance (30 µl) was placed directly atop the tissue. Length of exposition was 60 minutes. Nine tissues were used for the experiment, three per test substance (TS), three for positive control (PC) and three for negative control (NC).

After rinsing, tissues were post incubated for 42 hours due to leave of damage reparation. Three hours incubation with MTT and two hours extraction period with shaking followed then. Optical density (OD_{570}) of isopropanol extracts was measured on a spectrophotometer. Relative cell viability was calculated for each tissue as % of the mean viability of the negative control tissues.

Under the above-described experimental design, average viability of tissues treated by the test substance **S Face IS-1005P** was **94.7 %** of negative control average value (i.e. viability is >50 %). According to the criteria given in Method B.46. the test substance did not fall into any of quoted categories of skin irritation.

The test substance was **nonirritating in EpiDerm™ model.**

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1. INTRODUCTION

1.1. Purpose of study

To assess the potential of the **S Face IS-1005P** to cause cell damage in the human epidermal model designed to the detection of skin irritation properties of test substance. The study is a part of test substance health hazard evaluation.

1.2. Sponsor

Name and Address of Sponsor
Sakamoto Yakuhin Kogyo Co., Ltd.
20, Rinkai-cho 1-chome
Izumiotsu
Osaka 595-0075
JAPAN

Study Monitor
Hidenori Tachibana
Toyo Science Corp.
Kinsan Bldg. 1-21
Nihonbashi-Muromachi, 4-chome
Chuo-ku Tokyo 103-0022
JAPAN

1.3. Test Facility

Name and Address of Test Facility
Výzkumný ústav organických syntéz a.s.
Research Institute for Organic Syntheses, Inc.
Centre of Ecology, Toxicology and Analytics
GLP Test Facility
Rybitvi 296
533 54 Rybitvi
Czech Republic

Study Director
Martina Čapková, M.Sc.
Výzkumný ústav organických syntéz a.s.
Research Institute for Organic Syntheses, Inc.
Centre of Ecology, Toxicology and Analytics
GLP Test Facility
Genetic Toxicity/In vitro Testing Group
Rybitvi 296
533 54 Rybitvi
Czech Republic

Personnel Involved

Study performance:	Martina Čapková, M.Sc
Technical co-operation:	Simona Kozlovská
Quality assurance:	Rostislav Čihák, Ph.D.

Address of the personnel involved in this study is the same as the address of the test facility.

1.4. Legal items (Guidelines)

Quality Assurance
This study has been carried out in accordance with internationally valid GLP principles:
- Directive 2004/10/EC of the EP and of the Council, February 2004
- OECD Principles of Good Laboratory Practice (as revised in 1997) C (97) 186 (Final)

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Methods

The working procedure described in this final report is in accordance with the following methodical documents:

(1) Method B.46. *In vitro* skin irritation: Reconstructed human epidermis model test, Commision Regulation (EC) No. 761/2009, 23th July 2009

(2) OECD TG 439, *In Vitro* Skin Irritation: Reconstructed Human *Epidermis* Test Method, adopted 22 July, 2010

(3) Protocol for: In Vitro EpiDerm™ Skin Irritation Test For use with MatTek Corporation's Reconstructed Human Epidermal Model EpiDerm, Model EPI-200-SIT, MatTek Corporation January 1st, 2010, 1-37

1.5. Storage and retention of records and materials

The GLP Testing Section of CETA will archive all the documentation of the study at least for 15 years: contract documentation, study plan, raw data, final report with QA statement, and records of QA inspections.

The test substance sample will be archived till the end of expiry (1st August 2012).

The records of primary data from the study could be found on special data sheets archived together with final report.

1.6. Study time schedule

Study preparation (information completion, study plan) was performed from 08/09/2011 to 14/09/2011.
Experimental part of study was performed from 15/09/2011 to 24/09/2011
Examination of results and final report elaboration occurred from 15/09/2011 to dd/mm/2011.

2. TEST SUBSTANCE

Test substance name: **S Face IS-1005P**

a) ***Information from sponsor***

Chemical name:	Polyglyceryl-10 Pentaisostearate
CAS No.::	83138-62-9 , 126449-40-9
Batch No.::	10301
Purity (typical):	100 % (w/w)
Appearance:	pale yellow viscous liquid
Storage:	Store at the room temperature bellow 30°C.
Stability/Expiration date:	1st August 2012

b) Information from test facility

Appearance:	pale yellow viscous liquid
Storage during the study:	the sample of test substance has been stored at room temperature during the study

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3. STUDY PERFORMANCE

3.1. Definitions

Skin irritation Incurrence of reversible changes in skin after application of the test substance

3.2. Tissues

The reconstructed human epidermal model EpiDerm™ (EPI-200, MatTek, Ashland, USA); MTT test: EPI-200, Lot No. 15626, kit A

3.3. Chemicals and media

Chemicals:
PBS (phosphate buffered saline) – prepared in laboratory
MTT (3-(4,5-Dimethyl thiazol-2-yl)-2,5-diphenyltetrazolium bromide), research grade, Serva, Lot No. 081153
Isopropylakohol – p.a., Lach-Ner, Lot No. PP/2007/2176/0 exp. 4/12

Positive control:
5 % SDS (sodium dodecyl sulphate), MatTek, Lot No. 120110ACF

Media:
EPI-100-NMM-SIT/Maintenance Medium, MatTek, Lot No.091511TDD

3.4. Test method

The test methods are described in the guidelines referred in chap. 1.4. Details of routine are available in Protocol for: In Vitro EpiDerm™ Skin Irritation Test For use with MatTek Corporation's Reconstructed Human Epidermal Model EpiDerm, Model EPI-200-SIT, MatTek Corporation January 1st, 2010, 1-37.

3.5. Principle of the assay

The test consists of a topical exposure of the neat test chemical to a reconstructed human epidermis (RhE) model followed by a cell viability test. Cell viability is measured by dehydrogenase conversion of MTT [*(3-4,5-dimethyl thiazole 2-yl) 2,5-diphenyltetrazolium-bromide*], present in cell mitochondria, into a blue formazan salt that is quantitatively measured after extraction from tissues. The reduction of the viability of tissues exposed to chemicals in comparison to negative controls (treated with PBS) is used to predict the skin irritation potential.

Evaluation is determined by measuring of optical density (OD) of the formazan extracts using a spectrophotometer at 570 nm. Relative cell viability is calculated for each tissue as % of the mean OD value of the negative control tissues. Skin irritation potential of the test material is predicted if the remaining relative cell viability is below 50 %.

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3.6. Test system

The reconstructed human epidermal model EpiDerm™ (EPI-212, MatTek, Ashland, USA) consists of normal human-derived epidermal keratinocytes, which have been cultured to form a multilayered highly differentiated model of the human epidermis. The EpiDerm™ System is manufactured according to defined quality assurance procedures.

The EpiDerm™ tissues (surface 0.63 cm^2) are cultured on specially prepared cell culture inserts and shipped world-wide as kits, containing tissues on shipping agarose together with the necessary amount of culture media.

3.7. Test procedure

3.7.1. Direct MTT reduction - functional check in tubes

Some test substances may interfere with the MTT endpoint if it is able to directly reduce MTT. Therefore, before exposure, functional check for this possibility is performed as follows: 50 µl of the test substance is added to 1 mL MTT medium (red) and incubated in the incubator (37±1°C, 5±1 % CO_2, moistened) for 1 hour. At the end of the exposure time, the presence and intensity of the staining (if any) is observed. If the solution changes colour from red to blue, other steps to correction have to be done.

3.7.2. MTT test

a) Test substance application:

The test substance (30 µl) is placed directly atop the tissue. The material is spread on the tissue surface.

b) Procedure:

On the day of receipt, EpiDerm tissues are conditioned by incubation to release transport stress related compounds and debris. After pre-incubation, tissues are topically exposed to the test chemicals for 1 hour. Three tissues are used per test chemical and for the positive control (PC) and negative control (NC). Tissues are then thoroughly rinsed, blotted to remove the test substances, and transferred to fresh medium.

After 24 hour incubation period, the medium is replaced by fresh one. Tissues are incubated for another 18 hours. Afterwards, the MTT assay is performed by transferring the tissues to 24-well plates containing MTT medium (1 mg/ml). After 3 hour MTT incubation, the blue formazan salt formed by cellular mitochondria is extracted with 2.0 ml/tissue of isopropanol and the optical density of the extracted formazan is determined using a spectrophotometer at 570 nm. Detailed procedure is described in SOP M/48/3 (VUOS-CETA, 2011).

c) OD_{570} measuring:

OD_{570} was measured on a spectrophotometer Libra S22. Isopropanol served as a blank.

3.8. Assay acceptance criteria

1. Negative Control:

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The **absolute OD** of the negative control (NC) tissues (treated with sterile PBS) in the MTT-test is an indicator of tissue viability obtained in the testing laboratory after shipping and storing procedures and under specific conditions of use. **The assay meets the acceptance criterion if the mean OD_{570} of the NC tissues is = 1.0 and = 2.5.**

2. Positive Control:

A **5 % SDS** (in H_2O) solution is used as positive control (PC) and tested concurrently with the test chemicals. Concurrent means here the PC has to be tested in each assay, but not more than one PC is required per testing day. Viability of positive control should be within 95±1 % confidence interval of the historical data.

The assay meets the acceptance criterion if the mean viability of PC tissues expressed as % of the negative control tissues is = 20 %.

3. Standard Deviation (SD):

Since in each test skin irritancy potential is predicted from the mean viability determined on 3 single tissues, the variability of tissue replicates should be acceptably low. **The assay meets the acceptance criterion if the SD calculated from individual % tissue viabilities of the 3 identically treated replicates is < 18 %.**

3.9. Evaluation of results and classification

In vitro alternatives that have been validated and accepted may also be used to help in classification decisions making (see Regulation (EC) 1272/2008, 3.2. Skin corrosion/ irritation, 3.2.2. Classification criteria for substances).

For further classification the relative cell viability is calculated for each tissue as % of the mean of the negative control tissues viability, which is set at 100 %.

The cut-off values for the prediction of irritation are given below; these values are stated in EU Method B.46., part 2.2. INTERPRETATION OF RESULTS:

The test substance is considered to be **irritant to skin** in accordance with UN GHS [1] category 2:
(i) if the tissue viability after exposure and post-treatment incubation is less than or equal (=) to 50 %.
The test substance is considered to have **no category**:
(ii) if the tissue viability after exposure and post-treatment incubation is more than (>) 50 %.

[1] United Nations (UN) (2007). Globally Harmonized System of Classification and Labelling of Chemicals (GHS), Second revised edition, UN New York and Geneva, 2007

3.10. Deviations

No deviations from study plan or test guidelines/methods were observed during the study.

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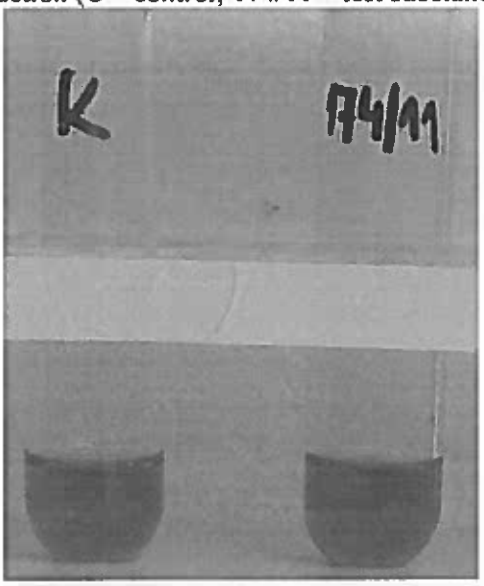
4. STUDY RESULTS

4.1. Direct MTT reduction- functional check in tubes

50 µl of the test substance was added to 2 ml of MTT medium. Suspension was incubated for 1 hour (37±1°C, 5±1 % CO_2, moistened). The test substance did not changed colour from red to blue (see Figure 1).

The test substance did not reduce MTT directly.

Figure 1: Direct MTT reduction (C – control; 174/11 – test substance)



4.2. MTT test

The test substance (30 µl) was placed directly atop the tissue. The substance was spread on the tissue surface. Tissues were exposed to the test substance for 1 hour. After exposition, tissues were thoroughly rinsed and blotted to remove the test substance. Small amount of the test substance remained in tissues. Then, tissues were let to post-incubate for 24 (medium exchange) + 18 hours (37±1°C, 5±1% CO_2, moistened).

Afterwards, the MTT assay was performed by transferring the tissues to 24-well plates containing MTT medium (1 mg/ml). After 3 hour MTT incubation, the blue formazan salt formed by cellular mitochondria was extracted with 2.0 ml/tissue of isopropanol and the optical density of the extracted formazan was determined using a spectrophotometer at 570 nm.

OD_{570} measuring was performed after 2-hour extraction with shaking. Results are given in the Table 1.

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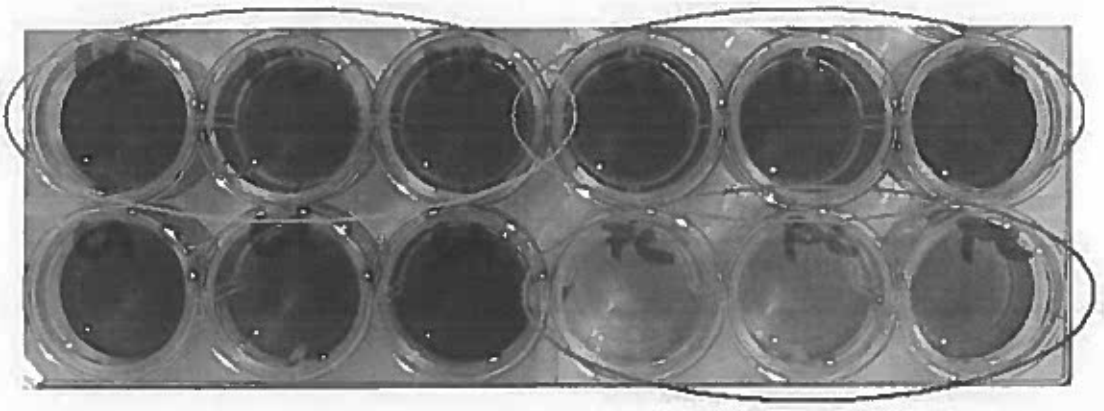
Table 1: OD_{570} values obtained at the MTT test, their averages, standard deviations (%) and relative viabilities

Treatment	Parameter	OD_{570}			mean	SD	Average viability (% NC)
		1	2	3			
NC (PBS)	OD_{570}	1.878	2.153	2.077	**2.036**	0.116	
	viability	92.24	105.74	102.01	**100.0**	**5.695**	**100.0**
C2 (Test substance)	OD_{570}	1.939	1.911	1.936	**1.929**	0.013	
	viability	95.24	93.86	95.09	**94.7**	**0.617**	**94.7**
PC (5% SDS)	OD_{570}	0.159	0.205	0.298	**0.221**	0.058	
	viability	7.81	10.07	14.64	**10.8**	**2.840**	**10.8**

Notes:

NC	negative control
PC	positive control
C2	test substance
mean	arithmetic mean
SD	standard deviation calculated from individual % tissue viabilities
viability (%)	viability of single tissues compared with negative control

Figure 2: Results of MTT test



——— - test substance ——— - positive control (SDS 5%)

——— - negative control (PBS)

4.3. Assay acceptance criteria fulfilment

Assay acceptance criteria 1-3 given in chap. 3.8 have been met.

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4.4. Evaluation of results and classification

Under the above-described experimental design, average viability of tissues treated by the test substance **S Face IS-1005P** was **94.7 %** of negative control average value, i.e. viability was >50 %.

According to the classification criteria given in chap. 3.9., the test substance did not fall into any of quoted categories of skin irritation. The test substance was **nonirritating in EpiDerm™ model.**

5. CONCLUSIONS

According to study results, the test substance was **nonirritating in EpiDerm™ model.**

6. APPENDICES

Appendix 1: Tissue Certificate

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Appendix 1: Tissue Certificate

MatTek Corporation

Certificate of Analysis

Product: EpiDerm™ Skin Tissue **Lot#** 15626

Part# : EPI-200

Description: Reconstructed skin tissue containing normal human keratinocytes.
This product is for research use only. Not for use in animals, humans or diagnostic purposes.

I. Cell source
All cells used to produce EpiDerm™ are purchased or derived from tissue obtained by MatTek Corporation from accredited institutions. In all cases, consent was obtained by these institutions from the donor or the donor's legal next of kin, for use of the cells or derivatives of the tissue for research purposes.

Keratinocyte Strain: 4F1188

II. Analysis for potential biological contaminants:
The cells used to produce EpiDerm™ tissue are screened for potential biological contaminants. Tests performed for each of the potential biological contaminant listed in the analysis that follows, were performed according to the test method given. The product resulted in "no detection" for the following potential biological contaminants determined by the stated test method:

Keratinocytes:	
HIV-1 virus – Oligonucleotide-directed amplification	Not detected
Hepatitis B virus – Oligonucleotide- directed amplification	Not detected
Hepatitis C virus – Oligonucleotide- directed amplification	Not detected
Bacteria, yeast, and other fungi – long term antibiotic, antimycotic free culture	Not detected

III. Analysis for tissue functionality:
Qualitative observation of MTT reduction: 1% Triton X-100, 5hr Exposure

Appearance: Pass

Initials:
Date: 9/21/11

Paul Kearney 9/21/11
Paul Kearney Date
Quality Assurance Manager

CAUTION: Whereas all information above is believed to be accurate and correct, no absolute guarantee that human derived material is non-infectious can be made or is implied by this certificate of analysis. All tissues should be treated as potential pathogens. The use of protective clothing and eyeware and appropriate disposal procedures are strongly recommended.

200 Homer Avenue
Ashland MA 01721 *Web Site:* **www.mattek.com** *Email:* **information@mattek.com**
(508) 881-6771
Fax (508) 879-1532

QC-10-012-0005

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ASSESSMENT OF THE SENSITIZING POTENTIAL OF A COSMETIC PRODUCT: FINAL CLINICAL SAFETY TEST UNDER DERMATOLOGICAL CONTROL

STUDY REF.	**DA11048-1**
PRODUCTS	**S Face IS-1005P** Polyglyceryl-10 Pentaisostearate
SPONSOR	**Sakamoto Yakuhin Kogyo Co., Ltd.** **20, Rinkai-cho, 1-chome,** **Izumiotsu, Osaka 595-0075** **JAPAN**
MONITOR	**Dermscan Asia**
INVESTIGATOR	**Dr Sujittra Napathorn, Dermatologist**

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SUMMARY

<table>
<tr><td colspan="3">Sponsor: Sakamoto Yakuhin Kogyo Co., Ltd.

Address:
20, Rinkai-cho, 1-chome,
Izumiotsu, Osaka 595-0075
JAPAN</td><td colspan="3">Investigator : Dermscan Asia Co., Ltd

Address:
3300/46-47 29th Flr., Tower A
Elephant Tower, Phaholyothin Rd., Chomphon,
Chatujak,Bangkok,10900
THAILAND</td></tr>
<tr><td>Study title</td><td colspan="5">ASSESSMENT OF THE SENSITIZING POTENTIAL OF A COSMETIC PRODUCT: FINAL CLINICAL SAFETY TEST UNDER DERMATOLOGICAL CONTROL</td></tr>
<tr><td rowspan="2">Product</td><td colspan="5">Reference:
S Face IS-1005P</td></tr>
<tr><td colspan="3">Galenic form:
Colorless viscous liquid</td><td colspan="2">Concentration:
Pure</td></tr>
<tr><td>Patch type:</td><td colspan="5">Occlusive</td></tr>
<tr><td>Study dates</td><td colspan="5">From : August 29 to October 7, 2011.</td></tr>
<tr><td>Objective</td><td colspan="5">To confirm that repeated applications of the product under maximized conditions, in healthy subjects, does not induce delayed contact allergy reactions.</td></tr>
<tr><td>Study design</td><td colspan="5">Monocentric and simple blind study.</td></tr>
<tr><td>Assessment criteria</td><td colspan="3">• Assessment of erythema and edema
• Irritation: MCII calculation
• Sensitization: ICDRG scoring</td><td>Zone</td><td>Scapular part of the back</td></tr>
<tr><td colspan="3"></td><td>Kinetics</td><td>Induction : 3 weeks
Rest : 2 weeks
Challenge : 1 week</td></tr>
<tr><td rowspan="3">Study population</td><td colspan="5">Number of analyzed subjects: 51.</td></tr>
<tr><td colspan="5">Average age : 44 ± 2 years old (between 18 and 65)</td></tr>
<tr><td colspan="5"><u>Main inclusion criteria</u>
• Asian Female with normal skin.
• Age ≥ 18 years old
• Phototype I to IV.</td></tr>
<tr><td>Results</td><td colspan="5">Irritating potential - Induction phase: Non irritating

Sensitizing potential - Challenge phase: Non-sensitizing

<u>CONCLUSION</u>

Under these study conditions, the product S Face IS-1005P can be considered "Hypoallergenic product".</td></tr>
</table>

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1. PROTOCOL

1.1. Aim of the study

The main objective of this study is to confirm that repeated applications of a cosmetic product under maximized conditions, in healthy subjects, does not induce delayed contact allergy reactions.

Its secondary objective is to determine the irritating potential of the product first after a single application and second after repeated applications.

1.2. Study design

This was an open study, without randomisation of the application site.

1.3. Study subjects

1.3.1. Inclusion criteria

- Healthy subjects.
- Female subjects with normal skin
- Age ≥ 18 years old
- Phototype I to IV.
- Subjects without scars, tattoos, any pigmentary marks, excessive pilosity and uneven skin tone or active dermal lesions, on the concerned areas of the back.
- Subjects having given their informed, written consent.
- Cooperative subjects, aware of the necessity and duration of controls so that perfect adhesion to the protocol established by DERMSCAN could be expected.

1.3.2. Non-Inclusion criteria

- Pregnant or nursing women or women without any medically efficient contraceptive method (for women of childbearing potential)..
- Sun or UV exposure during the 15 days before or during the study.
- Subject having taken part to a repeated patch tests study or having received photopatch-tests from less than 4 months (with a maximum of three studies of this kind each year).
- Hyper irritable skin.
- Known allergy or sensitivity to adhesive plaster and/or cosmetic products.
- History of abnormal response to sunlight or presence of active dermal lesions, scars, beauty spots, freckles or any abnormality, on the back.
- Serious or progressive disease (mainly skin cancer background).
- Subjects undergoing a topical or systemic treatment:
  - anti-inflammatories and/or anti-histamines during the previous week and during the study,
  - photo-allergic and/or phototoxic substances from less than one month and during the study,

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- immuno-suppressors and/or corticoids during the four previous weeks and during the study,
- retinoids during the six previous months and during the study

- Any condition considered, by the investigator, as incompatible with the study protocol

1.3.3. Restriction

- No exposure to sun or U.V rays during the whole study.
- Do not apply any cosmetic products nor topic drugs to the application zone. No swimming pool, sauna... during the whole study.
- Do not start any medical treatment without informing the investigator.

1.4. Study product

The product supplied by Sponsor, had the following characteristics:

Product name	Product aspect	Product code
S Face IS-1005P	Colorless viscous liquid	SFP

The product was received on 17/08/2011.

1.5. Methodology

1.5.1. Instruments, dose

The studied product was applied to a cleansed (with water) and dry skin under the following conditions:

Areas:	Scapular zones: homolateral (induction zone) and controlateral (challenge zone)
Patch tests type:	Finn Chamber® 8mm (50mm^2) - occlusive
Dose :	25 µl
Application conditions:	Pure
Application frequency:	Induction phase: 3 times a week during 48 hours. Challenge phase: once during 48 hours.
Contact time:	Induction phase: 3 weeks. Rest phase: 2 weeks. Challenge phase: 1 week
Control:	patch without product

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1.5.2. Clinical assessment

1.5.2.1. Clinical criteria regarding the irritating potential (induction phase)

After each application, the patch is removed and the clinical examination is performed by the investigator 30 minutes later in order to eliminate the pressure and the occlusion effects.

The result of examination is negative if the skin looks normal. The clinical examination is made on the back using the following criteria and scale (Quotation 0 to 3)

Score	Quotation	CRITERIA: description :	
		ERYTHEMA «E»	OEDEMA «O»
0	Absent	no erythema	no oedema
0.5	Very slight	barely perceptible: pinkish coloration of one part of the tested area	palpable, barely visible
1	Slight	pinkish coloration of the complete tested area or rather visible on one part of the tested area	palpable, visible
2	Obvious	obvious erythema covering the whole tested area	obvious oedema (thickness < 1 mm) with or without papule(s) or vesicle(s)
3	Important	severe erythema covering all the tested area or obvious erythema diffusing outside the tested area	severe oedema (thickness ≥ 1 mm or diffusing outside the tested area) with or without papule(s) or vesicle(s)

1.5.2.2. Clinical criteria regarding the sensitizing potential (challenge phase)

The occurrence of allergic reactions was assessed according to the following scale:

Criterion	ICDRG (*) Quotation	Numeric score Quotation
No reaction	0	0
Doubtful reaction	?	?
Erythema and edema	+	1
Erythema, edema and vesicles	++	2
Severe reaction with blisters or post-blisters ulcerations	+++	3

* (International Contact Dermatitis Research Group)

A score ≥ 2 represents a contact allergy reaction.

1.5.2.3. Behaviour in case of specific reactions

In case of reaction to the support (patch), this one is moved and applied next to the initial site. If a reaction is observed again, applications are definitely stopped.

During the induction phase, if the investigator observes, at the level of the product application site, as soon as the second application, a reaction like :

- erythema ≥ 2 or
- erythema ≥ 1 with edema or discreet infiltration with a few papules,

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applications continue on an adjacent site with readings on both sites until the end of induction.
If a new reaction occurs, applications are definitely stopped.

In case of allergy suspicion, a new product application is proposed to confirm the reaction et possibly identify the concerned substance(s). This new exposure takes place at least three weeks after disappearance of the previous reaction. The new exposure conditions have to be discussed between the investigator and the sponsor. The subject is followed until total disappearance of the signs.

1.5.3. Premature study termination

The subjects had the right to leave the study at any time whatever the reason.

The premature study termination could be due to multiple reasons:
- non-compliance with the visits schedule,
- adverse events (including intercurrent diseases),
- protocol non-adherence/departures from protocol,
- withdrawal of subject consent.

1.5.4. Study schedule

1.5.4.1. Induction phase

W1:

Day of the week	Mo	Tu	We	Th	Fr	Sa	Su
Study day	D1	D2	D3	D4	D5	D6	D7
Product application	↓		↓		↓		
Removal/Assessment			↓		↓		

W2:

Day of the week	Mo	Tu	We	Th	Fr	Sa	Su
Study day	D8	D9	D10	D11	D12	D13	D14
Product application	↓		↓		↓		
Removal/Assessment	↓		↓		↓		

W3:

Day of the week	Mo	Tu	We	Th	Fr	Sa	Su	Mo
Study day	D15	D16	D17	D18	D19	D20	D21	D22
Product application	↓		↓		↓			
Removal/Assessment	↓		↓		↓			↓

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1.5.4.2. Rest phase

W4 and W5: No application and no assessment.

1.5.4.3. Challenge phase

W6:

Day of the week	Mo	Tu	We	Th	Fr
Study day	D36	D37	D38	D39	D40
Product application	2 zones ↓				
Removal/Assessment			↓		↓

1.5.5. Results interpretation

1.5.5.1. Induction phase

At the end of the 9 readings of the induction phase, the average score of each subject (Cumulative Irritation Index (C.I.I.)) was calculated by adding the scores obtained for each of the readings and by dividing this sum by the actual number of readings (a reading was not taken into account if there was reaction on the control or a global irritation).

$$C.I.I. = \frac{\sum \text{of the grade (erythema + edema)}}{\text{Number of readings}}$$

The irritating potential of the product was estimated by calculating the mean of the reactions observed during the inductionh phase in order to obtain the Mean Cumulative Irritation Index (M.C.I.I.):

$$MCII = CII / \text{nb of subjects}$$

The obtained index allow to arbitrarily classify the studied product according to the following scale:

M.C.I.I.	Class
M.C.I.I. < 0.25	Non irritating (NI)
0.25 ≤ M.C.I.I. < 0.50	Very slightly irritating (VSI)
0.5 ≤ M.C.I.I. < 1	Slightly irritating (SI)
1 ≤ M.C.I.I. < 2	Moderately irritating (MI)
M.C.I.I. ≥ 2	Irritating (I)

Individual values and the product class were taken into account to write a suitable conclusion under the study conditions.

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1.5.5.2. Challenge phase

A possible allergic reaction, during the Induction or Challenge Phase, was rated from 0 to 3 according to ICDRG (International Contact Dermatitis Research Group) – see the table paragraph 1.5.2.2.
During the Challenge Phase, the reading took place 30 minutes after patch-tests removal and 48 hours later.

The sensitizing potential of the product was assessed by the readings on D38 and D40 (Challenge Phase) according to the following criteria: reaction ++ (2) or +++ (3) in the absence of added irritation phenomenon.

The presence of only one case of active sensitizing (**upper or equal score in ++ (2)**) on controlateral side leads to the conclusion "Potentially sensitive product".

2. STUDY FOLLOW-UP

2.1. Studied population

2.1.1. Inclusion

55 healthy subjects were selected according to the inclusion and the non-inclusion criteria, and 51 subjects completed study.
The table below presents the information concerning all the included subjects.

	Non included	Included	Drop out	Untraceable
Number of subjects	0	55	4*	0

Remark: * withdrawal of subject consent

2.1.2. Subjects characteristics

The summary table below presents a synthesis of the observations concerning exclusively the subjects taken into account for data analysis.

Number of subjects	Sex	Age (mean±SEM)	Phototype	Medical or surgical events and medical treatments	
				Before the study	During the study
51	F: 42 M: 9	44 ± 2	III: 11 IV: 40	see Tables in the APPENDIX II	

2.2. Protocol amendment and/or non-adherence

There was no protocol amendment and no non-adherence to the protocol was observed.

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3. RESULTS

3.1. Irritating potential: induction phase

The TABLE OF READINGS regarding the Induction Phase is presented in the APPENDIX III.

The readings done 30 minutes after having removed the patch-tests showed the following results:

Product	Score	D3		D5		D8		D10		D12		D15		D17		D19		D22		Global result
		n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	
S Face IS-1005P	T+	0	0	2	4	7	14	0	0	0	0	1	2	0	0	0	0	0	0	Non-irritating (0.032)
	0:	46	90	42	82	42	82	48	94	45	88	49	96	50	98	49	96	49	96	
	0.5:	5	10	7	14	2	4	3	6	6	12	1	2	1	2	2	4	2	4	
	1:	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	2:	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	3:	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	

n = number of subjects % = % of subjects
T+= positive control

Under these study conditions, product S Face IS-1005P showed a score, so it can be considered as <u>non-irritating.</u>

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3.2. Sensitizing potential: challenge phase

The TABLE OF READINGS regarding the Challenge Phase is presented in APPENDIX IV. These reading made 30 minutes and 48 hours after having removed the patch-tests showed the following results:

Product	Zones	score	Day of the reading				Global result
			D38		D40		
			n	%	n	%	
S Face IS-1005P	Homolateral	T+	2	4	0	0	Non-sensitizing
		0 :	44	86	51	100	
		? :	5	10	0	0	
		+ :	0	0	0	0	
		++ :	0	0	0	0	
		+++ :	0	0	0	0	
	Controlateral	T+	2	4	0	0	
		0 :	45	88	51	100	
		? :	4	8	0	0	
		+ :	0	0	0	0	
		++ :	0	0	0	0	
		+++ :	0	0	0	0	

n = number of subjects % = % of subjects
T+= positive control

Under these study conditions no reaction ++ (2) nor +++ (3) were observed, so the product S Face IS-1005P can be considered non-sensitizing.

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4. CONCLUSION

Under these study conditions, the product S Face IS-1005P can be considered non-irritating and non-sensitizing.

According to these two elements, this product can claim the label "HYPOALLERGENIC".

Bangkok
November 18, 2011

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5. BIBLIOGRAPHY

- COLIPA "Cosmetic product test guidelines for the assessment of human skin compatibility" 2nd edition – August 1997.

- AFSSAPS : "TEST CLINIQUE FINAL DE SECURITE D'UN PRODUIT COSMETIQUE EN VUE DE CONFIRMER SON ABSENCE DE POTENTIEL SENSIBILISANT CUTANE RETARDE : RECOMMANDATIONS AUX PROMOTEURS DE RECHERCHE ET AUX PRESTATAIRES DE SERVICE" - Recommandations TCFS – version finale de décembre 2008.

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APPENDIX I

AUTHENTIFICATION SHEET

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AUTHENTIFICATION PAGE

I am aware that the study N° DA11048-1,

has been conducted according to the STUDY PROTOCOL.

Dr Sujittra NAPATHORN	Date	Signature
Dermatologist	18/11/2011	

Arisa SURINPAO	Date	Signature
Technician	18/11/2011	

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APPENDIX II

SUBJECTS CHARACTERISTICS

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SUBJECTS CHARACTERISTICS

Subject	Name (the 3 first letters)	First name (the 2 first letters)	Age	Sex	Phototype	Skin type	Medical or surgical events and medical treatment	
							before the study	during the study
1	YIM	BA	47	F	IV	N	NO	NO
2	KOM	TA	39	F	III	N	NO	NO
3	SRI	OR	43	F	IV	N	NO	NO
4	RAT	SU	52	F	III	N	NO	NO
5	PRI	WI	59	F	IV	N	NO	NO
6	NAK	CH	45	F	IV	N	NO	NO
7	KIN	TA	64	M	IV	N	NO	NO
8	UPU	KA	45	M	IV	N	NO	NO
(9)*	(PHO)*	(WA)*	(49)*	(F)*	(IV)*	(N)*	(NO)*	(NO)*
10	PHO	PR	43	F	IV	N	NO	NO
(11)*	(PAH)*	(MA)*	(43)*	(F)*	(IV)*	(N)*	(NO)*	(NO)*
12	SIT	NA	57	F	IV	N	NO	NO
13	WAC	KA	65	F	IV	N	NO	NO
14	KAM	PI	63	F	IV	N	NO	NO
15	SOM	NI	18	F	III	N	NO	NO
16	SAI	PE	30	F	IV	N	NO	NO
17	KUN	UP	51	F	IV	N	NO	NO
18	KUN	MA	52	F	IV	N	NO	NO
(19)*	(LOH)*	(CH)*	(59)*	(F)*	(IV)*	(N)*	(NO)*	(NO)*
20	MAN	WA	22	M	IV	N	NO	NO
21	MAN	JI	60	F	IV	N	NO	NO
22	NGA	SA	60	F	IV	N	NO	NO
23	LIM	NO	52	F	IV	N	NO	NO
(24)*	(HMO)*	(KR)*	(49)*	(F)*	(IV)*	(N)*	(NO)*	(NO)*
25	KAM	NO	45	F	IV	N	NO	NO
26	CHO	UN	55	F	IV	N	NO	NO
27	PAR	BU	42	F	IV	N	NO	NO
28	UAN	TI	54	F	IV	N	NO	NO
29	KLO	WA	37	F	IV	N	NO	NO
30	SUW	CH	42	F	IV	N	NO	NO
31	RUN	JA	47	F	III	N	NO	NO
32	HMU	CH	22	F	III	N	NO	NO
33	SAN	SA	56	F	III	N	NO	NO
34	HMU	MA	45	F	IV	N	NO	NO
35	PRA	PH	49	F	IV	N	NO	NO
36	YOD	MA	46	F	IV	N	NO	NO
37	KAM	NI	64	M	IV	N	NO	NO
38	DAN	BA	42	F	IV	N	NO	NO
39	BUN	SU	47	F	IV	N	NO	NO
40	BOR	RA	23	F	IV	N	NO	NO
41	PUN	PR	60	F	IV	N	NO	NO
42	SAN	JA	24	M	IV	N	NO	NO
43	SIT	WA	57	F	III	N	NO	NO
44	CHU	KU	60	F	IV	N	NO	NO
45	NON	SA	48	F	IV	N	NO	NO
46	PON	KO	24	M	IV	N	NO	NO
47	SAK	AR	39	F	IV	N	NO	NO
48	JAI	PR	51	M	IV	N	NO	NO
49	JAI	NO	30	M	IV	N	NO	NO
50	UNP	TA	19	M	IV	N	NO	NO
51	PAN	TA	25	F	III	N	NO	NO
52	PEN	KA	26	F	III	N	NO	NO
53	MUK	PO	47	F	III	N	NO	NO
54	PON	KU	40	F	III	N	NO	NO
55	PRA	WI	30	F	IV	N	NO	NO

Mean	44	42	F	0	I	
Median	46	9	M	0	II	
Minimum	18			11	III	
Maximum	65			40	IV	
SEM	2			0	V	

()*: values not included in data analysis
F = Female
M = Male

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APPENDIX III

TABLES OF THE READINGS – INDUCTION PHASE

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TABLES OF THE READINGS – INDUCTION PHASE

No.of subject	D3		D5		D8		D10		D12		D15		D17		D19		D22	
	Induction site		Induction site		Induction site		Induction site		Induction site		Induction site		Induction site		Induction site		Induction site	
	SFP	C	SFP	C	SFP	C	SFP	C	SFP	C	SFP	C	SFP	C	SFP	C	SFP	C
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
(9)*	(0)*	(0)*	(0)*	(0)*	(0)*	(0)*	(0)*	(0)*	(0)*	(0)*	(0)*	(0)*	(0)*	(0)*	(0)*	(0)*	(0)*	(0)*
10	0	0	0	0	(0)*	(0.5)*	0	0	0	0	0	0	0	0	0	0	0	0
(11)*	(0)*	(0)*	(0)*	(0)*	Drop Out													
12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0.5	0	0	0	0	0	0	0	0	0
15	0	0	0	0	(0)*	(0.5)*	0	0	0	0	(0.5)*	(0.5)*	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17	0	0	0	0	0.5	0	0	0	0	0	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
(19)*	(0)*	(0)*	(0)*	(0)*	(0)*	(0)*	(0)*	(0)*	(0)*	(0)*	(0)*	(0)*	(0)*	(0)*	(0)*	(0)*	(0)*	(0)*
20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
22	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
23	0	0	0	0	(0)*	(0)*	0	0	0.5	0	0	0	0	0	0.5	0	0	0
(24)*	Drop Out																	
25	0.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
26	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
27	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
28	0	0	0	0	(0)*	(0.5)*	0	0	0	0	0	0	0	0	0	0	0	0
29	0	0	(0)*	(0.5)*	(0)*	(0.5)*	0	0	0.5	0	0	0	0	0	0	0	0	0
30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
31	0.5	0	0.5	0	0	0	0.5	0	0.5	0	0	0	0	0	0	0	0.5	0
32	0	0	0.5	0	0.5	0	0	0	0	0	0	0	0	0	0.5	0	0.5	0
33	0.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
34	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
35	0	0	0.5	0	(0.5)*	(0.5)*	0	0	0	0	0	0	0	0	0	0	0	0
36	0	0	0	0	0	0	0	0	0.5	0	0	0	0	0	0	0	0	0
37	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
38	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
39	0.5	0	0.5	0	0	0	0.5	0	0	0	0.5	0	0	0	0	0	0	0
40	0	0	0.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
41	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
42	0.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
43	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
44	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
45	0	0	0.5	0	0	0	0	0	0.5	0	0	0	0	0	0	0	0	0
46	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
47	0	0	0.5	0	(0)*	(0.5)*	0	0	0	0	0	0	0	0	0	0	0	0
48	0	0	0	0	0	0	0.5	0	0	0	0	0	0.5	0	0	0	0	0
49	0	0	(0.5)*	(0.5)*	0	0	0	0	0	0	0	0	0	0	0	0	0	0
50	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
51	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
52	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
53	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
54	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
55	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

()*: values not included in data analysis
SFP = Tested product
C = control

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APPENDIX IV

TABLES OF THE READINGS – CHALLENGE PHASE

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TABLES OF THE READINGS – CHALLENGE PHASE

No.of subject	D38				D40			
	induction site		challenge site		induction site		challenge site	
	SFP	C	SFP	C	SFP	C	SFP	C
1	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0
(9)*	Drop Out							
10	0	0	0	0	0	0	0	0
(11)*	Drop Out							
12	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0
(19)*	Drop Out							
20	0	0	?	0	0	0	0	0
21	0	0	0	0	0	0	0	0
22	0	0	0	0	0	0	0	0
23	0	0	0	0	0	0	0	0
(24)*	Drop Out							
25	0	0	0	0	0	0	0	0
26	0	0	0	0	0	0	0	0
27	?	0	0	0	0	0	0	0
28	0	0	0	0	0	0	0	0
29	(0)*	(?)*	(0)*	(?)*	0	0	0	0
30	0	0	0	0	0	0	0	0
31	?	0	?	0	0	0	0	0
32	0	0	0	0	0	0	0	0
33	0	0	0	0	0	0	0	0
34	0	0	0	0	0	0	0	0
35	0	0	0	0	0	0	0	0
36	0	0	?	0	0	0	0	0
37	0	0	0	0	0	0	0	0
38	0	0	0	0	0	0	0	0
39	(?)*	(?)*	(?)*	(?)*	0	0	0	0
40	0	0	0	0	0	0	0	0
41	0	0	0	0	0	0	0	0
42	0	0	0	0	0	0	0	0
43	0	0	0	0	0	0	0	0
44	0	0	0	0	0	0	0	0
45	?	0	?	0	0	0	0	0
46	0	0	0	0	0	0	0	0
47	0	0	0	0	0	0	0	0
48	0	0	0	0	0	0	0	0
49	?	0	0	0	0	0	0	0
50	0	0	0	0	0	0	0	0
51	0	0	0	0	0	0	0	0
52	0	0	0	0	0	0	0	0
53	?	0	0	0	0	0	0	0
54	0	0	0	0	0	0	0	0
55	0	0	0	0	0	0	0	0

()*: values not included in data analysis
SFP = Tested product
C = control

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Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: Lillian Gill, D.P.A.
Director - COSMETIC INGREDIENT REVIEW (CIR)

FROM: Beth A. Lange, Ph.D.
Industry Liaison to the CIR Expert Panel

DATE: March 25, 2016

SUBJECT: Information on Polyglyceryl-10 Nonaisostearate (SLR posted January 28, 2016)

SafePharm Laboratories. 2004. Acute eye irritation in the rabbit (Polyglyceryl-10 Nonaisostearate).

SafePharm Laboratories. 2004. Acute oral toxicity in the rat - acute toxic class method (Polyglyceryl-10 Nonaisostearate).

Life Science Laboratories, Ltd. 2004. Primary skin irritation study of S-Face IS-1009P (Polyglyceryl-10 Nonaisostearate) in rabbits.

Japan Hair Science Association. 2004. A study report "Patch test on a cosmetic ingredient" (Polyglyceryl-10 Nonaisostearate).

Phycher. 2006. Assessment of photosensitization on the albino guinea pig (Polyglyceryl-10 Nonaisostearate).

Phycher. 2006. Assessment of local cutaneous tolerance after repeated applications for two weeks in guinea pigs (Polyglyceryl-10 Nonaisostearate).

SafePharm Laboratories. 2004. Local lymph node assay in the mouse (Polyglyceryl-10 Nonaisostearate).

SafePharm Laboratories. 2004. Reverse mutation assay "Ames test" using *Salmonella typhimurium* and *Escherichia coli* (Polyglyceryl-10 Nonaisostearate).

Phycher. 2006. Assessment of contact phototoxicity on the albino guinea pig (Polyglyceryl-10 Nonaisostearate)

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SafePharm Laboratories

S-FACE IS-1009P:
Polyglyceryl-10 Nonaisostearate
ACUTE EYE IRRITATION IN THE RABBIT

SPL PROJECT NUMBER: 432/066

AUTHOR: A Pooles

STUDY SPONSOR:

Sakamoto Yakuhin Kogyo Co., Ltd.
1-20, Rinkai-cho
Izumiotsu-city
OSAKA 595-0075
JAPAN

TEST FACILITY:

Safepharm Laboratories Limited
Shardlow Business Park
Shardlow
Derbyshire
DE72 2GD

Telephone: +44 (0) 1332 792896

Facsimile: +44 (0) 1332 799018

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QUALITY ASSURANCE REPORT

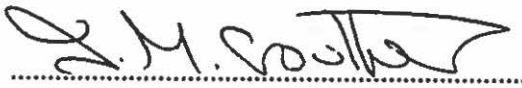
This study type is classed as short-term. The standard test method for this study type ("General Study Plan" in OECD terminology) was reviewed for compliance once only on initial production. Inspection of the routine and repetitive procedures that constitute the study is carried out as a continuous process 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
	16 February 2004	Test Material Preparation
	16 February 2004	Animal Preparation
	16 February 2004	Dosing
	19 February 2004	Assessment of Response
§	05 March 2004	Draft Report Audit
§	Date of QA Signature	Final Report Audit

§ Evaluation specific to this study



..... DATE: 25 MAR 2004

For Safepharm Quality Assurance Unit*

*Authorised QA Signatures:

Head of Department:	JR Pateman CBiol MIBiol DipRQA AIQA FRQA
Deputy Head of Department:	JM Crowther MIScT MRQA
Senior Audit Staff:	JV Johnson BSc MRQA; G Wren ONC MRQA

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GLP COMPLIANCE STATEMENT

The work described was performed in compliance with UK GLP standards (Schedule 1, Good Laboratory Practice Regulations 1999 (SI 1999/3106)). 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 87/18/EEC (as amended by Directive 1999/11/EC) and 88/320/EEC (as amended by Directive 1999/12/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, 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.

A. Pools DATE: 24/3/04

A Pooles BA (Hons)
Study Director

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S-FACE IS-1009P:

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)
- Commission Directive 92/69/EEC Method B5 Acute Toxicity (Eye Irritation)

Result. A single application of the test material to the non-irrigated eye of three rabbits produced minimal to moderate conjunctival irritation. Two treated eyes appeared normal at the 48-hour observation and the remaining treated eye appeared normal at the 72-hour observation.

Conclusion. The test material produced a maximum group mean score of 6.7 and was classified as a mild irritant (Class 4 on a 1 to 8 scale) to the rabbit eye according to a modified Kay and Calandra classification system.

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S-FACE IS-1009P:

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)
- Commission Directive 92/69/EEC Method B5 Acute Toxicity (Eye Irritation)

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 23 February 2004 and 29 February 2004.

2. TEST MATERIAL

2.1 Description, Identification and Storage Conditions

Sponsor's identification	:	S-FACE IS-1009P
Description	:	pale yellow slightly viscous liquid
Batch number	:	31112
Date received	:	04 December 2003
Storage conditions	:	room temperature in the dark

Data relating to the identity, purity and stability of the test material are the responsibility of the Sponsor.

A Certificate of Analysis supplied by the Sponsor is given in Appendix 5.

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.

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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
Undiluted as Supplied	approximately 6.2
90% v/v aqueous preparation of the test material	approximately 6.0
10% w/w aqueous preparation of the test material	10.1

3. METHODS

3.1 Animals and Animal Husbandry

Three New Zealand White rabbits were supplied by David Percival Ltd, Moston, Sandbach, Cheshire, UK. 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 metal cages. Free access to mains drinking water and food (Certified Rabbit Diet (Code 5322) supplied by BCM IPS Limited, London, UK) 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.

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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.

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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.

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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 to moderate conjunctival irritation was noted in all treated eyes one hour after treatment and at the 24-hour observation. Minimal conjunctival irritation persisted in one treated eye at the 48-hour observation.

Two treated eyes appeared normal at the 48-hour observation and the remaining treated eye appeared normal at the 72-hour observation.

6. CONCLUSION

The test material produced a maximum group mean score of 6.7 and was classified as a MILD IRRITANT (CLASS 4 ON A 1 TO 8 SCALE) to the rabbit eye according to a modified Kay and Calandra classification system.

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Table 1 Individual Scores and Individual Total Scores for Ocular Irritation

Rabbit Number and Sex	103 Male				155 Male				3 Male			
	IPR = 1				IPR = 0				IPR = 0			
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	1	0	0	1	2	1	0	1	1	0	0
B = Chemosis	1	0	0	0	1	1	0	0	1	0	0	0
C = Discharge	1	0	0	0	2	1	0	0	1	0	0	0
Score (A + B + C) x 2	6	2	0	0	8	8	2	0	6	2	0	0
Total Score	6	2	0	0	8	8	2	0	6	2	0	0

IPR = Initial pain reaction

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S-FACE IS-1009P : 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
103 Male	6	2	0	0
155 Male	8	8	2	0
3 Male	6	2	0	0
Group Total	20	12	2	0
Group Mean Score	6.7	4.0	0.7	0.0

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S-FACE IS-1009P : 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.

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S-FACE IS-1009P : 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

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S-FACE IS-1009P : 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)

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Appendix 4 Statement of GLP Compliance in Accordance with Directive 88/320/EEC

THE DEPARTMENT OF HEALTH OF THE GOVERNMENT OF THE UNITED KINGDOM

GOOD LABORATORY PRACTICE

STATEMENT OF COMPLIANCE
IN ACCORDANCE WITH DIRECTIVE 88/320 EEC

LABORATORY
SafePharm Limited
Shardlow Business Park,
London Road,
Shardlow,
Derbyshire,
DE72 2GD

TEST TYPE
Analytical/Clinical
Chemistry
Environmental tox.
Environmental fate
Mutagenicity
Phys./Chem. tests
Toxicology

DATE OF INSPECTION

2nd December 2002

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

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

13/2/03

Dr. Roger G. Alexander
Head, UK GLP Monitoring Authority

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Appendix 5 Certificate of Analysis

SAKAMOTO YAKUHIN KOGYO CO.,LTD.

HEAD OFFICE : 1-2-8 AWAJIMACHI,CHUO-KU,OSAKA CITY 541-0047,JAPAN
TEL (81)6-6231-1851 FAX (81)6-6222-0631
TOKYO BRANCH : 1-6-2 MARUNOUCHI,CHIYODA-KU,TOKYO 100-0005,JAPAN
TEL (81)3-3211-3613 FAX (081)3-3215-1789

date : Nov. 27 , 2003

Product : S-Face IS-1009P
Lot. No. : 31112
Quantity : 90g × 1

Certificate of analysis / Quality	Result
Description	A pale yellow viscous liquid with a faint characteristic odor
Color (Gardner)	less than 1
Acid value (mgKOH/g)	0.4
Saponification value (mgKOH/g)	159.6
Heavy metal (ppm)	less than 20
Arsenic (ppm)	less than 2
Residue on ignition (%)	less than 0.1

Y. Noguchi

Yasunori Noguchi
Quality Control Manager

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SAFEPHARM LABORATORIES LTD

S-FACE IS-1009P:

ACUTE EYE IRRITATION IN THE RABBIT

SPL PROJECT NUMBER: 432/066

I verify that this is an exact copy of the original report which is located in the Archives of Safepharm Laboratories Ltd., Derby, UK.

A Poob ..... DATE: 15/4/04

A Pooles BA (Hons)
Study Director

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SafePharm Laboratories

S-FACE IS-1009P:
Polyglyceryl-10 Nona isostearate
ACUTE ORAL TOXICITY IN THE RAT - ACUTE TOXIC CLASS METHOD

SPL PROJECT NUMBER: 432/064

AUTHOR: A Pooles

STUDY SPONSOR:

Sakamoto Yakuhin Kogyo Co., Ltd.
1-20, Rinkai-cho
Izumiotsu-city
OSAKA 595-0075
JAPAN

TEST FACILITY:

Safepharm Laboratories Limited
Shardlow Business Park
Shardlow
Derbyshire
DE72 2GD

Telephone: +44 (0) 1332 792896

Facsimile: +44 (0) 1332 799018

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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:

	07 June 2002	Standard Test Method Compliance Audit
	13, 14 January 2004	Test Material Preparation
	29 January 2004	Animal Preparation
	07 January 2004	Dosing
	12 January 2004	Assessment of Response
	12 January 2004	Necropsy
§	26 February 2004	Draft Report Audit
§	Date of QA Signature	Final Report Audit

§ Evaluation specific to this study

..... DATE: 25 MAR 2004

For Safepharm Quality Assurance Unit*

*Authorised QA Signatures:
Head of Department: JR Pateman CBiol MIBiol DipRQA FRQA
Deputy Head of Department: JM Crowther MIScT MRQA
Senior Audit Staff: JV Johnson BSc MRQA; G Wren ONC MRQA

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GLP COMPLIANCE STATEMENT

The work described was performed in compliance with UK GLP standards (Schedule 1, Good Laboratory Practice Regulations 1999 (SI 1999/3106)). 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 87/18/EEC (as amended by Directive 1999/11/EC) and 88/320/EEC (as amended by Directive 1999/12/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, 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.

A. Pools ..... DATE: 24/3/04

A Pooles BA (Hons)
Study Director

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S-FACE IS-1009P:

ACUTE ORAL TOXICITY IN THE RAT – ACUTE TOXIC CLASS METHOD

SUMMARY

Introduction. The study was performed to assess the acute oral toxicity of the test material following a single oral administration in the Sprague-Dawley CD strain rat. The method was designed to meet the requirements of the following:

- OECD Guidelines for the Testing of Chemicals No. 423 "Acute Oral Toxicity – Acute Toxic Class Method" (adopted 17 December 2001)

Method. A group of three fasted females was treated with the test material at a dose level of 300 mg/kg bodyweight. Based on the results from this dose level further groups of fasted females were treated at a dose level of 2000 mg/kg bodyweight. Dosing was performed sequentially.

The test material was administered orally undiluted for the 2000 mg/kg dose level and as a solution in arachis oil BP for the 300 mg/kg dose level. Clinical signs and bodyweight development were monitored during the study. All animals were subjected to gross necropsy.

Mortality. There were no deaths.

Clinical Observations. There were no signs of systemic toxicity.

Bodyweight. All animals showed expected gains in bodyweight over the study period.

Necropsy. No abnormalities were noted at necropsy.

Conclusion. The acute oral median lethal dose (LD_{50}) of the test material in the female Sprague-Dawley CD strain rat was estimated from the flow chart in Appendix 1 as being greater than 2500 mg/kg bodyweight.

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S-FACE IS-1009P:

ACUTE ORAL TOXICITY IN THE RAT - ACUTE TOXIC CLASS METHOD

1. INTRODUCTION

The study was performed to assess the acute oral toxicity of the test material following a single oral administration in the Sprague-Dawley CD strain rat. The method was designed to meet the requirements of the following:

- OECD Guidelines for the Testing of Chemicals No. 423 "Acute Oral Toxicity – Acute Toxic Class Method" (adopted 17 December 2001)

The rat was selected for this study as it is a readily available rodent species, historically used in safety evaluation studies, and is acceptable to appropriate regulatory authorities. The oral route was selected as the most appropriate route of exposure and the results are believed to be of value in predicting the likely toxicity of the test material to man.

The study was performed between 22 January 2004 and 18 February 2004.

2. TEST MATERIAL AND EXPERIMENTAL PREPARATION

2.1 Description, Identification and Storage Conditions

Sponsor's identification	:	S-FACE IS-1009P
Description	:	pale yellow slightly viscous liquid
Batch number	:	31112
Date received	:	04 December 2003
Storage conditions	:	room temperature in the dark

Data relating to the identity, purity and stability of the test material are the responsibility of the Sponsor.

A Certificate of Analysis supplied by the Sponsor is given in Appendix 3.

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2.2 Preparation of Test Material

For the purpose of the 300 mg/kg dose level the test material was freshly prepared, as required, as a solution at the appropriate concentration in arachis oil BP. Arachis oil BP was used because the test material did not dissolve/suspend in distilled water.

Determination by analysis of the concentration, homogeneity and stability of the test material preparations was not appropriate because it was not specified in the Study Plan and is not a requirement of the Test Guideline.

For the purpose of the 2000 mg/kg dose level the test material was used as supplied. The specific gravity was determined and used to calculate the appropriate dose volume for the required dose level.

3. METHODS

3.1 Animals and Animal Husbandry

Female Sprague-Dawley CD (Crl: CD® (SD) IGS BR) strain rats were supplied by Charles River (UK) Ltd, Margate, Kent, UK. On receipt the animals were randomly allocated to cages. The animals were nulliparous and non-pregnant. After an acclimatisation period of at least five days the animals were selected at random and given a number unique within the study by indelible ink-marking on the tail and a number written on a cage card. At the start of the study the animals were eight to twelve weeks of age. The bodyweights fell within an interval of ± 20% of the mean initial bodyweight of the first treated group.

The animals were housed in groups of three 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 (Certified Rat and Mouse Diet (Code 5LF2) supplied by BCM IPS Limited, London, UK) was allowed throughout the study. The diet, drinking water and bedding were routinely analysed 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. 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.

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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

Using all available information on the toxicity of the test material, 300 mg/kg was chosen as the starting dose.

Groups of fasted animals were treated as follows:

Dose Level (mg/kg)	Specific Gravity	Concentration (mg/ml)	Dose Volume (ml/kg)	Number of Rats
				Female
300	-	30	10	3
2000	0.952	-	2.11	3
2000	0.952	-	2.11	3

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 group and each dose level 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 fourteen 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.

The sequence of dosing may not always follow the Test Guideline as shown in the schematic diagram in Appendix 1. It is Company Policy to minimise the number of animals used on each study in accordance with UK Government Home Office guidelines. The sequence of testing does not affect the final classification of the test material.

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3.3 Evaluation of Data

Data evaluations included the relationship, if any, between the exposure of the animal to the test material and the incidence and severity of all abnormalities including behavioural and clinical observations, gross lesions, bodyweight 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 material was made as shown in the schematic diagram in Appendix 1.

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.

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5. RESULTS

5.1 Mortality Data

Individual mortality data are given in Table 1.

There were no deaths.

5.2 Clinical Observations

Individual clinical observations are given in Table 2 and Table 3.

There were no signs of systemic toxicity.

5.3 Bodyweight

Individual bodyweights and weekly bodyweight changes are given in Table 4 and Table 5.

All animals showed expected gains in bodyweight over the study period.

5.4 Necropsy

Individual necropsy findings are given in Table 6 and Table 7.

No abnormalities were noted at necropsy.

6. CONCLUSION

The acute oral median lethal dose (LD_{50}) of the test material in the female Sprague-Dawley CD strain rat was estimated from the flow chart in Appendix 1 as being greater than 2500 mg/kg bodyweight.

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Table 1 Mortality Data

Dose Level mg/kg	Sex	Number of Animals Treated	Deaths During Day of Dosing (Hours)				Deaths During Period After Dosing (Days)								Deaths
			½	1	2	4	1	2	3	4	5	6	7	8-14	
300	Female	3	0	0	0	0	0	0	0	0	0	0	0	0	0/3
2000	Female	3	0	0	0	0	0	0	0	0	0	0	0	0	0/3
	Female	3	0	0	0	0	0	0	0	0	0	0	0	0	0/3

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Table 2 Individual Clinical Observations - 300 mg/kg

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
300	1-0 Female	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	1-1 Female	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	1-2 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

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Table 3 Individual Clinical Observations - 2000 mg/kg

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
2000	2-0 Female	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2-1 Female	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2-2 Female	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
	3-1 Female	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3-2 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

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Table 4 Individual Bodyweights and Weekly Bodyweight Changes – 300 mg/kg

Dose Level mg/kg	Animal Number and Sex	Bodyweight (g) at Day			Bodyweight Gain (g) During Week	
		0	7	14	1	2
300	1-0 Female	196	222	241	26	19
	1-1 Female	192	216	223	24	7
	1-2 Female	199	234	252	35	18

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Table 5 Individual Bodyweights and Weekly Bodyweight Changes - 2000 mg/kg

Dose Level mg/kg	Animal Number and Sex	Bodyweight (g) at Day			Bodyweight Gain (g) During Week	
		0	7	14	1	2
2000	2-0 Female	207	248	262	41	14
	2-1 Female	204	234	234	30	0
	2-2 Female	200	232	248	32	16
	3-0 Female	190	231	242	41	11
	3-1 Female	210	256	275	46	19
	3-2 Female	212	246	265	34	19

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Table 6 Individual Necropsy Findings - 300 mg/kg

Dose Level mg/kg	Animal Number and Sex	Time of Death	Macroscopic Observations
300	1-0 Female	Killed Day 14	No abnormalities detected
	1-1 Female	Killed Day 14	No abnormalities detected
	1-2 Female	Killed Day 14	No abnormalities detected

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Table 7 Individual Necropsy Findings - 2000 mg/kg

Dose Level mg/kg	Animal Number and Sex	Time of Death	Macroscopic Observations
2000	2-0 Female	Killed Day 14	No abnormalities detected
	2-1 Female	Killed Day 14	No abnormalities detected
	2-2 Female	Killed Day 14	No abnormalities detected
	3-0 Female	Killed Day 14	No abnormalities detected
	3-1 Female	Killed Day 14	No abnormalities detected
	3-2 Female	Killed Day 14	No abnormalities detected

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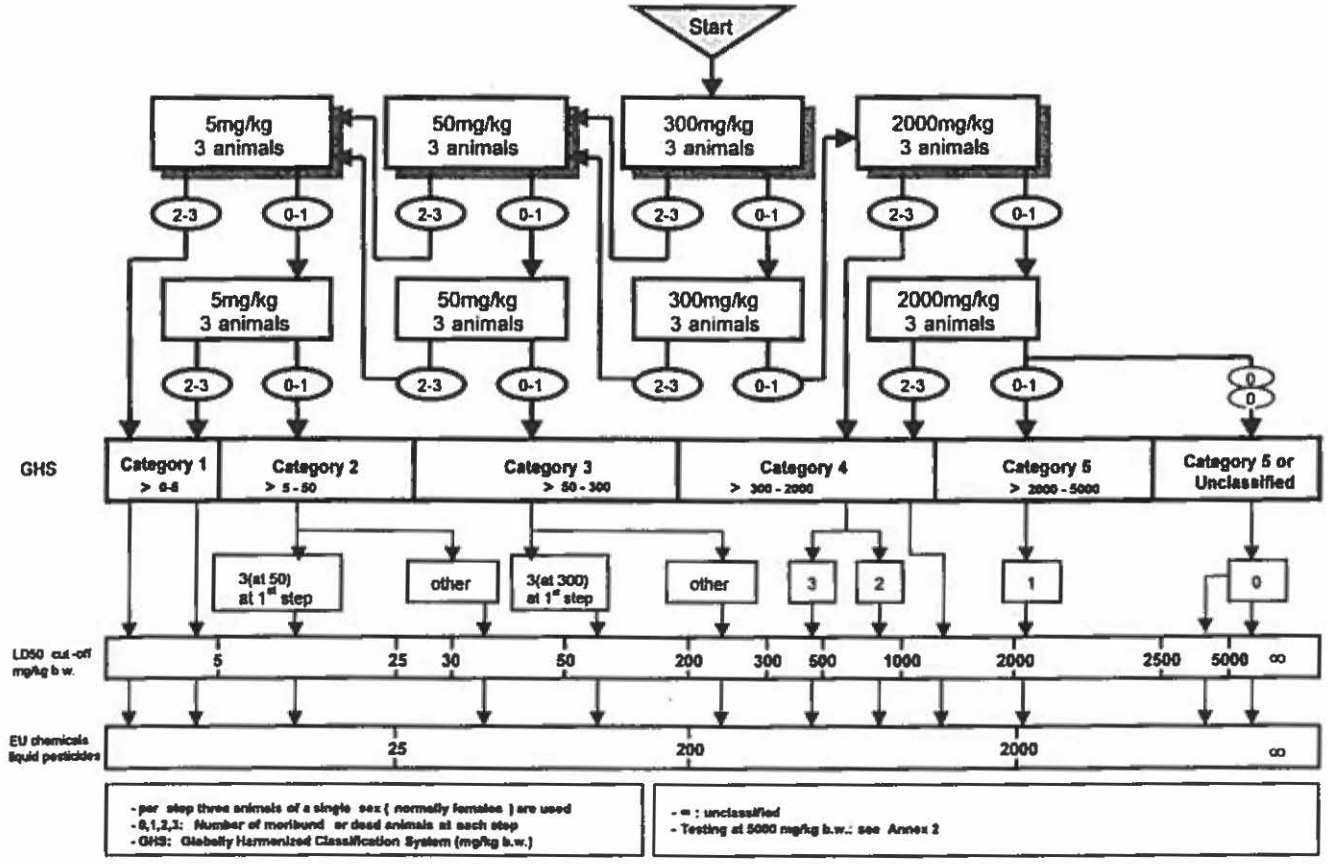
Appendix 1 Test Procedure with a Starting Dose of 300 mg/kg Bodyweight



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Appendix 2 Statement of GLP Compliance in Accordance with Directive 88/320/EEC

THE DEPARTMENT OF HEALTH OF THE GOVERNMENT OF THE UNITED KINGDOM

GOOD LABORATORY PRACTICE

STATEMENT OF COMPLIANCE
IN ACCORDANCE WITH DIRECTIVE 88/320 EEC

LABORATORY
SafePharm Limited
Shardlow Business Park,
London Road,
Shardlow,
Derbyshire,
DE72 2GD

TEST TYPE
Analytical/Clinical
Chemistry
Environmental tox.
Environmental fate
Mutagenicity
Phys./Chem. tests
Toxicology

DATE OF INSPECTION

2nd December 2002

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

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

13/2/03

Dr. Roger G. Alexander
Head, UK GLP Monitoring Authority

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Appendix 3 Certificate of Analysis

SAKAMOTO YAKUHIN KOGYO CO.,LTD.

HEAD OFFICE : 1-2-8 AWAJIMACHI,CHUO-KU,OSAKA CITY 541-0047,JAPAN
TEL (81)6-6231-1851 FAX (81)6-6222-0631
TOKYO BRANCH : 1-8-2 MARUNOUCHI,CHIYODA-KU,TOKYO 100-0005,JAPAN
TEL (81)3-3211-3813 FAX (081)3-3216-1789

date : Nov. 27 , 2003

Product : S-Face IS-1009P
Lot. No. : 31112
Quantity : 90g × 1

Certificate of analysis / Quality

	Result
Description	A pale yellow viscous liquid with a faint characteristic odor
Color (Gardner)	less than 1
Acid value (mgKOH/g)	0.4
Saponification value (mgKOH/g)	159.6
Heavy metal (ppm)	less than 20
Arsenic (ppm)	less than 2
Residue on ignition (%)	less than 0.1

Y. Noguchi
Yasunori Noguchi
Quality Control Manager

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SAFEPHARM LABORATORIES LTD

S-FACE IS-1009P:

ACUTE ORAL TOXICITY IN THE RAT – ACUTE TOXIC CLASS METHOD

SPL PROJECT NUMBER: 432/064

I verify that this is an exact copy of the original report which is located in the Archives of Safepharm Laboratories Ltd., Derby, UK.

A Pooles ..... DATE: 15/4/04

A Pooles BA (Hons)
Study Director

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LIFE SCIENCE LABORATORIES,LTD.
5-19,2-chome,Nishihonmachi
Nishi-ku,OSAKA,JAPAN.
Tel.06-6531-1881

株式会社 生活科学研究所
大阪府大阪市西区西本町2丁目5番19号
Tel.06-6531-1881 (代)

Test Code No. 04-IXA4-0301

STUDY REPORT

Primary skin irritation study of S-Face IS-1009P in rabbits

Polyglyceryl-10 Nonaisostearate

Report issued: April 22, 2004
English translation issued: July 27, 2010

LIFE SCIENCE LABORATORIES, LTD.
New Okazakibashi Bldg.
5-19 Nishihonmachi 2-chome, Nishi-ku,
Osaka 550-0005, Japan
Tel: 06-6531-1881

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04-IXA4-0301

Title

Primary skin irritation study of S-Face IS-1009P in rabbits

Summary

S-Face IS-1009P, loaded on the cloth disc of adhesive plaster for patch test, was applied to the intact and abraded skin of three rabbits by closed patch for 24 hours and its primary skin irritation was observed for 72 hours after application. As control, adhesive plaster only (nothing was loaded on the cloth disc) was applied to the intact and abraded skin in a similar way. Application of the test substance to the intact skin caused very slight erythema in all three animals after 24 hours, and the irritant effect remained still in two animals after 72 hours. Application of the test substance to the abraded skin caused very slight to slight erythema in all three animals after 24 hours. The adverse effect was reduced with time, but very slight erythema remained still in all animals after 72 hours. No edema was observed in all animals by application of the test substance during the observation period. The primary irritation index (P.I.I.) was 1.08 for the test substance and 0.42 for the control (applied only adhesive plaster). According to the criteria by Draize[2], S-Face IS-1009P was considered to be very slightly irritating under our experimental conditions, taking into consideration that very slight erythema was also observed in the control applied only adhesive plaster to the abraded skin.

1. Purpose

As a part of safety evaluation for S-Face IS-1009P, primary skin irritation study was performed using three rabbits with intact and abraded skin to examine whether or not the test substance has a primary skin irritation potential when applied to the skin by closed patch for 24 hours.

This study was conducted by reference to "Guidance for Cosmetic Safety Evaluation 2001 (Edited by Japan Cosmetic Industry Association)"[1] and Draize method[2].

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大阪府大阪市西区西本町2丁目5番19号
Tel 06-6531-1881 (代)

2. Testing Facility

Contract Laboratory:
LIFE SCIENCE LABORATORIES, LTD.
5-19 Nishihonmachi 2-chome, Nishi-ku, Osaka 550-0005, Japan
Testing Facility:
The Second Chihaya-akasaka Laboratory, LIFE SCIENCE LABORATORIES, LTD.
900, Aza-Chihayadani, Chihaya-akasaka-mura, Minamikawachi-gun,
Osaka 585-0051, Japan

3. Sponsor

SAKAMOTO YAKUHIN KOGYO CO., LTD.
1-20 Rinkai-cho, Izumiotsu-City, Osaka Prefecture 595-0075, Japan
Person in charge of the study: Yasunori Noguchi

4. Testing Period

From March 30, 2004 to April 22, 2004

5. Persons Involved in the Study

Study director:
Hitoshi Yamamoto
Persons engaged in the study:
Hitoshi Yamamoto (animal care, administration and observation)
and Nobukazu Matsumura (animal care and observation)

6. Test Substance and Control Substance

1) Test substance

Name:
S-Face IS-1009P
Lot No.:
31112

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大阪府大阪市西区西本町2丁目5番19号
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Chemical Name:

decaglycerol nonaisostearate

Structure:

RO-(-CH_2-CH(OR)-CH_2-O-)n-R

(n=10、R= Hydrogen or Isostearic Acid moiety)

Purity:

100%

Appearance:

Pale yellow clear liquid, slight specific odor

Solubility:

Insoluble in water, soluble in various oils used for cosmetics (such as hydrocarbon, triglyceride, ester oil, etc.)

Stability:

Stable at room temperature

Storage condition:

Store at room temperature

Supplied by:

SAKAMOTO YAKUHIN KOGYO CO., LTD.

1-20 Rinkai-cho, Izumiotsu-City, Osaka Prefecture 595-0075, Japan

Person in charge of the study: Yasunori Noguchi

2) Control substance

Not used.

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7. Preparation of the Test Substance Solution

The test substance was used undiluted for the study as delivered by the sponsor.

8. Stability and Uniformity of the Test Substance under the Conditions of Administration

The test substance was considered to be stable and uniform under the conditions of administration because it was used undiluted.

9. Test System

1) Animals

Four male Japanese white rabbits (conventional) weighing 2.20 to 2.35kg, 9 to 10 weeks of age, were purchased from Hokusetsu Sangyo Co. (20-4 Beppu 1-chome, Settsu-City, Osaka Prefecture 566-0046) on April 5, 2004. After 7 days of acclimation, 3 animals in good conditions with healthy intact skin were used for the study.

2) Procedure for identification

Animals were identified individually by marking of temporary animal numbers on right ears with black felt pen at arrival and of experimental animal numbers on left ears with red felt pen at grouping, and also with cage- and rack-labeling.

3) Grouping

Three animals in good conditions with healthy intact skin were selected randomly from four animals. Their body weights were 2.48 to 2.62kg at the beginning of the experiment.

4) Animal care

Rabbits were housed individually in aluminum bracket cages (320W × 550D × 350Hmm) and maintained in the clean room for middle-sized animals No. 1 (in Building B) at 20 to 23℃ room temperature and 43 to 67% relative humidity with 10 changes or more of room air per hour on an 8-hour light cycle (0900 to 1700 hour). Three times a week the room and cage racks were cleaned and the floor was disinfected with a disinfectant (Neochlor Clean, Shikoku Chemicals Corporation).

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Standard laboratory chow (RC4, Oriental Yeast Co., Ltd.) in feed baskets and tap water (from Chihaya-akasaka-mura water supply) by automatic water system were freely available.

10. Unforeseeable Circumstances That Might Have Ill Effects on the Reliability of the Study and Deviation from the Protocol

There were no unforeseeable circumstances that might have ill effects on the reliability of the study and no deviation from the protocol.

11. Methods

1) Administration

Three rabbits were used for the study. Fur was removed carefully from the dorsal area of the trunk of each animal by clipping with an electric clipper on the day of the test substance application. As shown in Chart, four sites of the dorsal area were designated for the position of the test substance application. The two were abraded by making four epidermal incisions (two perpendicular to twp others like #) with a needle of 18G not so as to injure dermis (abraded skin) and the other two remained intact (intact skin).

A dose of 0.5 mL of the test substance was loaded on the cloth disc (2.5cm in diameter) of adhesive plaster for patch test and applied to the two sites (abraded skin and intact skin) of each animal by closed patch for 24 hours. As control, adhesive plaster only (nothing was loaded on the cloth disc) was applied to the remaining two sites in a similar way. The application sites were secured with hypo-allergenic rayon tape (Dermicel, Johnson & Johnson Medical Inc.).

Applied site No. 1 (intact skin):
The test substance: 0.5 mL
Applied site No. 2 (abraded skin):
The test substance: 0.5 mL
Applied site No. 3 (intact skin):

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Adhesive plaster only
Applied site No. 4 (abraded skin):
Adhesive plaster only

2) Reason for selection of dosage levels, and method, frequency and duration of administration of the test substance

In consideration of skin contact with the test substance, dosage levels, and method, frequency and duration of administration of the test substance were according to the primary skin irritation test by Draize method[2].

3) Observation

Animals were observed for general conditions every day during the observation period and weighed on the day of application and the last day of the observation period (at 72 hours after application).

The patches were removed from the application sites 24 hours after application. Skin reactions including erythema/eschar formation and edema formation were examined at 24, 48 and 72 hours after application. The evaluation criteria of skin reactions were according to Draize method[2] (shown below).

Skin reactions	Grade
A 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) to slight eschar formation (injuries in depth)	4
B Edema formation	
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well defined by definite raising)	2

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Moderate edema (area raised approximately 1mm)	3
Severe edema (raised more than 1mm and extending beyond area of exposure)	4

Photographs of the skin at the applied sites of the test substance and adhesive plaster only were taken at 24, 48 and 72 hours after application.

12. Evaluation

The skin reactions were evaluated by primary irritation index (P. I. I., shown below), namely, the combined average of the averages of the 24- and 72-hour scores (erythema/eschar formation and edema formation) for the intact and for the abraded skin.

Primary irritation index	Evaluation
0 ~ 2	non irritating to mildly irritating
3 ~ 5	moderate irritant
6 ~ 8	severe irritant

13. Results

1) General conditions and body weights

The body weights on the day of application and the last day of the observation period are shown in Table 1.

No abnormal findings were observed in general conditions of all three rabbits and their body weights were increased uneventfully during the observation period.

2) Skin reactions

The scores of skin reactions in the application sites of each rabbit during the observation period (for 72 hours) are shown in Tables 2 to 5 and the primary irritation index (P. I. I.) in Table 6.

(1) Application site No. 1 (intact skin): S-Face IS-1009P (Table 2, Photo 1)

Very slight erythema was observed in all animals at 24 hours after application and disappeared in one animal within 72 hours, but it still remained in the other two

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animals after 72 hours. No edema was found in all animals during the observation period.

(2) Application site No. 2 (abraded skin): S-Face IS-1009P (Table 3, Photo 2)

Very slight to slight erythema was observed in all animals at 24 hours after application. The adverse effect was reduced with time, but very slight erythema remained still in all animals after 72 hours. There was no edema observed in all animals during the observation.

(3) Application site No. 3 (intact skin): adhesive plaster only (Table 4, Photo 3)

No skin irritations such as erythema/eschar and edema were found in all animals through 72 hours after application.

(4) Application site No. 4 (abraded skin): adhesive plaster only (Table 5, Photo 4)

Very slight erythema was found in all animals at 24 hours after application and disappeared in one animal within 72 hours, but it remained still in the other two animals after 72 hours. No edema was formed in all animals during the observation period.

3) Primary irritation index (P. I. I.) (Table 6)

The mean P. I. I. was 1.08 for the test substance (S-Face IS-1009P) and 0.42 for the control (only adhesive plaster).

14. Discussion and Conclusion

S-Face IS-1009P, loaded on the cloth disc of adhesive plaster for patch test, was applied to the intact and abraded skin of three rabbits by closed patch for 24 hours and its primary skin irritation was examined for 72 hours after application. As control, adhesive plaster only (nothing was loaded on the cloth disc) was applied to the intact and abraded skin in a similar way.

There were no abnormal findings in general conditions and body weight gain of all three animals during the observation period.

When S-Face IS-1009P was applied to the intact and abraded skin of three animals, very slight erythema was observed on the intact skin in all animals after 24 hours and still remained in the two animals after 72 hours. Very slight to slight erythema was

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Tel.06-6531-1881

株式会社 生活科学研究所
大阪府大阪市西区西本町2丁目5番19号
Tel.06-6531-1881 (代)

found on the abraded skin in all animals after 24 hours. The adverse effect was reduced with time, but very slight erythema still remained in all animals after 72 hours. There was no edema observed in all animals during the observation period.

On the other hand, when adhesive plaster (as control) was applied to the intact and abraded skin of three animals, no skin irritation was found on the intact skin in all animals, but very slight erythema was observed on the abraded skin in all animals. It disappeared in one animal, but it still remained in the other two animals after 72 hours. No edema was observed on the intact and abraded skin during the observation period.

The mean primary irritation index (P. I. I.) was 1.08 for S-Face IS-1009P and 0.42 for control.

From these results, S-Face IS-1009P was considered to be very slightly irritating according to the criteria of Draize[2] under our experimental conditions, taking into consideration that very slight erythema was also observed on the abraded skin applied only adhesive plaster as control.

15. Archives

The protocol, raw data, documentation records and the study report are retained in the archival room specified by Life Science Laboratory (Room No. 126, 2nd Floor in Okazakibashi Bldg., 9-11 Utsubohonmachi 2-chome, Nishi-ku, Osaka 550-0004, Japan) for 10 years after the completion of the study.

16. References

1) Guidance for cosmetic safety evaluation 2001, Edited by Japan Cosmetic Industry Association, Yakuji Nippo, Ltd., Tokyo (2001) (in Japanese)
2) J. H. Draize, C. Woodard and H. O. Calvery: Methods for the study of irritation and toxicity of substance applied topically to the skin and mucous membranes. J. Pharmacol. Exp. Ther. 82, 377－390 (1944)

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大阪府大阪市西区西本町2丁目5番19号
Tel.06-6531-1881 (代)

04-IXA4-0301

Study director
Hitoshi Yamamoto (retired)
April 22, 2004

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Table 1 Body weight changes after application of S-Face IS-1009P to the skin by closed patch for 24 hours

Experimental Animal No.	Body weight after application (kg)	
	Day of application	After 72 hours
M1	2.51	2.56
M2	2.48	2.53
M3	2.62	2.68
Mean ±SD	2.54 ± 0.074	2.59 ± 0.079

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Table 2 Scores for primary skin irritation in individual rabbits after application of the test substance to the intact skin by closed patch for 24 hours

Application site No. 1 (intact skin) : S-Face IS-1009P

Animal No.	Erythema/eschar formation			Edema formation		
	24 hours	48 hours	72 hours	24 hours	48 hours	72 hours
M1	1	1	1	0	0	0
M2	1	1	1	0	0	0
M3	1	1	0	0	0	0
Total	3	3	2	0	0	0

Table 3 Scores for primary skin irritation in individual rabbits after application of the test substance to the abraded skin by closed patch for 24 hours

Application site No. 2 (abraded skin) : S-Face IS-1009P

Animal No.	Erythema/eschar formation			Edema formation		
	24 hours	48 hours	72 hours	24 hours	48 hours	72 hours
M1	1	1	1	0	0	0
M2	2	1	1	0	0	0
M3	2	1	1	0	0	0
Total	5	3	3	0	0	0

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Table 4 Scores for primary skin irritation in individual rabbits after application of adhesive plaster only to the intact skin by closed patch for 24 hours

Application site No.3 (intact skin) : Adhesive plaster only

Animal No.	Erythema/eschar formation			Edema formation		
	24 hours	58 hours	72 hours	24 hours	48 hours	72 hours
M1	0	0	0	0	0	0
M2	0	0	0	0	0	0
M3	0	0	0	0	0	0
Total	0	0	0	0	0	0

Table 5 Scores for primary skin irritation in individual rabbits after application of adhesive plaster only to the abraded skin by closed patch for 24 hours

Application site No.4 (abraded skin) : Adhesive plaster only

Animal No.	Erythema/eschar formation			Edema formation		
	24 hours	48 hours	72 hours	24 hours	48 hoours	72 hours
M1	1	1	1	0	0	0
M2	1	1	1	0	0	0
M3	1	1	0	0	0	0
Total	3	3	2	0	0	0

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Table 6 Primary irritation index (P. I. I.) of S-Face IS-1009P when applied to the rabbit skin by closed patch for 24 hours

Experimental Animal No.	Test substance S-Face IS-1009P	Control Adhesive plaster only
M1	1.00	0.50
M2	1.25	0.50
M3	1.00	0.25
Mean	1.08	0.42

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-TRANSLATION- A STUDY REPORT "PATCH TEST ON A COSMETIC INGREDIENT"

Nichimokyo No. 15053-(3)
29th January, 2004

To Sakamoto Yakuhin Kogyo Co., Ltd.

Yasushi Watanabe
Chairman of Directors
Japan Hair Science Association
(Incorporated Association)

This report presents the result of patch test on a cosmetic ingredient, performed by Japan Hair Science Association. It was sponsored by Sakamoto Yakuhin Kogyo Co., Ltd. on January 6, 2004.

TEST FOR IRRITATION EFFECTS ON THE COSMETIC INGREDIENT ON HUMAN SKIN

1. Purpose of the test:
This patch test was performed to assess possible irritation potential of a cosmetic ingredient on healthy human skin.
2. Test sample: The supplied sample is the following material.
S FACE IS-1009P Lot# 31112 Polyglyceryl-10 Nonaisostearate
3. Test procedure
(1) Volunteers: 17 males and 18 females (35 people in total), age 19 to 62, were selected as volunteers.
(2) Applied amount: 0.01 g of test sample was applied to each volunteer
(3) Method of patch test:
0.01g of test sample was placed on FINN CHAMBER (EPITEST Ltd. Oy) and applied to the crooked side of upper arm of each volunteer for 24 hours in occlusive condition for evaluation.
At the end of 24 hours exposure period, the test sample was removed. Skin reactions were evaluated 1 hour and 24 hours after removing the test sample.
(4) Criteria for evaluation
The reaction was evaluated using the following ICDRG criteria.

Negative	−: No reaction ±: Very slight erythema
Positive	+ : Well-defined erythema ++: Erythema with swelling or with papule +++: Blisters in addition to the above symptom

Takashi Nagashima, M.D., a Vice-chairman of Directors of Japan Hair Science Association (dermatologist) was in overall charge of the tests and observations reported herein.

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4. Results

The results of this test on 35 volunteers were as shown in the Table. In all volunteers, no reaction was observed in 1 hour after removing the test sample (shown in Table as 24 hours Evaluation time). Similarly, no reaction was observed in 24 hours after removing the test sample (shown in Table as 48 hours Evaluation time), in all volunteers.

5. Conclusion

This test was performed in order to assess possible irritation potential of the test sample on healthy human skin, sponsored by Sakamoto Yakuhin Kogyo Co., Ltd.

No reaction was observed in all volunteers. Therefore, there was concluded that the supplied test sample [S FACE IS-1009P Lot# 31112] has minimal potential to elicit irritative reaction.

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Table Results of patch test

No.	Volunteer	Sex	Age	Evaluation time (hrs.)	Result	Note (anamnesis)
1	S. A.	F	45	24 48	− −	Healthy
2	S. I.	M	51	24 48	− −	Healthy
3	Y. O.	M	20	24 48	− −	Healthy
4	K. O.	M	61	24 48	− −	Healthy
5	K. K.	M	52	24 48	− −	Healthy
6	J. K.	F	30	24 48	− −	Healthy
7	E. K.	F	19	24 48	− −	Healthy
8	A. K.	F	45	24 48	− −	Healthy
9	M. S.	F	29	24 48	− −	Healthy
10	T. S.	F	19	24 48	− −	Healthy
11	I. S.	M	40	24 48	− −	Healthy
12	Y. S.	M	32	24 48	− −	Healthy
13	T. S.	F	57	24 48	− −	Healthy
14	M. T.	F	32	24 48	− −	Healthy
15	J. T.	M	51	24 48	− −	Healthy
16	K. T.	M	51	24 48	− −	Healthy
17	M. T.	F	55	24 48	− −	Healthy
18	K. T.	F	43	24 48	− −	Healthy
19	J. T.	F	40	24 48	− −	Healthy
20	S. T.	F	19	24 48	− −	Healthy
21	Y. N.	M	52	24 48	− −	Healthy
22	T. N.	M	19	24 48	− −	Healthy
23	T. H.	F	31	24 48	− −	Healthy
24	N. B.	F	46	24 48	− −	Healthy
25	M. H.	F	28	24 48	− −	Healthy
26	S. F.	F	33	24 48	− −	Healthy
27	K. H.	M	62	24 48	− −	Healthy
28	S. M.	M	54	24 48	− −	Healthy
29	T. M.	M	32	24 48	− −	Healthy
30	S. M.	F	19	24 48	− −	Healthy
31	M. Y.	F	35	24 48	− −	Healthy
32	T. Y.	M	37	24 48	− −	Healthy
33	S. Y.	M	57	24 48	− −	Healthy
34	S. Y.	M	48	24 48	− −	Healthy
35	Y. Y.	M	49	24 48	− −	Healthy

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ACCORDING TO THE G.L.P.
DESCRIBED in the directive 2004/9/EC
AND C(81)30 council decision O.E.C.D.
BY G.I.P.C. 10/23/98

Phycher
Bio développement

Assessment of photosensitization on the albino guinea pig

Final report: PAC-PH-06/0283
SPL project: 0432/0092

TEST ITEM
S-Face IS-1009P
Batch n° 509103

Polyglyceryl-10 Nonaisostearate

❑ **STUDY MONITOR**	:	**SAFEPHARM Laboratories** **Mr A. POOLES** **P.O. Box 45** **Derby DE1 2BT** **England**
❑ **SPONSOR**	:	**Sakamoto Yakuhin Kogyo Co. Ltd** **1-20, Rinkaicho** **Izumiotsu-city** **OSAKA 595-01075** **Japan**
❑ **AUTHOR**	:	**Mr F. RICHEUX**
❑ **TEST FACILITY**	:	**PHYCHER Bio-Développement** **18, Chemin Lou Tribail** **ZI de Toctoucau** **F-33611 CESTAS Cedex**

Report of 18 pages

Cestas, 06 November 2006

Siège social : 18, chemin de Lou Tribail ZI de Toctoucau 33611 CESTAS Cedex - ✆ 05 57 97 16 80 - Fax 05 57 97 16 81
info@phycher.com - www.phycher.com
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PART 2

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QUALITY ASSURANCE ATTESTATION

I, the undersigned Bernard Benech, Quality Assurance unit of PHYCHER *Bio développement*, attest that the study **PAC-PH-06/0283** was submitted to the inspection of Quality Assurance.

The routine inspections of the toxicity tests performed within the laboratory of PHYCHER *Bio développement*, are carried out as a control process of different major technical phases concerning at least one similar test. The frequency is once a term or more. Dates of technical phases inspections relating to this study type are given below:

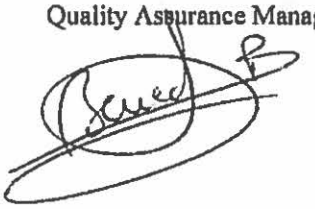
Date of inspection	: 04 October 2006 (PAC-PH-06/0283) *Challenge phase: macroscopic examination at 48 hours*
Date of reporting to Study Director	: 05 October 2006
Date of reporting to Management	: 05 October 2006

This report has been audited by PHYCHER *Bio développement* Quality Assurance unit. It is considered to be an accurate account of the data generated and the application of the operating procedures in use within the laboratory:

	Draft report	Final report
Date of audit report inspection	: 17 October 2006	06 November 2006
Date of reporting to Study Director	: 17 October 2006	06 November 2006
Date of reporting to Management	: 17 October 2006	06 November 2006

Date: 06 November 2006

Bernard BENECH
Quality Assurance Manager



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G.L.P. COMPLIANCE STATEMENT

I, the undersigned, François Richeux, Study director, certify that the study **PAC-PH-06/0283** was performed on the premises of the Laboratory PHYCHER *Bio développement*.

I, certify that the objectives laid down in the technical protocol were achieved and any undesirable event occurred to affect the quality or the integrity of the study. I consider the data generated to be valid. This report fully and accurately reflects the operating procedures used and data generated.

The entire work was devised and performed in compliance with the principles of the Good Laboratory Practice (G.L.P.), as defined in *the directive 2004/9/EC, the O.E.C.D. ruling relative to the mutual acceptance of data in the evaluation of chemical substances (C(81) 30 (final) Appendix 2 - May 12th, 1981; C (97) 186, November 26th, 1997)*, and in the French decree *n° 98-1312 dated December 31st, 1998 of the Journal Officiel de la République Française (see certificate of G.L.P. in appendix).*

Date: 06 November 2006

Dr. François Richeux
Study Director
Expert Toxicologist (PhD, ERT)

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SUMMARY AND CONCLUSION OF THE STUDY

In order to assess the photoallergenic potential of **S-Face IS-1009P** a photoallergenicity test was performed in 17 female albino Dunkin-Hartley guinea pigs (11 test and 6 control) in accordance with *Ichikawa H., Armstrong RB. and Harber LC. J. Invest. Dermatol. 1981 : 76(6).p. 498-501. Maurer TH. Photodermatol. 1984. 1 p.221-231. Lovell W.W. and Sanders D.J. J. Intern. J. Sci. 1988. 10, p271-279.*

For the induction of senzitisation of the treated group, 0.5 mL of the test item **S-Face IS-1009P** was applied to an interscapular skin area of about 25cm² (marked previously with two pair of intradermal injections of the Freund's Complete Adjuvant/physiological saline solution at 50%). The test sites were then exposed to 7 J/cm² UV-A irradiation. This was repeated two more times with 2 days intervals within one week of the induction phase. Control animals were performed according to the same experimental conditions, with the control item (liquid paraffin).

16 days after the last induction, a challenge was performed by treating the experimental animals (test and control) on about 50 cm² (dorso-lumbar zone) with 0.5 mL of the test item at 100% on one flank while the opposite flank remained untreated. After 30 min, both flanks were exposed to 7 J/cm² UVA irradiation.

Cutaneous reactions, i.e. erythema and oedema formation were evaluated 24 and 48 h after the challenge.

No macroscopic cutaneous reactions attributable to photosensitization were recorded during the examination following the challenge phase.

In view of these results, under these experimental conditions, we are able to conclude that the photosensitization reaction risk is minim with the test item **S-Face IS-1009P** at 100%. Moreover, in view of these results, the test item **S-FACE IS-1009P** belongs to the category **class 0, no photosensitising potential.**

Date: 06 November 2006

Scientific Direction PHYCHER *Bio développement*

Dr. Christian TOUSSAINT
Pharmacologist
(Hdr)

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TEST REPORT

1 - TEST ITEM

- Sponsor's identification: **S-Face IS-1009P**
- Container : plastic flask (n=1)
- Quantity : 128.37 g (container + contents)
- Batch n° : 509103
- CAS n° : 83138-62-9
- Composition: Polyglyceryl-10 Nonaisostearate
- Date received: 16 August 2006
- Form : liquid
- Colour : pale yellow
- Storage : Room temperature
- Purity : >99%
- Expiry date: -

It was identified under the code number: **PH-06/0283.**

The test item was considered as 100% for the study.
Informations concerning the identity, purity and stability of the test item are the responsibility of the Sponsor. An information data sheet concerning the item was provided by the study monitor.

2 - METHODOLOGY

The experimentation phase was carried out between 04 September 2006 and 04 October 2006, in *PHYCHER Bio Développement*– 18 chemin de Lou Tribail – 33611 Cestas Cedex according to the experimental protocol *Ref. PAC-version September 1999,* established in accordance with:
Ichikawa H., Armstrong RB. and Harber LC. J. Invest. Dermatol. 1981: 76(6).p. 498-501. Maurer TH. Photodermatol. 1984. 1 p.221-231. Lovell W.W. and Sanders D. J. Intern. J. Sci. 1988. 10, p271-279.

2.1 – Animals

For the main study, seventeen female albino guinea-pigs of Dunkin-Hartley strain, supplied from by Charles RIVER (F-69592 L'Arbresle) were exposed to the test item after of at least a 5-day acclimatisation period. The animals weighed between 308 g and 353 g at the beginning of the test and were 5 weeks old.

2.1.1 - Preliminary test

Determination of the Maximal Non Irritant Concentration (MNIC):
Three female guinea pigs identified C6102 & C6104 were used.

After inductions by topical application with liquid paraffin and a 16-day rest phase, the challenge phase consists in a single topical application of the test item at the following concentrations: 100%, diluted at 50% (v/v) and 25% (v/v) in liquid paraffin, 30 minutes before UV-A irradiation. In the same time, an irradiated site on the left flank, without any item, served as control.

2.1.2 - Main study

GROUP 1 (negative control) : 6 female guinea pigs identified n° C6142 to C6146 & C6158

GROUP 2 (treated) : 11 female guinea pigs identified n° C6147 to C6157.

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The environmental parameters for the main study were:
- temperature : between 19 °C and 27 °C
- relative humidity : between 42% and 70%

2.2 – Chronological development

Preliminary test

24 August 2006	Arrival of animals
04 Sept 06 to 26 Sept 06	Preliminary test (MNIC determination)

Main study

31 August 2006	Arrival of animals Induction phase (Group 2)
11 September 2006	1st induction: topical application of the test item at 100% on the scapular area 30 minutes before the irradiation (7 J/cm² UV-A).
11 September 2006	Weighing of the animals
13 September 2006	2nd induction: topical application of the test item at 100% on the scapular area 30 minutes before the irradiation (7 J/cm² UV-A).
15 September 2006	3rd induction: topical application of the test item at 100% on the scapular area 30 minutes before the irradiation (7 J/cm² UV-A).
16 Sept 06 to 01 Oct 06	Rest phase: 16 days
02 October 2006	Challenge phase (Groups 1 & 2): topical application of the test item at 100% on one flank, 30 minutes before the irradiation (7 J/cm² UV-A).
03 October 2006	24-hour reading time
04 October 2006	48-hour reading time and weighing

3 - RESULTS

3.1 – Concentration selected

Preliminary test:

MNIC determination

24 hours after the challenge phase, no cutaneous reaction was recorded (*table 1*, page 9).
In view of this result, the concentration selected was 100% (MNIC) for the challenge phase.

Main study

Induction phase

The test item was topically applied at 100%, 30 minutes before UV-A irradiation to an interscapular skin area (about 25 cm²), at D0, D2 and D4, as planned in the experimental protocol, paragraph 4.5.2.
No cutaneous reaction was recorded, during the induction phase.

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Challenge phase

The test item was topically applied, at 100%, on about 50 cm² (dorso-lumbar zone) on one flank of the animals while the opposite flank remained untreated. After 30 minutes, both flanks were exposed to 7 J/cm² UV-A irradiation.

3.2 – Photosensitization potential assessment

Overall results (readings at 24 and 48 hours) are given in *table 2* (page 10).
Individuals scores of macroscopic evaluations performed during challenge phase are given in *table 3* (page 11).

No macroscopic cutaneous reaction attributable to photosensitization was recorded during the examinations following the challenge phase.

3.3 – Weight evolution

The weight gain of control animals (Group 1) and treated animals (Group 2) are respectively presented in *tables 4 and 5*, (pages 12 and 13).

Not any abnormality was recorded in the weight gain of both groups.

3.4 – Mortality

No mortality occurred during the main study.

4 - CONCLUSION

In view of these results, under these experimental conditions, we are able to conclude that the photosensitization reaction risk is minim with the test item **S-Face IS-1009P** at 100%. Moreover, in view of these results, the test item **S-Face IS-1009P** belongs to the category **class 0, no photosensitising potential.**

5 - ARCHIVES

All the original data related and the final report related to the study will be stored for a period of 10 years in the company under the reference **PAC-PH-06/0283**. After this period, the sponsor's instructions will be applied.

6 - PROTOCOL ADHERENCE

Number of animals: in accordance with the animal welfare, the preliminary study has been carried out in a first step with 3 animals instead of 6 animals as planned in the experimental protocol.
Environmental parameter: a temperature of 27°C was registered during this test instead of 25°C (maximal limit) as planned in the experimental protocol.

These deviations did not, in any case, influence the development and the results of the study.

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PHOTOSENSITIZATION TEST: Preliminary test

TEST ITEM: S-Face IS-1009P

Application: topical

Application date (D0): 04 September 2006 Animals: 3

Table 1

MNIC DETERMINATION

Macroscopic evaluation of cutaneous reactions (erythema and Oedema)

Vehicle: liquid paraffin

Application mode	N° of ANIMALS	CONCENTRATIONS					
		100%		50%		25%	
		Er	Oe	Er	Oe	Er	Oe
Topical application 30 minutes before UV-A irradiation	C6102	0	0				
	C6103			0	0		
	C6104					0	0

Maximal Non Irritant Concentration	MNIC	100%

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PHOTOSENSITIZATION TEST: Main study

TEST ITEM: S-Face IS-1009P

Application date (D0): 11 September 2006

Animals: 17
- Negative control group: 6
- Treated group: 11

Table 2

ASSESSMENT OF THE PHOTOSENSITIZATION POTENTIAL

Overall results:
Macroscopic evaluation (readings at 24 and 48 hours) of cutaneous reactions

Groups	Reading time	Concentrations	QUOTATIONS				% of positive responses ≥1	% of animal sensitized
			0	1	2	3 or >		
Negative Control Group	24 h	100%	6	0	0	0	0%	
	48 h	100%	6	0	0	0	0%	
Treated Group	24 h	100%	11	0	0	0	0%	0%
	48 h	100%	11	0	0	0	0%	0%

Grading scales:

Erythema
0.....No visible modification
1.....Slight or patches of erythema
2.....Moderate confluent erythema
3.....Internal erythema and swelling

Oedema
0..... No visible modification
1..... Slight oedema
2..... Moderate oedema
3..... Important oedema

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PHOTOSENSITIZATION TEST: Main study

TEST ITEM: S-Face IS-1009P

Application date (D0): 11 September 2006

Animals: 17
- Negative control group: 6
- Treated group: 11

Table 3

ASSESSMENT OF THE PHOTOSENSITIZATION POTENTIAL

Individual results:
Macroscopic evaluation (readings at 24 and 48 hours) of cutaneous reactions (Erythema + Œdema)

ANIMALS No			IRRADIATED AREAS – Treated area			
			24 hours		48 hours	
GROUP 1 (Control)			Erythema	Oedema	Erythema	Oedema
N° C	6142	F	0	0	0	0
N° C	6143	F	0	0	0	0
N° C	6144	F	0	0	0	0
N° C	6145	F	0	0	0	0
N° C	6146	F	0	0	0	0
N° C	6158	F	0	0	0	0
GROUP 2 (Treated)			Erythema	Oedema	Erythema	Oedema
N° C	6147	F	0	0	0	0
N° C	6148	F	0	0	0	0
N° C	6149	F	0	0	0	0
N° C	6150	F	0	0	0	0
N° C	6151	F	0	0	0	0
N° C	6152	F	0	0	0	0
N° C	6153	F	0	0	0	0
N° C	6154	F	0	0	0	0
N° C	6155	F	0	0	0	0
N° C	6156	F	0	0	0	0
N° C	6157	F	0	0	0	0

Treated area: pure test item + UV-A irradiation
F: Female

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PHOTOSENSITIZATION TEST: Main study

TEST ITEM: S-Face IS-1009P

Application date (D0): 11 September 2006 Animals: 6

Table 4

Weight evolution
(in grams)

GROUP 1 – CONTROL

ANIMALS	SEX	Beginning of the study	End of the study	Body weight Gain
N°C 6142	F	324	406	82
N°C 6143	F	327	405	78
N°C 6144	F	330	438	108
N°C 6145	F	313	418	105
N°C 6146	F	330	427	97
N°C 6158	F	330	404	74
MEAN		**325.7**	**416.3**	**90.7**
Standard deviation		**6.7**	**14.0**	**14.6**

F: Female

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PHOTOSENSITIZATION TEST: Main study

TEST ITEM: S-Face IS-1009P

Application date (D0): 11 September 2006 Animals: 11

Table 5

Weight evolution
(in grams)

GROUP 2 – TREATED

ANIMALS	SEX	Beginning of the study	End of the study	Body weight Gain
N°C 6147	F	320	415	95
N°C 6148	F	347	450	103
N°C 6149	F	334	434	100
N°C 6150	F	330	419	89
N°C 6151	F	338	434	96
N°C 6152	F	322	430	108
N°C 6153	F	353	446	93
N°C 6154	F	308	401	93
N°C 6155	F	309	400	91
N°C 6156	F	334	469	135
N°C 6157	F	340	474	134
MEAN		**330.5**	**433.8**	**103.4**
Standard deviation		**14.5**	**24.6**	**16.3**

F: Female

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APPENDICES

Appendix 1: G.L.P. Certificate (1 page)

Appendix 2: Animals diet sheet (1 page r/v)

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Appendix 1: G.L.P. Certificate (1 page)

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Liberté • Égalité • Fraternité
RÉPUBLIQUE FRANÇAISE

GROUPE INTERMINISTERIEL DES PRODUITS CHIMIQUES

Paris, le 13 DEC. 2005

OBJET : Evaluation de la conformité aux B.P.L. selon la directive 88/320/C.E.E.

Consécutivement à votre engagement vis à vis du GIPC et du COFRAC et en application du décret n° 81-278 du 25 mars 1981 portant création d'un groupe interministériel des produits chimiques, modifié notamment par le décret 90-206 du 7 mars 1990 et par le décret n° 98-1312 du 31 décembre 1998 concernant les bonnes pratiques de laboratoires, je vous confirme que le GIPC, au vu des résultats du contrôle exercé par le Comité français d'accréditation (COFRAC) - Section Laboratoires a décidé pour votre installation du statut suivant :

respect des principes de B.P.L.
Domaines de reconnaissance :
☐ 2 - **études de toxicité**
☐ 3 - **études de mutagénicité**

Date d'inspection : 3 octobre 2005

☐ **inspection de contrôle périodique (i.p)**
☐ **inspection d'extension (i.e)**

Date de décision du G.I.P.C. : 9 décembre 2005
Date de prise d'effet : 3 octobre 2005
Année de première conformité : 1998
Durée de validité : 18 mois

Le Président, P. O.

Pierre CREYSSEL
Conseiller d'Etat h.

PHYCHER BIO Developpement
ZI de Toctoucau
18, chemin de Lou Tribail
33611 CESTAS Cedex

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ACCORDING TO THE G.L.P.
DESCRIBED in the directive 2004/9/EC
AND C(81)30 council decision O.E.C.D.
BY G.I.P.C. 10/23/98

Phycher
Bio développement

Assessment of local cutaneous tolerance after repeated applications for two weeks in guinea pigs

Study Report: TCL-PH-06/0283
SPL project: 0432/0090

TEST ITEM
S-Face IS-1009P
Batch n°: 509103

Polyglyceryl-10 Nonaisostearate

❑ **STUDY MONITOR**	:	**SAFEPHARM Laboratories** **Mr A. POOLES** **P.O. Box 45** **Derby DE1 2BT** **England**
❑ **SPONSOR**	:	**SAKAMOTO Yakuhin Kogyo Co., Ltd** **1-20, Rinkaicho** **Izumiotsu-city** **OSAKA 595-01075** **Japan**
❑ **AUTHOR**	:	**Mr F. RICHEUX**
❑ **TEST FACILITY**	:	**PHYCHER Bio-Développement** **18, Chemin Lou Tribail** **ZI de Toctoucau** **F-33611 CESTAS Cedex**

Report of 22 pages **Cestas, 12 December 2006**

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Appendix 2: Animals diet sheet (1 page r/v)	Page 21

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QUALITY ASSURANCE ATTESTATION

I, the undersigned Bernard Benech, Quality Assurance unit of PHYCHER *Bio développement*, attest that the study **TCL-PH-06/0283** was submitted to the inspection of Quality Assurance.

The routine inspections of the toxicity tests performed within the laboratory of PHYCHER *Bio développement*, are carried out as a control process of different main technical phases concerning at least one similar test. The frequency is once a term or more. Dates of technical phases inspections relating to this study type are given below:

Date of inspection	: 20 September 2006 (TCL-PH-06/0283) *Macroscopic examinations ant treatment at D1*
Date of reporting to Study Director	: 22 September 2006
Date of reporting to Management	: 22 September 2006

This report has been audited by PHYCHER *Bio développement* Quality Assurance unit. It is considered to be an accurate account of the data generated and the application of the operating procedures in use within the laboratory:

	Draft report	Final report
Date of inspection	: 09 November 2006	12 December 2006
Date of reporting to Study Director	: 09 November 2006	12 December 2006
Date of reporting to Management	: 09 November 2006	12 December 2006

Date: 12 December 2006

Bernard BENECH
Quality Assurance Manager

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G.L.P. COMPLIANCE STATEMENT

I, the undersigned, François Richeux, Study director, certify that the study **TCL-PH-06/0283** was performed on the premises of the Laboratory PHYCHER *Bio développement.*

I, certify that the objectives laid down in the technical protocol were achieved and no undesirable event occurred to affect the quality or the integrity of the study. I consider the data generated to be valid. This report fully and accurately reflects the operating procedures used and data generated.

The entire work was devised and performed in compliance with the principles of the Good Laboratory Practice (G.L.P.), as defined in *the directive 2004/9/EC, the O.E.C.D. ruling relative to the mutual acceptance of data in the evaluation of chemical substances (C(81) 30 (final) Appendix 2 - May 12th, 1981; C (97) 186, November 26th, 1997)*, and in the french decree *n° 98-1312 dated December 31st, 1998 of the Journal Officiel de la République Française (see certificate of G.L.P. in appendix).*

Date: 12 December 2006

Dr. François Richeux
Study director
Expert Toxicologist (PhD, ERT)

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SUMMARY AND CONCLUSION OF THE STUDY

The test item **S-Face IS-1009P**, has been applied as supplied, on a site of the back level of 8 albino Guinea-pigs skin (4 males and 4 females), during 14 consecutive days. In parallel, a control site has been treated with the control item (liquid paraffin); each animal is his own control. This test was performed in accordance with *Japanese Ministry of Health and Welfare, Pharmaceutical Affairs Bureau, Toxicity test guidelines, 1989.*

No clinical sign due to a systemic toxicity after a possible transdermal passage of the item was observed during the 2 weeks of observation.
The body weight evolution of the animals remained normal throughout the study.

No macroscopic cutaneous reaction (erythema, oedema) was noted after the daily application for 14 days, on the control area which received liquid paraffin.

It was only noted a slight erythema on the treated area, in three animals, the 2nd day of the test. These reactions were totally reversible the 3rd day of the test.

The maximum weekly irritation index was 0.06, at the level of the treated area.

In conclusion, in view of these results, under these experimental conditions, the item **S-Face IS-1009P** is **practically non irritant** to the skin, in accordance with the classification.

Date: 12 December 2006

Scientific Direction PHYCHER *Bio développement*

Dr. Christian TOUSSAINT
Pharmacologist
(Hdr)

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TEST REPORT

1 - TEST ITEM

- Sponsor's identification: **S-Face IS-1009P**
- Container : plastic flask (n=1)
- Quantity : 128.37 g (container + contents)
- Batch n° : 509103
- CAS n° : 83138-62-9
- Composition: Polyglyceryl-10 Nonaisostearate
- Date received: 16 August 2006
- Form : liquid
- Colour : pale yellow
- Storage : Room temperature
- Purity : >99%
- Expiry date: -

It was identified under the code number: **PH-06/0283.**

The test item was considered as 100% for the study.
Informations concerning the identity, purity and stability of the test item are the responsibility of the Sponsor. An information data sheet concerning the item was provided by the study monitor.

2 - METHODOLOGY

The test was carried out between 19 September 2006 and 03 October 2006 in *PHYCHER Bio développement - ZI de Toctoucau - 18 chemin de Lou Tribail - 33611 Cestas Cedex*, according to the experimental protocol *Ref. TCL-version August 2005*, established according to *Japanese Ministry of Health and Welfare, Pharmaceutical Affairs Bureau, Toxicity test guidelines, 1989.*

2.1 - Animals

Hight albino guinea pigs (4 males and 4 females) of Dunkin-Hartley strain, supplied by Charles RIVER (F-69592 L'Arbresle) were exposed to the test item after an acclimatisation period of at least five days. For the main study, the animals weighed between 260 g and 303 g (males) and between 266 g and 282 g (females) at the beginning of the test.

A unique group of 8 animals has been constituted; each animal is his own control.

- 4 males identified n° C6194 to C6197
- 4 females identified n° C6198 to C6201

The environmental parameters were:

- Temperature: between 20 °C and 25 °C
- Relative humidity: between 38% and 61%

2.2 - Treatment

All the dorsal surface area of the animals were clipped and shaved at D-1 then at D3, D7, D10 and D13.

A volume of 50 µl of the test item is applied daily on predefined area (2 cm x 2 cm) by gentle digital massage on the right flank.
The control item (paraffin oil) was applied under the same experimental conditions on the left flank.

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2.3 - Grading of reactions

Each day, the skin reactions were appreciated using the following scales:

Grading scales :

Erythema and Eschar formation	Oedema
0 .....No erythema	0.....No oedema
1 .....Slight (barely perceptible) erythema	1.....Very slight (barely perceptible) oedema
2 .....Definite erythema	2.....Slight oedema (contour clearly defined)
3 .....Moderate to severe erythema	3.....Moderate oedema (thickness approx. 1mm)
4 .....Severe erythema (purple) with formation of eschars (deep lesions) preventing erythema from being grading	4.....Severe oedema (thickness greater than 1mm, an surface larger than zone of application)

2.4 - Chronological development

14 September 2006 : Arrival of animals

D_{-1}: 18 Sept 06 : Clipping and shaving
D_0 : 19 Sept 06 : Photographs, scoring, 1st treatment, and weighing
D_1 : 20 Sept 06 : Scoring, treatment
D_2 : 21 Sept 06 : Scoring, treatment
D_3 : 22 Sept 06 : Scoring, treatment, clipping and shaving 6 hours after the treatment
D_4 : 23 Sept 06 : Photography, scoring, treatment
D_5 : 24 Sept 06 : Scoring, treatment
D_6 : 25 Sept 06 : Scoring, treatment
D_7 : 26 Sept 06 : Scoring, treatment, weighing, clipping and shaving 6 after the treatment
D_8 : 27 Sept 06 : Photography, scoring, treatment
D_9 : 28 Sept 06 : Scoring, treatment
D_{10}: 29 Sept 06 : Scoring, treatment, clipping and shaving 6 hours after the treatment
D_{11}: 30 Sept 06 : Photography, scoring, treatment
D_{12}: 01 Oct 06 : Scoring, treatment
D_{13}: 02 Oct 06 : Scoring, treatment, clipping and shaving 6 hours after the treatment
D_{14}: 03 Oct 06 : Photography, scoring 24 hours after the treatment, weighing and euthanasia of the animals

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3 - RESULTS

3.1 - Clinical examinations

No clinical sign due to a systemic toxicity after a possible transdermal passage of the item was observed during the 2 weeks of observation.

3.2 - Weight evolution

The individual results, mean and standard deviation of animals is respectively presented in *table 1 (page 10)*.

The weight evolution has been normal for this strain.

3.3 - Macroscopic examinations of treated areas (test item, control item)

It was only noted a slight erythema on the treated area, in three animals, the 2nd day of the test. These reactions were totally reversible the 3rd day of the test.
The presented photographs (pages 13-17) for the animal C6197 were performed immediately before the treatment on D0, D4, D8, D11 and D14.

4 - INTERPRETATION OF CUTANEOUS TOLERANCE RESULTS

Individual results of the daily macroscopic evaluation, daily irritation indices and weekly irritation indices were presented in *tables 2 to 5 (pages 11 to 14)*.

In accordance with the calculation retain (as planned in the experimental protocol)

The mean weekly irritation indices were at the level of the site 1 (control area with paraffin oil):

- 0 (W.I.I.1) the first week and 0 the second week (W.I.I.2).

The maximum weekly irritation indices were at the level of the control area with paraffin oil:

- **0 (W.I.I.max) as in the first week than in the second one**

The mean weekly irritation indices were at the level of the site 2 (treated area with test item)

- 0.06 (W.I.I.1) the first week and 0 the second week (W.I.I.2).

The maximum weekly irritation index was at the level of the treated area:

- **0.06 (W.I.I.max) as in the first week**

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5 - CONCLUSION

In view of these results, under these experimental conditions, the item **S-Face IS-1009P is practically non irritant** to the skin, in accordance with the classification.

6 - ARCHIVES

All the original data related and the final report related to this study will be stored for a period of 10 years in the company under the reference **TCL-PH-06/0283**. After this period, the Sponsor's instructions will be applied.

7 - PROTOCOL ADHERENCE

Number of animals: The study has been carry out with 8 animals instead at 6 animals has planned in the experimental protocol.

This deviation did not, in any case, influence the development and the results of the study.

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ASSESSMENT OF LOCAL CUTANEOUS TOLERANCE

TEST ITEM: S-Face IS-1009P

Application: 50 µL per day

D0: 19 September 2006 Animals: 6

Table 1

WEIGHT EVOLUTION
(in grams)

ANIMALS	SEX	Beginning of the test	End of the test	Body Weight gain
N°C 6194	M	303	433	130
N°C 6195	M	287	396	109
N°C 6196	M	282	418	136
N°C 6197	M	260	355	95
MEAN		**283.0**	**400.5**	**117.5**
Standard-deviation		**17.8**	**33.9**	**18.9**
N°C 6198	F	282	372	90
N°C 6199	F	266	368	102
N°C 6200	F	270	373	103
N°C 6201	F	266	341	75
MEAN		**271.0**	**363.5**	**92.5**
Standard-deviation		**7.6**	**15.2**	**13.1**

F: Female
M: Male

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ASSESSMENT OF LOCAL CUTANEOUS TOLERANCE

CONTROL ITEM: PARAFFIN OIL

Application: 50 µL per day

D0: 19 September 2006

Table 2
Macroscopic evaluation of cutaneous reactions on left flank – Site 1

	Animals	Days 1	2	3	4	5	6	7	8	9	10	11	12	13	14
ERYTHEMA	C6194	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	C6195	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	C6196	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	C6197	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	C6198	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	C6199	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	C6200	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	C6201	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OEDEMA	C6194	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	C6195	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	C6196	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	C6197	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	C6198	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	C6199	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	C6200	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	C6201	0	0	0	0	0	0	0	0	0	0	0	0	0	0

D.I.I.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
W.I.I.	**W.I.I. 1= 0**							**W.I.I. 2= 0**							

W.I.I. max. = 0

Classification: Non irritant

D.I.I. : **Daily Irritation Index**
W.I.I. : **Weekly Irritation Index**
W.I.I. max. : **Maximum Weekly Irritation Index**

Note
Weekly Mean irritation indices (W.I.I.) are calculated from the seven daily indices of each week. The maximum weekly mean irritation index (max. W.I.I.) is used to arbitrary classify the test item according to the following table:

Max. W.I.I.	CLASSIFICATION
0	Non irritant
>0	Practically Non irritant
>0.5	Slightly irritant
>2	Irritant
>5	Very irritant

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ASSESSMENT OF LOCAL CUTANEOUS TOLERANCE

TEST ITEM: S-Face IS-1009P

Application: 50 µL per day

D0: 19 September 2006

Table 3

Macroscopic evaluation of cutaneous reactions on the right flank – Site 2

	Animals	Days 1	2	3	4	5	6	7	8	9	10	11	12	13	14
ERYTHEMA	C6194	0	1	0	0	0	0*	0*	0*	0*	0*	0*	0*	0	0
	C6195	0	0	0	0	0	0*	0*	0	0	0	0	0	0	0
	C6196	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	C6197	0	1	0	0	0	0*	0*	0*	0*	0*	0*	0*	0	0
	C6198	0	0	0	0	0	0*	0*	0	0	0	0*	0*	0	0
	C6199	0	0	0	0	0	0*	0*	0	0	0	0	0	0	0
	C6200	0	1	0	0	0	0*	0*	0	0	0	0	0	0	0
	C6201	0	0	0	0	0	0*	0*	0	0	0	0	0	0	0
OEDEMA	C6194	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	C6195	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	C6196	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	C6197	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	C6198	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	C6199	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	C6200	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	C6201	0	0	0	0	0	0	0	0	0	0	0	0	0	0

(*) : dryness

D.I.I.	0	0.4	0	0	0	0	0	0	0	0	0	0	0	0	0
W.I.I.	**W.I.I. 1= 0.06**							**W.I.I. 2= 0**							

W.I.I. max. = 0.06

Classification: Practically Non irritant

D.I.I. : Daily Irritation Index
W.I.I. : Weekly Irritation Index
W.I.I. max. : Maximum Weekly Irritation Index

Note
Weekly Mean irritation indices (W.I.I.) are calculated from the seven daily indices of each week. The maximum weekly mean irritation index (max. W.I.I.) is used to arbitrary classify the test item according to the following table:

Max. W.I.I.	CLASSIFICATION
0	Non irritant
>0	Practically Non irritant
>0.5	Slightly irritant
>2	Irritant
>5	Very irritant

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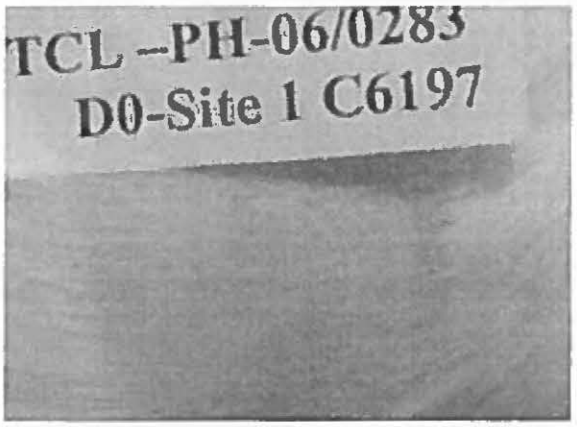
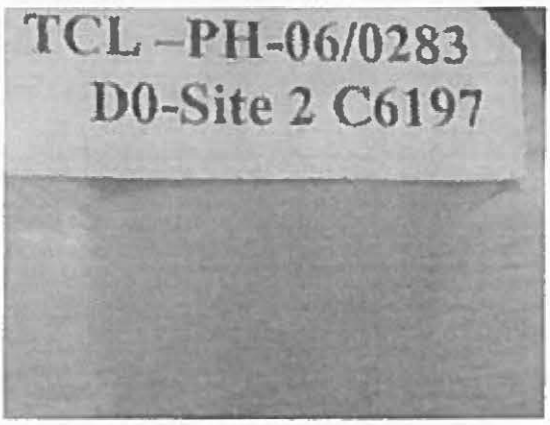
Photographies: 1st day

C6197 – Sites 1 & 2





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Photographies: 5th day

C6197 – Sites 1 & 2

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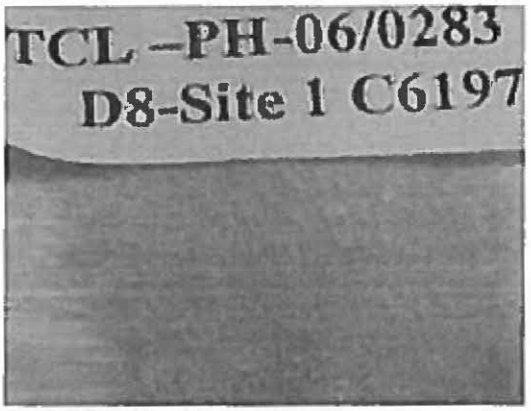
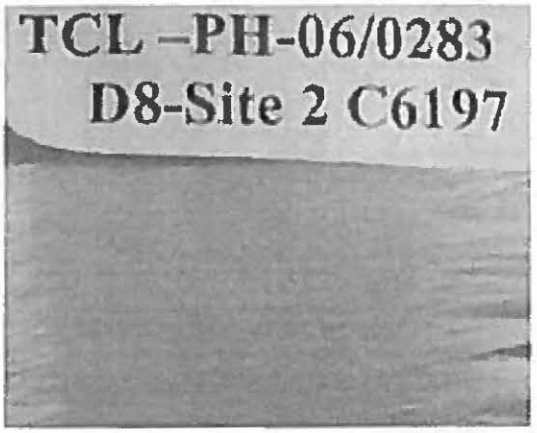
Photographies: 9th day

C6197 – Sites 1 & 2





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Photographies: 12th day

C6197 – Sites 1 & 2

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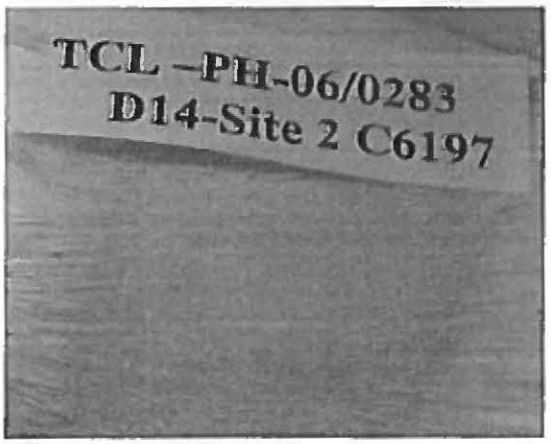
Photographies: 15th day

C6197 – Sites 1 & 2



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APPENDICES

Appendix 1: Certificate of G.L.P. (1 page)

Appendix 2: Animals diet sheet (1 page r/v)

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Appendix 1: Certificate of G.L.P. (1 page)

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RÉPUBLIQUE FRANÇAISE

GROUPE INTERMINISTERIEL DES PRODUITS CHIMIQUES

Paris, le 13 DEC. 2005

OBJET : Evaluation de la conformité aux B.P.L. selon la directive 88/320/C.E.E.

Consécutivement à votre engagement vis à vis du GIPC et du COFRAC et en application du décret n° 81-278 du 25 mars 1981 portant création d'un groupe interministériel des produits chimiques, modifié notamment par le décret 90-206 du 7 mars 1990 et par le décret n° 98-1312 du 31 décembre 1998 concernant les bonnes pratiques de laboratoires, je vous confirme que le GIPC, au vu des résultats du contrôle exercé par le Comité français d'accréditation (COFRAC) - Section Laboratoires a décidé pour votre installation du statut suivant :

respect des principes de B.P.L.
Domaines de reconnaissance :
☐ **2 - études de toxicité**
☐ **3 - études de mutagénicité**

Date d'inspection : 3 octobre 2005

☐ **inspection de contrôle périodique (i.p)**
☐ **inspection d'extension (i.e)**

Date de décision du G.I.P.C. : 9 décembre 2005
Date de prise d'effet : 3 octobre 2005
Année de première conformité : 1998
Durée de validité : 18 mois

Le Président, P. O.

Pierre CREYSSEL
Conseiller d'Etat h.

PHYCHER BIO Developpement
ZI de Toctoucau
18, chemin de Lou Tribail
33611 CESTAS Cedex

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SafePharm Laboratories

S-FACE IS-1009P:
Polyglyceryl-10 Nonaisostearate
LOCAL LYMPH NODE ASSAY IN THE MOUSE

SPL PROJECT NUMBER: 432/067

AUTHOR: A Pooles

STUDY SPONSOR:

Sakamoto Yakuhin Kogyo Co., Ltd.
1-20, Rinkai-cho
Izumiotsu-city
OSAKA 595-0075
JAPAN

TEST FACILITY:

Safepharm Laboratories Limited
Shardlow Business Park
Shardlow
Derbyshire
DE72 2GD

Telephone: +44 (0) 1332 792896

Facsimile: +44 (0) 1332 799018

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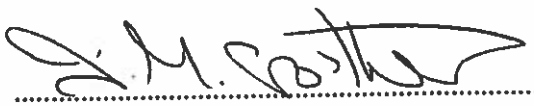
QUALITY ASSURANCE REPORT

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
	08 January 2004	Test Material Preparation
	20 January 2004	Test System Preparation
	23 January 2004	Animal Preparation
	07 January 2004	Dosing
	13 January 2004	Assessment of Response
§	19 February 2004	Draft Report Audit
§	Date of QA Signature	Final Report Audit
§	Evaluation specific to this study	



DATE: 25 MAR 2004

For Safepharm Quality Assurance Unit*

*Authorised QA Signatures:
Head of Department: JR Pateman CBiol MIBiol DipRQA FRQA
Deputy Head of Department: JM Crowther MIScT MRQA
Senior Audit Staff: JV Johnson BSc MRQA; G Wren ONC MRQA

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GLP COMPLIANCE STATEMENT

The work described was performed in compliance with UK GLP standards (Schedule 1, Good Laboratory Practice Regulations 1999 (SI 1999/3106)). 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 87/18/EEC (as amended by Directive 1999/11/EC) and 88/320/EEC (as amended by Directive 1999/12/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, 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.

A. Poob ..... DATE: 24/3/04

A Pooles BA (Hons)
Study Director

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S-FACE IS-1009P:

LOCAL LYMPH NODE ASSAY IN THE MOUSE

SUMMARY

Introduction. A study was performed to assess the skin sensitisation potential of the test material in the CBA/Ca strain mouse following topical application to the dorsal surface of the ear. The method was designed to meet the requirements of the following:

- OECD Guideline for the Testing of Chemicals No. 429 "Skin Sensitisation: Local Lymph Node Assay" (adopted 24 April 2002)

Methods. Following a preliminary screening test, three groups, each of four animals, were treated with 50 µl (25 µl per ear) of the undiluted test material and the test material as a solution in acetone/olive oil 4:1 at concentrations of 25% and 50% v/v. A further group of four animals was treated with acetone/olive oil 4:1 alone.

Results. The Stimulation Index (SI) expressed as the mean radioactive incorporation for each treatment group divided by the mean radioactive incorporation of the vehicle control group are as follows:

Concentration (% v/v) in acetone/olive oil 4:1	Stimulation Index (SI)	Result
25	0.68	Negative
50	0.70	Negative
100	0.87	Negative

Conclusion. The test material was considered to be a non-sensitiser under the conditions of the test.

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S-FACE IS-1009P:

LOCAL LYMPH NODE ASSAY IN THE MOUSE

1. INTRODUCTION

A study was performed to assess the skin sensitisation potential of the test material in the CBA/Ca strain mouse following topical application to the dorsal surface of the ear. The method was designed to meet the requirements of the following:

- OECD Guideline for the Testing of Chemicals No. 429 "Skin Sensitisation: Local Lymph Node Assay" (adopted 24 April 2002)

The assay has undergone extensive inter-laboratory validation and has been shown to reliably detect test materials that are moderate to strong sensitisers.

The strain of mouse used in these laboratories has been shown to produce satisfactory responses using known sensitisers and non-sensitisers during the in-house validation. The results of routine positive control studies are shown in Appendix 1 and Appendix 2. The results of the study are believed to be of value in predicting the sensitisation potential of the test material to man.

The study was performed between 14 January 2004 and 04 February 2004.

2. TEST MATERIAL

2.1 Description, Identification and Storage Conditions

Sponsor's identification	:	S-FACE IS-1009P
Description	:	pale yellow slightly viscous liquid
Batch number	:	31112
Date received	:	04 December 2003
Storage conditions	:	room temperature in the dark

Data relating to the identity, purity and stability of the test material are the responsibility of the Sponsor.

A Certificate of Analysis supplied by the Sponsor is given in Appendix 4.

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2.2 Preparation of Test Material

For the purpose of the study the test material was used undiluted and freshly prepared in acetone/olive oil 4:1. This vehicle was chosen as it produced the highest concentration that was suitable for dosing. The concentrations used are given in the procedure section.

Determination, by analysis, of the concentration, homogeneity and stability of the test material preparations was not appropriate because it was not specified in the Study Plan and is not a requirement of the Test Guideline.

3. METHODS

3.1 Animals and Animal Husbandry

Female CBA/Ca (CBA/CaBkl) strain mice were supplied by B & K Universal Ltd, Hull, UK. On receipt the animals were randomly allocated to cages. The animals were nulliparous and non-pregnant. After an acclimatisation period of at least five days the animals were selected at random and given a number unique within the study by indelible ink-marking on the tail and a number written on a cage card. At the start of the study the animals were in the weight range of 15 to 23 g, and were eight to twelve weeks old.

The animals were individually housed in suspended solid-floor polypropylene cages furnished with softwood woodflakes. Free access to mains tap water and food (Certified Rat and Mouse Diet (Code 5LF2) supplied by BCM IPS Limited, London, UK) was allowed throughout the study.

The temperature and relative humidity were controlled to remain within target ranges of 19 to 25°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 approximately 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.

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3.2 Procedure

3.2.1 Preliminary Screening Test

As no toxicological information was available regarding the systemic toxicity/irritancy potential of the test material a preliminary screening test was performed using one mouse. The mouse was treated by daily application of 25 µl of the undiluted test material to the dorsal surface of each ear for three consecutive days (Days 1, 2, 3). The mouse was observed twice daily on Days 1, 2 and 3 and once daily on Days 4, 5 and 6. Any signs of toxicity or excessive local irritation noted during this period were recorded. The bodyweight of the mouse was recorded on Day 1 (prior to dosing) and on Day 6.

3.2.2 Main Test

3.2.2.1 Test Material Administration

Groups of four mice were treated with the undiluted test material or the test material at concentrations of 25% or 50% v/v in acetone/olive oil 4:1. The preliminary screening test suggested that the test material would not produce systemic toxicity or excessive local irritation at the highest suitable concentration. The mice were treated by daily application of 25 µl of the appropriate concentration of the test material to the dorsal surface of each ear for three consecutive days (Days 1, 2, 3). The test material formulation was administered using an automatic micropipette and spread over the dorsal surface of the ear using the tip of the pipette.

A further group of four mice received the vehicle alone in the same manner.

3.2.2.2 ^{3}H-Methyl Thymidine Administration

Five days following the first topical application of the test material (Day 6) all mice were injected via the tail vein with 250 µl of phosphate buffered saline containing ^{3}H-methyl thymidine (^{3}HTdR: 80 µCi/ml, specific activity 2.0 Ci/mmol, Amersham Biosciences UK Ltd) giving a total of 20 µCi to each mouse.

3.2.2.3 Observations

Clinical Observations: All animals were observed twice daily on Days 1, 2 and 3 and on a daily basis on Days 4, 5 and 6. Any signs of toxicity or signs of ill health during the study were recorded.

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Bodyweights: The bodyweight of each mouse was recorded on Day 1 (prior to dosing) and Day 6 (prior to termination).

3.2.2.4 Terminal Procedures

Termination: Five hours following the administration of ^{3}HTdR all mice were killed by carbon dioxide asphyxiation. The draining auricular lymph nodes from the four mice were excised and pooled for each experimental group. For each group 1 ml of phosphate buffered saline (PBS) was added to the pooled lymph nodes.

Preparation of Single Cell Suspension: A single cell suspension of pooled lymph node cells was prepared by gentle mechanical disaggregation through a 200-mesh stainless steel gauze. The lymph node cells were rinsed through the gauze with 4 ml of PBS into a petri dish labelled with the project number and dose concentration. The lymph node cell suspension was transferred to a 10 ml centrifuge tube. The petri dish was washed with an additional 5 ml of PBS to remove all remaining lymph node cells and these were added to the centrifuge tube. The pooled lymph node cells were pelleted at 1400 rpm (approximately 190 *g*) for ten minutes. The pellet was resuspended in 10 ml of PBS and re-pelleted. To precipitate out the radioactive material, the pellet was resuspended in 3 ml of 5% Trichloroacetic acid (TCA).

Determination of ^{3}HTdR Incorporation: After overnight incubation at 4°C, the precipitates were recovered by centrifugation at 2100 rpm (approximately 450 *g*) for ten minutes, resuspended in 1 ml of TCA and transferred to 10 ml of scintillation fluid (Optiphase 'Trisafe'). ^{3}HTdR incorporation was measured by β-scintillation counting. The "Poly Q^{TM}" vials containing the samples and scintillation fluid were placed in the sample changer of the scintillator and left for approximately twenty minutes. The purpose of this period of time in darkness was to reduce the risk of luminescence, which has been shown to affect the reliability of the results. After approximately twenty minutes, the vials were shaken vigorously. The number of radioactive disintegrations per minute was then measured using the Beckman LS6500 scintillation system (Beckman Instruments Inc, Fullerton, CA).

3.3 Interpretation of Results

The proliferation response of lymph node cells was expressed as the number of radioactive disintegrations per minute per lymph node (dpm/node) and as the ratio of ^{3}HTdR incorporation into lymph node cells of test nodes relative to that recorded for the control nodes (Stimulation Index).

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The test substance will be regarded as a sensitiser if at least one concentration of the test material results in a threefold or greater increase in ^{3}HTdR incorporation compared to control values. Any test material failing to produce a threefold or greater increase in ^{3}HTdR incorporation, will be classified as a "non-sensitiser".

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.

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5. RESULTS

5.1 Preliminary Screening Test

Clinical observations, bodyweight and mortality data are given in Table 1.

There were no deaths or signs of systemic toxicity noted. Mild redness on the neck and around the base of the ear was noted one hour post dosing on Day 3.

Based on this information the dose levels selected for the main test were 25% and 50% v/v in acetone/olive oil 4:1 and 100%.

5.2 Main Test

5.2.1 Estimation of the Proliferative Response of Lymph Node Cells

The radioactive disintegrations per minute (dpm) per lymph node and the stimulation index (SI) are given in Table 2.

A stimulation index of less than 3 was recorded for the three concentrations of the test material (25% and 50% v/v and 100%).

5.2.2 Clinical Observations and Mortality Data

Individual clinical observations and mortality data for test and control animals are given in Table 3.

There were no deaths. No signs of systemic toxicity were noted in the test or control animals during the study.

5.2.3 Bodyweight

Individual bodyweights and bodyweight changes for test and control animals are given in Table 4.

Bodyweight changes of the test animals between Day 1 and Day 6 were comparable to those observed in the corresponding control group animals over the same period.

6. CONCLUSION

The test material was considered to be a non-sensitiser under the conditions of the test.

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S-FACE IS-1009P : LOCAL LYMPH NODE ASSAY IN THE MOUSE

Table 1 **Clinical Observations, Bodyweight and Mortality Data – Preliminary Screening Test**

Concentration (%)	Animal Number	Bodyweight (g)		Day								
				1		2		3		4	5	6
		Day 1	Day 6	Pre-Dose	1 Hr Post Dose	Pre-Dose	1 Hr Post Dose	Pre-Dose	1 Hr Post Dose			
100	S-1	20	20	0	0	0	0	0	0*	0	0	0

Hr = Hour
0 = No signs of systemic toxicity
* = Mild redness on the neck and around the base of the ear

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S-FACE IS-1009P : LOCAL LYMPH NODE ASSAY IN THE MOUSE

Table 2 Dpm, Dpm/Node and Stimulation Index (SI)

Concentration (% v/v) in acetone/olive oil 4:1	Dpm	Dpm/Node [a]	Stimulation Index (SI) [b]	Result
Vehicle	6870.90	858.86	N/A	N/A
25	4640.75	580.09	0.68	Negative
50	4833.16	604.15	0.70	Negative
100	5956.87	744.61	0.87	Negative

a = Dpm/node obtained by dividing the Dpm value by 8 (total number of lymph nodes)
b = Stimulation Index of 3.0 or greater indicates a positive result
N/A = Not applicable

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S-FACE IS-1009P : LOCAL LYMPH NODE ASSAY IN THE MOUSE

Table 3 Individual Clinical Observations and Mortality Data

Concentration (% v/v) in acetone/olive oil 4:1	Animal Number	Day 1		Day 2		Day 3		Day 4	Day 5	Day 6
		Pre-Dose	1-Hr Post Dose	Pre-Dose	1-Hr Post Dose	Pre-Dose	1-Hr Post Dose			
Vehicle	1-1	0	0	0	0	0	0	0	0	0
	1-2	0	0	0	0	0	0	0	0	0
	1-3	0	0	0	0	0	0	0	0	0
	1-4	0	0	0	0	0	0	0	0	0
25	2-1	0	0	0	0	0	0	0	0	0
	2-2	0	0	0	0	0	0	0	0	0
	2-3	0	0	0	0	0	0	0	0	0
	2-4	0	0	0	0	0	0	0	0	0
50	3-1	0	0	0	0	0	0	0	0	0
	3-2	0	0	0	0	0	0	0	0	0
	3-3	0	0	0	0	0	0	0	0	0
	3-4	0	0	0	0	0	0	0	0	0
100	4-1	0	0	0	0	0	0	0	0	0
	4-2	0	0	0	0	0	0	0	0	0
	4-3	0	0	0	0	0	0	0	0	0
	4-4	0	0	0	0	0	0	0	0	0

Hr = Hour
0 = No signs of systemic toxicity

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S-FACE IS-1009P : LOCAL LYMPH NODE ASSAY IN THE MOUSE

Table 4 Individual Bodyweights and Bodyweight Changes

Concentration (% v/v) in acetone/olive oil 4:1	Animal Number	Bodyweight (g)		Bodyweight Change (g)
		Day 1	Day 6	
Vehicle	1-1	20	20	0
	1-2	19	19	0
	1-3	20	20	0
	1-4	18	19	1
25	2-1	20	20	0
	2-2	22	21	-1
	2-3	19	19	0
	2-4	20	20	0
50	3-1	17	18	1
	3-2	23	22	-1
	3-3	22	21	-1
	3-4	19	19	0
100	4-1	22	20	-2
	4-2	22	22	0
	4-3	22	20	-2
	4-4	20	21	1

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S-FACE IS-1009P : LOCAL LYMPH NODE ASSAY IN THE MOUSE

Appendix 1 Latest Positive Control Study for the Local Lymph Node Assay

Introduction. A study was performed to assess the sensitivity of the strain of mouse used at these laboratories to a known sensitiser. The method was designed to meet the requirements of the following:

- OECD Guideline for the Testing of Chemicals No. 429 "Skin Sensitisation: Local Lymph Node Assay" (adopted 24 April 2002)

Test Material: α-HEXYLCINNAMALDEHYDE

SPL Project number: 039/656

Study dates: 10 October 2003 to 16 October 2003

Methods. Three groups, each of four animals, were treated with 50 µl (25 µl per ear) of α-HEXYLCINNAMALDEHYDE as a solution in acetone/olive oil 4:1 at concentrations of 5%, 10% and 25% v/v. A further control group of four animals was treated with acetone/olive oil 4:1 alone.

Results. The Stimulation Index (SI) expressed as the mean radioactive incorporation for each treatment group divided by the mean radioactive incorporation of the vehicle control group are as follows:

Concentration (% v/v) in Acetone/Olive Oil 4:1	Stimulation Index (SI)	Result
5	1.76	Negative
10	2.78	Negative
25	5.06	Positive

Conclusion. α-HEXYLCINNAMALDEHYDE was considered to be a sensitiser under the conditions of the test.

Appendix 2 Summary of Positive Control Data for the Local Lymph Node Assay

Project Number	Start Date	Finish Date	Test Material	Concentration	Vehicle	Stimulation Index[a]	Classification[b]
039/503*	03/07/01	09/07/01	2-Mercapto-benzothiazole	1, 5, 25% w/v	Dimethyl Formamide	2.0, 2.8, 5.4	Positive
039/586*	13/08/02	19/08/02	α-Hexylcinnamaldehyde	5, 10, 50% w/v	4:1 acetone/olive oil	5.7, 5.5, 33.5	Positive
039/629*	13/03/03	19/03/03	α-Hexylcinnamaldehyde	5, 10, 25% v/v	4:1 acetone/olive oil	2.8, 2.3, 5.5	Positive
039/630•	13/03/03	19/03/03	α-Hexylcinnamaldehyde	5, 10, 25% v/v	4:1 acetone/olive oil	2.0, 1.9, 6.8	Positive
039/656*	10/10/03	16/10/03	α-Hexylcinnamaldehyde	5, 10, 25% v/v	4:1 acetone/olive oil	1.76, 2.78, 5.06	Positive
039/658•	16/10/03	22/10/03	α-Hexylcinnamaldehyde	5, 10, 25% v/v	4:1 acetone/olive oil	1.49, 1.73, 5.26	Positive

a = Ratio of test to control lymphocyte proliferation
b = Stimulation index greater than 3.0 indicates a positive result
* = Standard Test Method 595 ('Pooled' nodes)
• = Standard Test Method 599 ('Individual' nodes)

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Appendix 3 Statement of GLP Compliance in Accordance with Directive 88/320/EEC

THE DEPARTMENT OF HEALTH OF THE GOVERNMENT OF THE UNITED KINGDOM

GOOD LABORATORY PRACTICE

STATEMENT OF COMPLIANCE
IN ACCORDANCE WITH DIRECTIVE 88/320 EEC

LABORATORY
SafePharm Limited
Shardlow Business Park,
London Road,
Shardlow,
Derbyshire,
DE72 2GD

TEST TYPE
Analytical/Clinical
Chemistry
Environmental tox.
Environmental fate
Mutagenicity
Phys./Chem. tests
Toxicology

DATE OF INSPECTION

2nd December 2002

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

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

Roger G. Alexander
13/2/03

Dr. Roger G. Alexander
Head, UK GLP Monitoring Authority

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Appendix 4 Certificate of Analysis

SAKAMOTO YAKUHIN KOGYO CO.,LTD.

HEAD OFFICE : 1-2-6 AWAJIMACHI,CHUO-KU,OSAKA CITY 541-0047,JAPAN
TEL (81)6-6231-1851 FAX (81)6-6222-0631
TOKYO BRANCH : 1-6-2 MARUNOUCHI,CHIYODA-KU,TOKYO 100-0005,JAPAN
TEL (81)3-3211-3813 FAX (081)3-3215-1788

date : Nov. 27 , 2003

Product : S-Face IS-1009P
Lot. No. : 31112
Quantity : 90g × 1

Certificate of analysis / Quality

	Result
Description	A pale yellow viscous liquid with a faint characteristic odor
Color (Gardner)	less than 1
Acid value (mgKOH/g)	0.4
Saponification value (mgKOH/g)	159.6
Heavy metal (ppm)	less than 20
Arsenic (ppm)	less than 2
Residue on ignition (%)	less than 0.1

Y. Noguchi
Yasunori Noguchi
Quality Control Manager

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SAFEPHARM LABORATORIES LTD

S-FACE IS-1009P:

LOCAL LYMPH NODE ASSAY IN THE MOUSE

SPL PROJECT NUMBER: 432/067

I verify that this is an exact copy of the original report which is located in the Archives of Safepharm Laboratories Ltd., Derby, UK.

A Poole DATE: 15/4/04

A Pooles BA (Hons)
Study Director

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SafePharm Laboratories

S-Face IS-1009P:
Polyglyceryl-10 Nonaisostearate
REVERSE MUTATION ASSAY "AMES TEST"
USING *SALMONELLA TYPHIMURIUM*
AND *ESCHERICHIA COLI*

SPL PROJECT NUMBER: 432/068

AUTHOR: P W Thompson

STUDY SPONSOR:

Sakamoto Yakuhin Kogyo Co., Ltd.
1-20, Rinkai-cho
Izumiotsu-city
OSAKA 595-0075
JAPAN

TEST FACILITY:

Safepharm Laboratories Limited
Shardlow Business Park
Shardlow
Derbyshire
DE72 2GD

Telephone: +44 (0) 1332 792896

Facsimile: +44 (0) 1332 799018

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QUALITY ASSURANCE REPORT

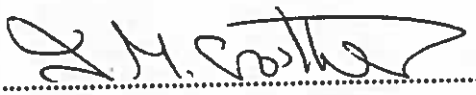
This study type is classed as short-term. The standard test method for this study type ("General Study Plan" in OECD terminology) was reviewed for compliance once only on initial production. Inspection of the routine and repetitive procedures that constitute the study is carried out as a continuous process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

	19 August 2002	Standard Test Method Compliance Audit
	02, 03 February 2004	Test Material Preparation
	02 February 2004	Test System Preparation
	03 February 2004	Exposure
	05 February 2004	Assessment of Response
§	22 March 2004	Draft Report Audit
§	Date of QA Signature	Final Report Audit

§ Evaluation specific to this study



DATE: 30 MAR 2004

For Safepharm Quality Assurance Unit*

*Authorised QA Signatures:
Head of Department: JR Pateman CBiol MIBiol DipRQA AIQA FRQA
Deputy Head of Department: JM Crowther MIScT MRQA
Senior Audit Staff: JV Johnson BSc MRQA; G Wren ONC MRQA

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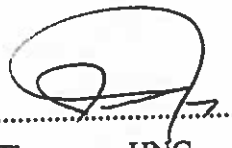
GLP COMPLIANCE STATEMENT

The work described was performed in compliance with UK GLP standards (Schedule 1, Good Laboratory Practice Regulations 1999 (SI 1999/3106)). 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, 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: 30 MAR 2004

P W Thompson HNC
Study Director

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S-Face IS-1009P:

REVERSE MUTATION ASSAY "AMES TEST" USING *SALMONELLA TYPHIMURIUM* AND *ESCHERICHIA COLI*

SUMMARY

Introduction. The method was designed to conform to the guidelines for bacterial mutagenicity testing published by the major Japanese Regulatory Authorities including METI, MHLW and MAFF. It also meets the requirements of the OECD Guidelines for Testing of Chemicals No. 471 "Bacterial Reverse Mutation Test", Method B13/14 of Commission Directive 2000/32/EC and the USA, EPA (TSCA) OPPTS harmonised guidelines.

Methods. *Salmonella typhimurium* strains TA1535, TA1537, TA98 and TA100 and *Escherichia coli* strain WP2uvrA$^-$ were treated with the test material using the Ames plate incorporation method at five dose levels, in triplicate, both with and without the addition of a rat liver homogenate metabolising system (10% liver S9 in standard co-factors). The dose range for the range-finding test was determined in a preliminary toxicity assay and was 50 to 5000 µg/plate. The experiment was repeated on a separate day using the same dose range as the range-finding test, fresh cultures of the bacterial strains and fresh test material formulations.

Results. The vehicle (tetrahydrofuran) control plates gave counts of revertant colonies within the normal range. All of the positive control chemicals used in the test induced marked increases in the frequency of revertant colonies, both with or without metabolic activation. Thus, the sensitivity of the assay and the efficacy of the S9-mix were validated.

The test material caused no visible reduction in the growth of the bacterial background lawn at any dose level. The test material was, therefore, tested up to the maximum recommended dose level of 5000 µg/plate. An oily precipitate was observed at and above 1500 µg/plate, this did not prevent the scoring of revertant colonies.

No significant increases in the frequency of revertant colonies were recorded for any of the bacterial strains, with any dose of the test material, either with or without metabolic activation.

Conclusion. The test material was considered to be non-mutagenic under the conditions of this test.

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S-Face IS-1009P:

REVERSE MUTATION ASSAY "AMES TEST" USING *SALMONELLA TYPHIMURIUM* AND *ESCHERICHIA COLI*

1. INTRODUCTION

This study was designed to assess the mutagenic potential of the test material using a bacterial/microsome test system. The study was based on the *in vitro* technique described by Ames and his co-workers (1, 2, 3) and Garner *et al* (4) in which mutagenic activity is assessed by exposing histidine auxotrophs of *Salmonella typhimurium* and tryptophan auxotrophs of *Escherichia coli* to various concentrations of the test material. The method conforms to the guidelines for bacterial mutagenicity testing published by the major Japanese Regulatory Authorities including METI, MHLW and MAFF. This method also conforms with the OECD Guidelines for the Testing of Chemicals, Protocol No. 471, Method B13/14 in EC Commission Directive 2000/32/EC and the USA, EPA (TSCA) OPPTS harmonised guidelines. A copy of the Certificate of Compliance with GLP, issued by the UK Department of Health, is included as Appendix 3.

The mutant strains of *Salmonella* are incapable of synthesising histidine and are, therefore, dependent for growth on an external source of this particular amino acid. When exposed to a mutagenic agent these bacteria may undergo a reverse mutation to histidine independent forms which are detected by their ability to grow on a histidine deficient medium. Using various strains of this organism, revertants produced after exposure to a chemical mutagen may arise as a result of base-pair substitution in the genetic material (miscoding) or frame-shift mutation in which genetic material is either added or deleted. In order to make the bacteria more sensitive to mutation by chemical and physical agents, several additional traits have been introduced. These include a deletion through the excision repair gene (uvrB$^-$ *Salmonella* strains) which renders the organism incapable of DNA excision repair and deep rough mutation (rfa) which increases the permeability of the cell wall. A mutant strain of *Escherichia coli* (WP2uvrA$^-$), which requires tryptophan and which can be reverse mutated by base substitution to tryptophan independence, was used to complement the *Salmonella* strains. This strain also has a deletion in an excision repair gene (uvrA$^-$).

Since many compounds do not exert a mutagenic effect until they have been metabolised by enzyme systems not available in the bacterial cell, the test material and the bacteria are also

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incubated in the presence of a liver microsomal preparation (S9-mix) prepared from rats pre-treated with a compound known to induce an elevated level of these enzymes.

The experimental phase of this study was performed between 14 January 2004 and 05 March 2004.

2. TEST MATERIAL

Sponsor's identification	:	S-Face IS-1009P
Description	:	Pale yellow slightly viscous liquid
Batch number	:	31112
Date received	:	04 December 2003
Storage conditions	:	Room temperature in the dark

Data relating to the identity, purity and stability of the test material are the responsibility of the Sponsor.

3. METHODS

3.1 Tester Strains

Salmonella typhimurium	TA1535, TA1537, TA98 and TA100
Escherichia coli	WP2uvrA$^-$

The *Salmonella typhimurium* strains were obtained from the University of California at Berkeley on culture discs on 4 August 1995 whilst *Escherichia coli* strain WP2uvrA$^-$ was obtained from the British Industrial Biological Research Association on 17 August 1987. All of the strains were stored at -196°C. Prior to the master strains being used, characterisation checks were carried out to confirm the amino-acid requirement, presence of rfa, R factors, uvrB or uvrA mutation and the spontaneous reversion rate.

In this assay, overnight sub-cultures of the appropriate coded stock cultures were prepared in nutrient broth and incubated at 37°C for approximately 10 hours. Each culture was monitored spectrophotometrically for turbidity with titres determined by viable count analysis on nutrient agar plates.

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3.2 Preparation of Test and Control Materials

The test material was accurately weighed and approximate half-log dilutions prepared in tetrahydrofuran by mixing on a vortex mixer on the day of each experiment. Tetrahydrofuran is toxic to the bacterial cells at and above 50 µl, therefore all of the formulations were prepared at concentrations four times greater than required on Vogel-Bonner agar plates. To compensate, each formulation was dosed using 25 µl aliquots. Tetrahydrofuran is an acceptable solvent vehicle for use in this test system (6). Analysis for concentration, homogeneity and stability of the test material formulations is not a requirement of the test guidelines and was, therefore, not determined. Prior to use, the solvent was dried using molecular sieves (sodium alumino-silicate) *ie* 2 mm pellets with a nominal pore diameter of 4×10^{-4} microns.

Vehicle and positive controls were used in parallel with the test material. A solvent treatment group was used as the vehicle control and the positive control materials were as follows:

N-ethyl-N'-nitro-N-nitrosoguanidine (ENNG):	2 µg/plate for WP2uvrA$^-$, 3 µg/plate for TA100 and 5 µg/plate for TA1535
9-Aminoacridine (9AA):	80 µg/plate for TA1537
4-Nitroquinoline-1-oxide (4NQO):	0.2 µg/plate for TA98

In addition, 2-Aminoanthracene (2AA) and Benzo(a)pyrene (BP), which are non-mutagenic in the absence of metabolising enzymes, were used in the series of plates with S9-mix at the following concentrations:

2AA at 1 µg/plate for TA100
2AA at 2 µg/plate for TA1535 and TA1537
2AA at 10 µg/plate for WP2uvrA$^-$
BP at 5 µg/plate for TA98

3.3 Microsomal Enzyme Fraction

S9 was prepared in-house on 25 October 2003 from the livers of male Sprague-Dawley rats weighing ~ 250g. These had each orally received three consecutive daily doses of phenobarbitone/β-naphthoflavone (80/100 mg per kg per day) prior to S9 preparation on Day 4. Before use, each batch of S9 was assayed for its ability to metabolise appropriate indirect mutagens used in the Ames Test. The S9 was stored at -196°C.

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3.4 S9-Mix and Agar

The S9-mix was prepared immediately before use using sterilised co-factors and maintained on ice for the duration of the test.

S9	5.0 ml
1.65 M KCl/0.4 M $MgCl_2$	1.0 ml
0.1 M Glucose-6-phosphate	2.5 ml
0.1 M NADPH	2.0 ml
0.1 M NADH	2.0 ml
0.2 M Sodium phosphate buffer (pH 7.4)	25.0 ml
Sterile distilled water	12.5 ml

A 0.5 ml aliquot of S9-mix and 2 ml of molten, trace histidine or tryptophan supplemented, top agar was overlaid onto a sterile Vogel-Bonner Minimal agar plate in order to assess the sterility of the S9-mix. This procedure was repeated, in triplicate, on the day of each experiment.

Top agar was prepared using 0.6% Difco Bacto agar and 0.5% sodium chloride with 5 ml of 1.0 mM histidine and 1.0 mM biotin or 1.0 mM tryptophan solution added to each 100 ml of top agar. Vogel-Bonner Minimal agar plates were purchased from ILS Ltd.

3.5 Test Procedure

3.5.1 Preliminary Toxicity Test

In order to select appropriate dose levels for use in the main test, a preliminary assay was carried out to determine the toxicity of the test material. The concentrations tested were 0, 0.15, 0.5, 1.5, 5, 15, 50, 150, 500, 1500 and 5000 µg/plate. The assay was performed by mixing 0.1 ml of bacterial culture (TA100 or WP2uvrA$^-$), 0.025 ml of test material formulation, 0.5 ml of S9-mix or phosphate buffer and 2 ml of molten, trace histidine or tryptophan supplemented, top agar and overlaying onto sterile plates of Vogel-Bonner Minimal agar (30 ml/plate). Ten concentrations of the test material and a vehicle control (tetrahydrofuran) were tested. In addition, 0.025 ml of the maximum concentration of the test material and 2 ml of molten, trace histidine or tryptophan supplemented, top agar was overlaid onto a sterile Nutrient agar plate in order to assess the sterility of the test material. After approximately 48 hours incubation at 37°C the plates were assessed for numbers of revertant colonies using a Domino colony counter and examined for effects on the growth of the bacterial background lawn.

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3.5.2 Mutation Test – Experiment 1 (Range-finding Test)

Five concentrations of the test material (50, 150, 500, 1500 and 5000 µg/plate) were assayed in triplicate against each tester strain, using the direct plate incorporation method.

Measured aliquots (0.1 ml) of one of the bacterial cultures were dispensed into sets of test tubes followed by 2.0 ml of molten, trace histidine or tryptophan supplemented, top agar, 0.025 ml of the test material formulation, vehicle or 0.1 ml of positive control and either 0.5 ml of S9-mix or phosphate buffer. The contents of each test tube were mixed and equally distributed onto the surface of Vogel-Bonner Minimal agar plates (one tube per plate). This procedure was repeated, in triplicate, for each bacterial strain and for each concentration of test material both with and without S9-mix.

All of the plates were incubated at 37°C for approximately 48 hours and the frequency of revertant colonies assessed using a Domino colony counter.

3.5.3 Mutation Test – Experiment 2 (Main Test)

The second experiment was performed using methodology as described for the range-finding test, using fresh bacterial cultures, test material and control solutions. The test material dose range was the same as the range-finding test (50 to 5000 µg/plate).

3.6 Acceptance Criteria

The reverse mutation assay may be considered valid if the following criteria are met:

All tester strain cultures exhibit a characteristic number of spontaneous revertants per plate in the vehicle and untreated controls. Acceptable ranges are presented in the standard test method section 3 with historical control ranges for 2002 and 2003 presented in Appendix 2.

The appropriate characteristics for each tester strain have been confirmed, eg rfa cell-wall mutation and pKM101 plasmid R-factor etc.

All tester strain cultures should be in the approximate range of 1 to 9.9 x 10^9 bacteria per ml.

Each mean positive control value should be at least two times the respective vehicle control value for each strain, thus demonstrating both the intrinsic sensitivity of the tester strains to mutagenic

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exposure and the integrity of the S9-mix. The positive control historical ranges for 2002 and 2003 are presented in Appendix 2.

There should be a minimum of four non-toxic test material dose levels.

There should not be an excessive loss of plates due to contamination.

3.7 Evaluation Criteria

The test material may be considered positive in this test system if the following criteria are met:

The test material should have induced a reproducible, dose-related and statistically (Dunnett's method of linear regression (5)) significant increase in the revertant count in at least one strain of bacteria.

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.

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5. RESULTS

5.1 Preliminary Toxicity Test

The test material was non-toxic to the strains of bacteria used (TA100 and WP2uvrA$^-$). The test material formulation and S9-mix used in this experiment were both shown to be effectively sterile.

The number of revertant colonies for the toxicity assay were:

With (+) or without (-) S9-mix	Strain	Dose (µg/plate)										
		0	0.15	0.5	1.5	5	15	50	150	500	1500	5000
-	TA100	97	94	103	97	90	81	88	84	84	98P	83P
+	TA100	100	88	82	93	93	88	98	69	106	86P	86P
-	WP2uvrA$^-$	26	28	25	21	24	30	30	27	28	19P	29P
+	WP2uvrA$^-$	36	34	27	33	30	24	34	35	30	36P	32P

P: Precipitate

5.2 Mutation Test

Prior to use, the master strains were checked for characteristics, viability and spontaneous reversion rate (all were found to be satisfactory). These data are not given in the report. The S9-mix used in both experiments of the main test was shown to be sterile.

Results for the negative controls (spontaneous mutation rates) are presented in Table 1 and were considered to be acceptable. These data are for concurrent untreated control plates performed on the same day as the Mutation Test.

The individual plate counts, the mean number of revertant colonies and the standard deviations for the test material, vehicle and positive controls both with and without metabolic activation, are presented in Table 2 to Table 5 with the results also expressed graphically in Figure 1 to Figure 4.

Information regarding the equipment and methods used in these experiments as required by the Japanese Ministry of Economy, Trade and Industry and Japanese Ministry of Health, Labour and Welfare are presented in Appendix 1.

A history profile of vehicle and positive control values is presented in Appendix 2.

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SAFEPHARM LABORATORIES LIMITED

AUTHENTICATION OF AMENDMENT TO FINAL REPORT:

S-Face IS-1009P
REVERSE MUTATION ASSAY "AMES TEST"
USING *SALMONELLA TYPHIMURIUM*
AND *ESCHERICHIA COLI*
SPL PROJECT NUMBER: 432/068

REASON FOR AMENDMENT

A hand amendment to the table key on page 12 was discovered as the study was submitted to the archive.

DETAILS OF AMENDMENT

The report has been amended to clarify the meaning of the postfix attached to the Preliminary Toxicity Test.

Page 12 is hereby amended.

This amendment does not affect the validity or interpretation of the data.

..... DATE: 23 APR 2004

P W Thompson HNC
Study Director

This amendment has been audited by Safepharm Laboratories Quality Assurance Unit and is considered to be an accurate account of the project.

..... DATE: 26 APR 2004

For Safepharm Quality Assurance Unit*

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*Authorised QA Signatures:

Head of Department:	JR Pateman CBiol MIBiol DipRQA FRQA
Deputy Head of Department:	JM Crowther MIScT MRQA
Senior Audit Staff:	JV Johnson BSc MRQA; G Wren ONC MRQA

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5. RESULTS

5.1 Preliminary Toxicity Test

The test material was non-toxic to the strains of bacteria used (TA100 and WP2uvrA$^-$). The test material formulation and S9-mix used in this experiment were both shown to be effectively sterile.

The number of revertant colonies for the toxicity assay were:

With (+) or without (-) S9-mix	Strain	Dose (µg/plate)										
		0	0.15	0.5	1.5	5	15	50	150	500	1500	5000
-	TA100	97	94	103	97	90	81	88	84	84	98P	83P
+	TA100	100	88	82	93	93	88	98	69	106	86P	86P
-	WP2uvrA$^-$	26	28	25	21	24	30	30	27	28	19P	29P
+	WP2uvrA$^-$	36	34	27	33	30	24	34	35	30	36P	32P

P: Precipitate

5.2 Mutation Test

Prior to use, the master strains were checked for characteristics, viability and spontaneous reversion rate (all were found to be satisfactory). These data are not given in the report. The S9-mix used in both experiments of the main test was shown to be sterile.

Results for the negative controls (spontaneous mutation rates) are presented in Table 1 and were considered to be acceptable. These data are for concurrent untreated control plates performed on the same day as the Mutation Test.

The individual plate counts, the mean number of revertant colonies and the standard deviations for the test material, vehicle and positive controls both with and without metabolic activation, are presented in Table 2 to Table 5 with the results also expressed graphically in Figure 1 to Figure 4.

Information regarding the equipment and methods used in these experiments as required by the Japanese Ministry of Economy, Trade and Industry and Japanese Ministry of Health, Labour and Welfare are presented in Appendix 1.

A history profile of vehicle and positive control values is presented in Appendix 2.

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The test material caused no visible reduction in the growth of the bacterial background lawn at any dose level. The test material was, therefore, tested up to the maximum recommended dose level of 5000 µg/plate. An oily precipitate was observed at and above 1500 µg/plate, this did not prevent the scoring of revertant colonies.

No significant increases in the frequency of revertant colonies were recorded for any of the bacterial strains, at any dose level either with or without metabolic activation.

All of the positive control chemicals used in the test induced marked increases in the frequency of revertant colonies thus confirming the activity of the S9-mix and the sensitivity of the bacterial strains.

6. CONCLUSION

The test material was considered to be non-mutagenic under the conditions of this test.

7. REFERENCES

1. Ames B N, Durston W E, Yamasaki E and Lee F D (1973b) *Proc. Natl. Acad. Sci. (USA)*, **70**, 2281-2285.

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S-Face IS-1009P: REVERSE MUTATION ASSAY "AMES TEST"
USING *SALMONELLA TYPHIMURIUM* AND *ESCHERICHIA COLI*

Table 1 Spontaneous Mutation Rates (Concurrent Negative Controls)

Range-finding Test

Number of revertants (mean number of colonies per plate)									
Base-pair substitution type						Frameshift type			
TA100		TA1535		WP2uvrA⁻		TA98		TA1537	
120		17		23		17		15	
142	(131)	17	(21)	26	(30)	17	(22)	20	(16)
130		28		40		32		13	

Main Test

Number of revertants (mean number of colonies per plate)									
Base-pair substitution type						Frameshift type			
TA100		TA1535		WP2uvrA⁻		TA98		TA1537	
82		13		27		22		7	
80	(80)	17	(15)	24	(23)	32	(32)	8	(8)
77		15		17		41		9	

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S-Face IS-1009P: REVERSE MUTATION ASSAY "AMES TEST"
USING *SALMONELLA TYPHIMURIUM* AND *ESCHERICHIA COLI*

Table 2 Test Results: Range-Finding Test– Without Metabolic Activation

Test Period		From: 19 February 2004						To: 22 February 2004			
With or without S9-Mix	Test substance concentration (µg/plate)	Number of revertants (mean number of colonies per plate)									
		Base-pair substitution type						Frameshift type			
		TA100		TA1535		WP2uvrA-		TA98		TA1537	
-	0	84 115 137	(112) 26.6#	27 15 17	(20) 6.4	22 18 24	(21) 3.1	28 24 29	(27) 2.6	10 11 19	(13) 4.9
-	50	123 138 113	(125) 12.6	10 25 25	(20) 8.7	21 18 18	(19) 1.7	18 18 18	(18) 0.0	13 17 17	(16) 2.3
-	150	113 112 122	(116) 5.5	10 15 33	(19) 12.1	21 23 22	(22) 1.0	23 24 25	(24) 1.0	13 15 13	(14) 1.2
-	500	100 117 138	(118) 19.0	20 21 16	(19) 2.6	13 20 20	(18) 4.0	33 34 31	(33) 1.5	11 20 9	(13) 5.9
-	1500	133 P 134 P 122 P	(130) 6.7	10 P 10 P 17 P	(12) 4.0	26 P 21 P 16 P	(21) 5.0	24 P 27 P 27 P	(26) 1.7	13 P 4 P 28 P	(15) 12.1
-	5000	114 P 116 P 131 P	(120) 9.3	15 P 26 P 18 P	(20) 5.7	32 P 33 P 8 P	(24) 14.2	33 P 41 P 24 P	(33) 8.5	18 P 26 P 10 P	(18) 8.0
Positive controls	Name	ENNG		ENNG		ENNG		4NQO		9AA	
	Concentration (µg/plate)	3		5		2		0.2		80	
S9-Mix	No. colonies per plate	539 509 484	(511) 27.5	697 501 242	(480) 228.2	831 904 982	(906) 75.5	238 251 224	(238) 13.5	818 821 746	(795) 42.5
-											

ENNG N-ethyl-N'-nitro-N-nitrosoguanidine
4NQO 4-Nitroquinoline-1-oxide
9AA 9-Aminoacridine
P Precipitate
Standard deviation

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S-Face IS-1009P: REVERSE MUTATION ASSAY "AMES TEST" USING *SALMONELLA TYPHIMURIUM* AND *ESCHERICHIA COLI*

Table 3 Test Results: Range-Finding Test– With Metabolic Activation

Test Period		From: 19 February 2004						To: 22 February 2004					
With or without S9-Mix	Test substance concentration (µg/plate)	Number of revertants (mean number of colonies per plate)											
		Base-pair substitution type						Frameshift type					
		TA100			TA1535			WP2uvrA-			TA98			TA1537		
+	0	120 143 108	(124) 17.8#		11 9 11	(10) 1.2		28 19 28	(25) 5.2		39 30 22	(30) 8.5		20 24 15	(20) 4.5	
+	50	113 115 144	(124) 17.3		8 12 10	(10) 2.0		22 26 33	(27) 5.6		27 29 33	(30) 3.1		19 16 10	(15) 4.6	
+	150	115 110 118	(114) 4.0		12 11 7	(10) 2.6		29 25 26	(27) 2.1		25 32 38	(32) 6.5		21 20 16	(19) 2.6	
+	500	125 138 110	(124) 14.0		12 10 20	(14) 5.3		37 31 26	(31) 5.5		28 28 32	(29) 2.3		19 26 20	(22) 3.8	
+	1500	130 P 113 P 136 P	(126) 11.9		11 P 11 P 5 P	(9) 3.5		31 P 27 P 29 P	(29) 2.0		17 P 29 P 37 P	(28) 10.1		13 P 12 P 17 P	(14) 2.6	
+	5000	104 P 119 P 145 P	(123) 20.7		15 P 10 P 19 P	(15) 4.5		36 P 29 P 27 P	(31) 4.7		33 P 32 P 28 P	(31) 2.6		8 P 22 P 29 P	(20) 10.7	
Positive controls	Name	2AA			2AA			2AA			BP			2AA		
	Concentration (µg/plate)	1			2			10			5			2		
S9-Mix +	No. colonies per plate	1436 1136 985	(1186) 229.6		303 262 288	(284) 20.7		437 435 378	(417) 33.5		217 247 202	(222) 22.9		400 276 267	(314) 74.3	

BP Benzo(a)pyrene
2AA 2-Aminoanthracene
P Precipitate
Standard deviation

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S-Face IS-1009P: REVERSE MUTATION ASSAY "AMES TEST" USING *SALMONELLA TYPHIMURIUM* AND *ESCHERICHIA COLI*

Table 4 Test Results: Main Test– Without Metabolic Activation

Test Period		From: 02 March 2004						To: 05 March 2004			
With or without S9-Mix	Test substance concentration (µg/plate)	Number of revertants (mean number of colonies per plate)									
		Base-pair substitution type						Frameshift type			
		TA100		TA1535		WP2uvrA-		TA98		TA1537	
-	0	106 107 116	(110) 5.5#	17 17 15	(16) 1.2	27 32 23	(27) 4.5	32 35 33	(33) 1.5	18 21 16	(18) 2.5
-	50	100 104 97	(100) 3.5	17 11 17	(15) 3.5	27 35 22	(28) 6.6	37 37 31	(35) 3.5	16 15 24	(18) 4.9
-	150	89 84 101	(91) 8.7	11 12 19	(14) 4.4	26 26 26	(26) 0.0	35 37 21	(31) 8.7	28 25 10	(21) 9.6
-	500	117 110 81	(103) 19.1	13 13 16	(14) 1.7	38 41 23	(34) 9.6	34 51 43	(43) 8.5	15 16 15	(15) 0.6
-	1500	90 P 92 P 87 P	(90) 2.5	30 P 16 P 22 P	(23) 7.0	28 P 26 P 23 P	(26) 2.5	20 P 37 P 22 P	(26) 9.3	21 P 20 P 12 P	(18) 4.9
-	5000	79 P 76 P 118 P	(91) 23.4	13 P 18 P 15 P	(15) 2.5	21 P 30 P 26 P	(26) 4.5	30 P 27 P 27 P	(28) 1.7	13 P 15 P 21 P	(16) 4.2
Positive controls	Name	ENNG		ENNG		ENNG		4NQO		9AA	
S9-Mix	Concentration (µg/plate)	3		5		2		0.2		80	
-	No. colonies per plate	554 709 543	(602) 92.8	200 183 143	(175) 29.3	705 702 548	(652) 89.8	135 205 163	(168) 35.2	407 693 658	(586) 156.0

ENNG N-ethyl-N'-nitro-N-nitrosoguanidine
4NQO 4-Nitroquinoline-1-oxide
9AA 9-Aminoacridine
P Precipitate
Standard deviation

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S-Face IS-1009P: REVERSE MUTATION ASSAY "AMES TEST" USING *SALMONELLA TYPHIMURIUM* AND *ESCHERICHIA COLI*

Table 5 Test Results: Main Test– With Metabolic Activation

Test Period		From: 02 March 2004						To: 05 March 2004			
With or without S9-Mix	Test substance concentration (µg/plate)	Number of revertants (mean number of colonies per plate)									
		Base-pair substitution type						Frameshift type			
		TA100		TA1535		WP2uvrA-		TA98		TA1537	
+	0	116 110 97	(108) 9.7#	20 13 19	(17) 3.8	32 29 36	(32) 3.5	33 33 44	(37) 6.4	28 17 17	(21) 6.4
+	50	112 110 90	(104) 12.2	18 7 9	(11) 5.9	43 40 30	(38) 6.8	39 34 35	(36) 2.6	26 26 24	(25) 1.2
+	150	113 98 85	(99) 14.0	8 6 12	(9) 3.1	24 25 38	(29) 7.8	36 37 34	(36) 1.5	16 24 13	(18) 5.7
+	500	85 85 92	(87) 4.0	12 8 23	(14) 7.8	40 43 30	(38) 6.8	37 35 38	(37) 1.5	22 22 27	(24) 2.9
+	1500	113 P 114 P 112 P	(113) 1.0	9 P 20 P 10 P	(13) 6.1	40 P 25 P 28 P	(31) 7.9	40 P 36 P 35 P	(37) 2.6	18 P 18 P 23 P	(20) 2.9
+	5000	88 P 109 P 85 P	(94) 13.1	19 P 9 P 13 P	(14) 5.0	34 P 35 P 33 P	(34) 1.0	39 P 39 P 48 P	(42) 5.2	18 P 18 P 25 P	(20) 4.0
Positive controls	Name	2AA		2AA		2AA		BP		2AA	
	Concentration (µg/plate)	1		2		10		5		2	
S9-Mix +	No. colonies per plate	1197 1083 1107	(1129) 60.1	235 249 211	(232) 19.2	508 675 C	(592) 118.1	150 170 162	(161) 10.1	195 192 220	(202) 15.4

BP	Benzo(a)pyrene
2AA	2-Aminoanthracene
C	Contaminated
P	Precipitate
#	Standard deviation

Figure 1 **Dose Response Curve: Range-Finding Test– Without Metabolic Activation**

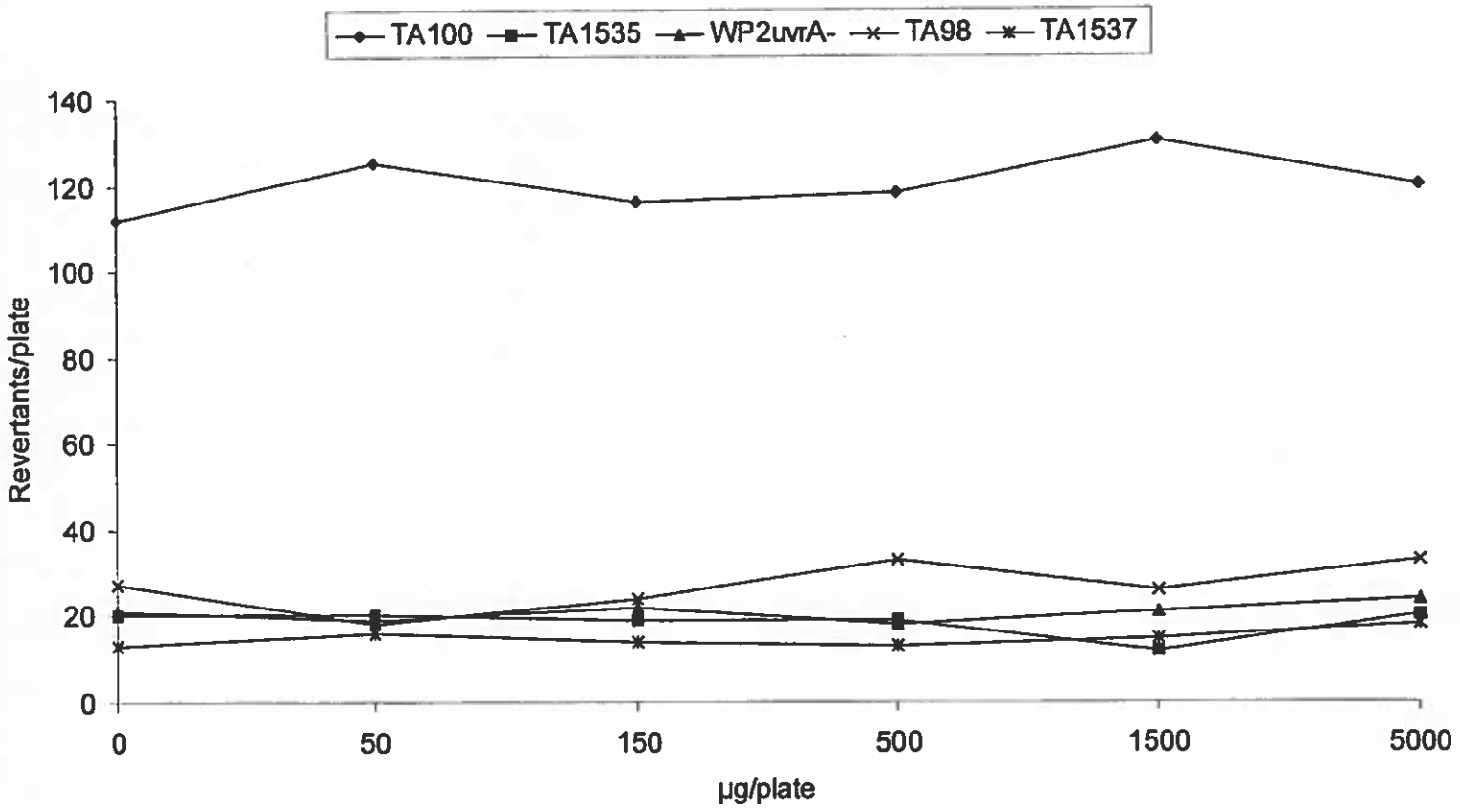

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Figure 2 **Dose Response Curve: Range-Finding Test– With Metabolic Activation**

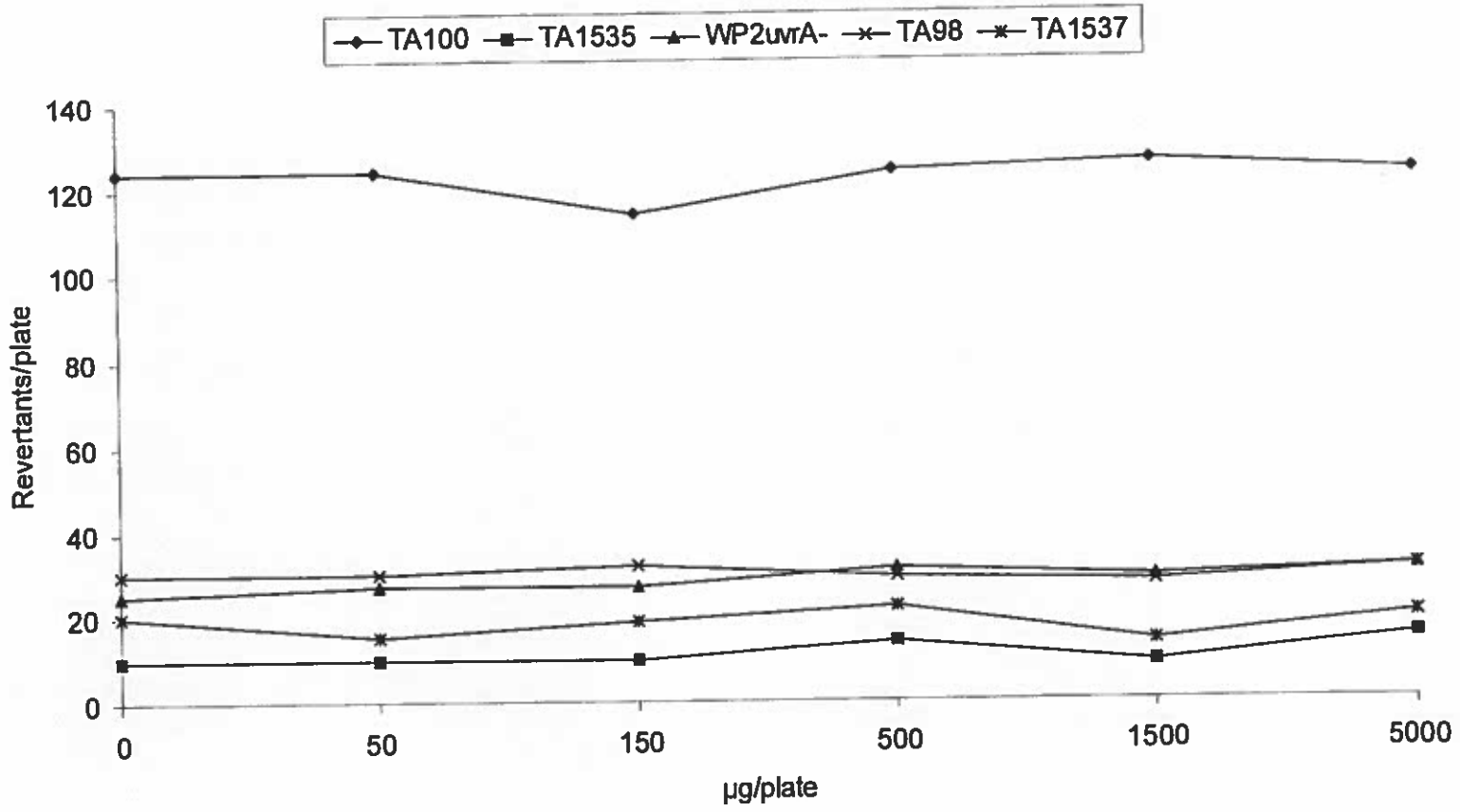

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Figure 3 **Dose Response Curve: Main Test– Without Metabolic Activation**

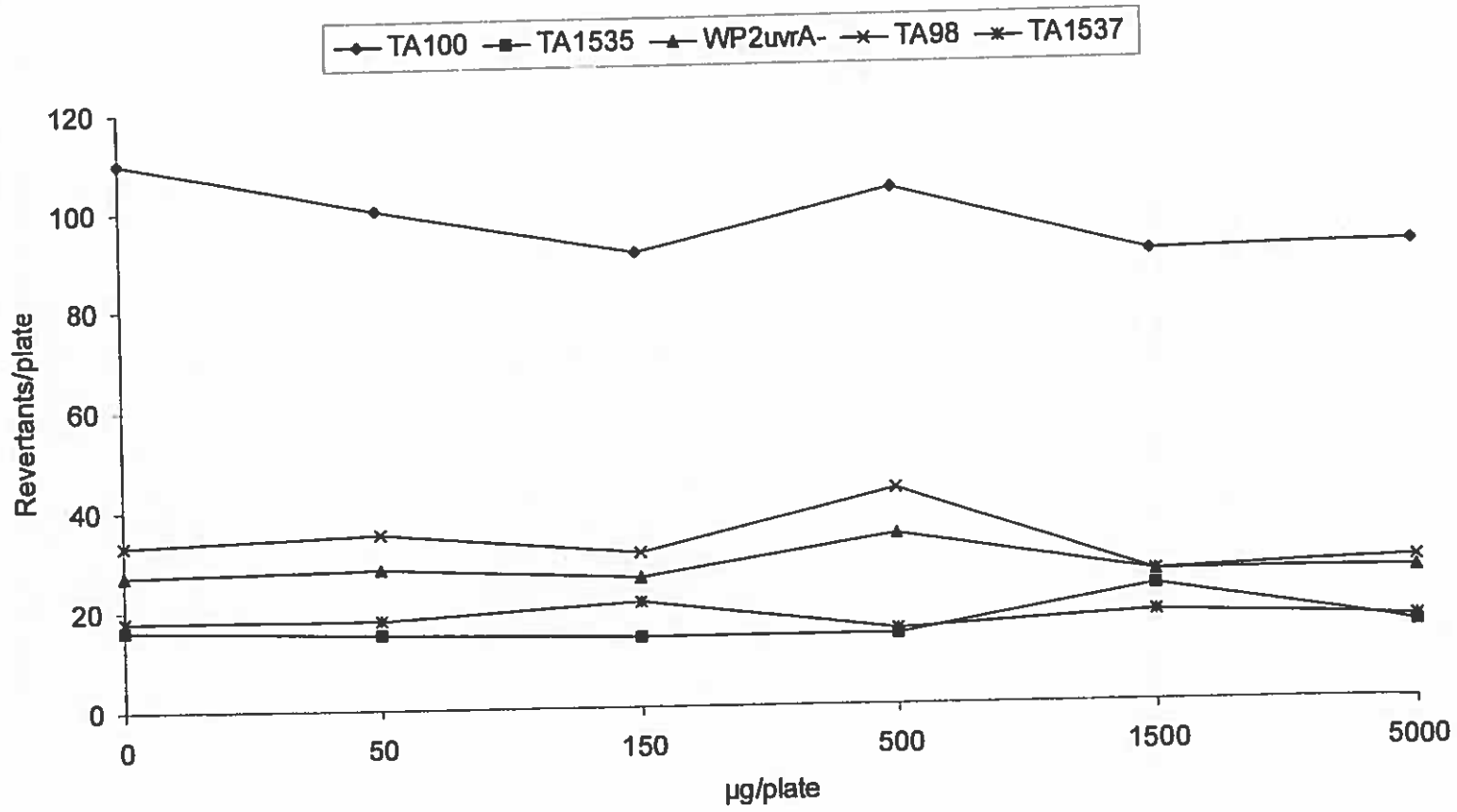

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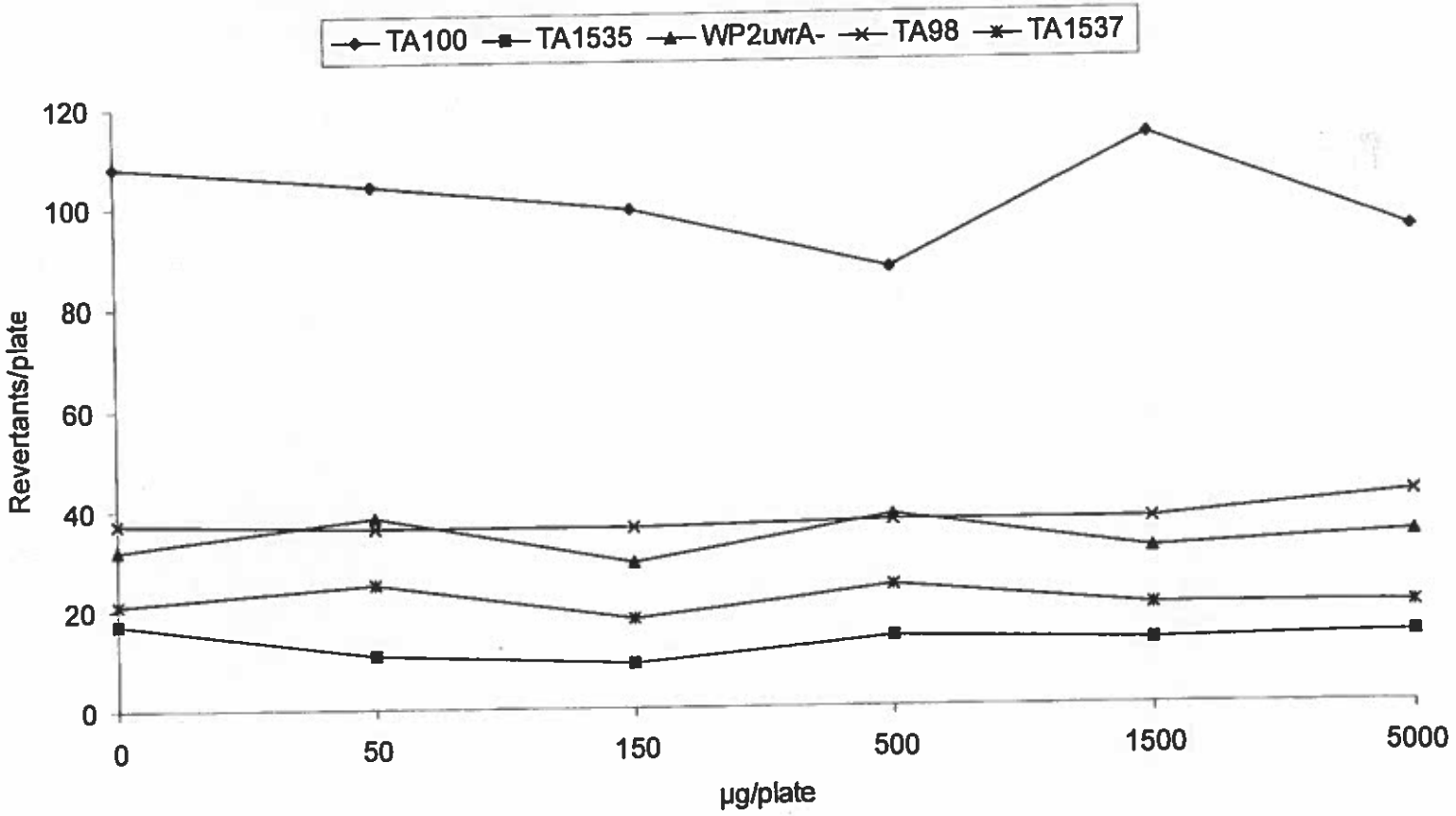
Figure 4 **Dose Response Curve: Main Test– With Metabolic Activation**



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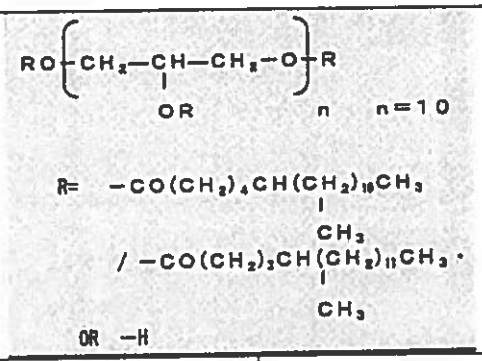
**S-Face IS-1009P: REVERSE MUTATION ASSAY "AMES TEST"
USING *SALMONELLA TYPHIMURIUM* AND *ESCHERICHIA COLI***

Appendix 1 Report of Results in Mutagenicity Test using Micro-organisms

1. GENERAL ITEMS (to be completed by the sponsor)

Name of the new chemical substance (IUPAC nomenclature)	Polyglyceryl-10 Nona Isostearate					
Other name	S-Face IS-1009P					
Structural formula or rational formula (or outline of manufacturing method, in case both are unknown)						
Purity of the new chemical substance tested	Over 99%		Lot No. of the new chemical substance tested		31112	
Name of impurities and concentration	Under 1% (no impurity listed)					
CAS No.	83-138-62-9		Vapour pressure		-	
Molecular weight	3150		Partition coefficient		-	
Melting point (°C)	-20		Appearance at ordinary temperature		Pale yellow slightly viscous liquid	
Boiling point (°C)	Over 350					
Stability	Stable under normal temperatures and pressure					
Degree of solubility in solvent	Solvent	Degree of solubility at 50 mg/ml	Stability in solvent	Solvent	Degree of solubility at 50 mg/ml	Stability in solvent
	Water	Immiscible	-	DMSO	Immiscible	-
	Acetone	Immiscible	-	DMF	Immiscible	-
	Ethanol	Immiscible	-	THF	Miscible at 200 mg/ml	*

Solubility data generated by the testing facility

* No visible change in test material formulations throughout each experiment, therefore, assumed stable

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S-Face IS-1009P: REVERSE MUTATION ASSAY "AMES TEST"
USING *SALMONELLA TYPHIMURIUM* AND *ESCHERICHIA COLI*

Appendix 1 (continued)

2. TESTER STRAINS

Tester strain	Obtained from	Date obtained
TA100	UCB*	4 August 1995
TA1535	UCB*	4 August 1995
WP2uvrA$^-$	BIBRA**	17 August 1987
TA98	UCB*	4 August 1995
TA1537	UCB*	4 August 1995

3. S-9 MIX

(1) Source of S9

(encircle the applicable number and fill in the relevant entries)

Made in-house or purchase	(1.) Made in-house	2. Purchase (Supplier)
Prepared on	25 October 2003	
Lot No. (in case of purchase)	-	
Storage temperature	-196°C	

(2) Preparation of S9

Animal used		Inducing substance	
Species strain	Rat Sprague-Dawley	Name	Phenobarbitone/β-naphthoflavone
Sex	Male	Administration method	Oral
Age (in weeks)	7 weeks	Administration period and amount (mg/kg bodyweight/day)	3 days 80/100 mg per kg per day
Weight	~ 250g		

(3) Composition of S9-Mix

Constituents	Amount in 1 ml S9-Mix	Constituents	Amount in 1 ml S9-Mix
S9	0.1 ml	NADPH	4.0 µmol
$MgCl_2$	8.0 µmol	NADH	4.0 µmol
KCl	33.0 µmol	Na-phosphate buffer	100.0 µmol
Glucose-6-phosphate	5.0 µmol	Others ( )	
Glucose-6-phosphate dehydrogenase			

* UCB: University of California at Berkeley
** BIBRA: British Industrial Biological Research Association

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S-Face IS-1009P: REVERSE MUTATION ASSAY "AMES TEST"
USING *SALMONELLA TYPHIMURIUM* AND *ESCHERICHIA COLI*

Appendix 1 (continued)

4. PREPARATION OF THE TEST MATERIAL IN SOLUTION

(encircle the applicable response)

	Name	Supplier	Lot No.	Grade	Purity (%)
Solvent used	THF	Fisher Scientific	0397765	HPLC	99.99
Reason for selection of solvent	Good solution at 200 mg/ml				
Appearance of the test material preparation	(Solution)	Suspension		Others ( )	
Suspension and other methods when test substance difficult to dissolve	Solution achieved by action on an autovortex mixer				
Storage time and temperature of solution from preparation until use	1 hour and 20 minutes at room temperature (range-finding test) 1 hour and 25 minutes at room temperature (main test)				
Conversion of purity	Yes	(No)			

5. CONDITIONS OF PRE-CULTURE ETC.

(1) Conditions

	Name	Manufacturer	Lot No.
Nutrient Broth	Oxoid NB No.2	Oxoid Ltd.	301083 04/08 (range-finding test) 323907 11/08 (main test)
Period of pre-culture	10 hr		
Culture flask (form, size)	Costar 75 cm^2 tissue culture flasks		
Amount of culture medium	5 ml	Amount of strain inoculated	20µl

(2) Cell viability at the end of pre-culture

		Base-pair substitution type			Frameshift type		
		TA100	TA1535	WP2uvrA⁻	TA98	TA1537	
Cell number (x 10^9/ml)	Range-finding test	2.4	4.5	5.7	4.0	3.7	
	Main test	1.7	3.4	7.4	3.4	5.2	
Count method (encircle the applicable number)	1. Conversion from O.D. value (2.) Stepwise dilution method 3. Others ( )						

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S-Face IS-1009P: REVERSE MUTATION ASSAY "AMES TEST"
USING *SALMONELLA TYPHIMURIUM* AND *ESCHERICHIA COLI*

Appendix 1 (continued)

6. MINIMUM GLUCOSE AGAR PLATE MEDIUM

(encircle the applicable number and fill in the relevant entries)

Made in-house or purchase	1. Made in-house (2.) Purchase (ILS Ltd)
Prepared on	10 February 2004 (range-finding test) 24 February 2004 (main test)
Lot No. in case of purchase	850942-02 07/08

7. TEST METHOD (encircle the applicable number and fill in the relevant entries)

(1) Test Method and the reason why it was selected

Test method used	1. Pre-incubation method (2.) Plate incorporation method 3. Others ( )
Reason in case of others	-

(2) Test Conditions

		Pre-incubation	Plate Method
Composition	Bacterial suspension	ml	0.1 ml
	Test substance solution	ml	0.025 ml
	Na-phosphate buffer	ml	0.5 ml
	S9-mix (in case of metabolic activation method)	ml	0.5 ml
	Top agar solution	ml	2.0 ml
	Others ( )	ml	-
Pre-incubation	Temperature	°C	-
	Time	min	-
Incubation	Temperature	°C	37°C
	Time	hours	48 hours

8. COUNT METHOD OF COLONIES (Encircle 1 and 2 if both methods used)

Count method	1. By hand (2.) Colony counter
Conduction of correction	1. No (2.) Yes (Correction method: Area and miscount correction)

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S-Face IS-1009P: REVERSE MUTATION ASSAY "AMES TEST"
USING *SALMONELLA TYPHIMURIUM* AND *ESCHERICHIA COLI*

Appendix 1 (continued)

9. TEST RESULTS

(1) The results should be reported on the attached form

(2) Judgement of the results

Judgement (encircle one)	Positive	(Negative)
Reason for judgement: No significant increase in the frequency of revertant colonies was recorded for any bacterial strain used with any dose of the test material in two separate experiments either with or without metabolic activation.		

(when judged as positive, attach a table for relative activity)

(3) Referential matters

An oily precipitate was observed at and above 1500 µg/plate, this did not prevent the scoring of revertant colonies. The test material was insoluble in a range of solvent vehicles at 50 mg/ml but was soluble in Tetrahydrofuran at 200 mg/ml in solubility checks performed in-house. Tetrahydrofuran was therefore selected as the vehicle of choice. This solvent vehicle is also a suitable solvent for use in the Ames Test (6). Prior to use, the solvent was dried using molecular sieves (sodium alumino-silicate) ie 2 mm pellets with a nominal pore diameter of 4×10^{-4} microns.

For a substance to be considered positive in this test system, it should have induced a reproducible, dose-related and statistically (Dunnett's method of linear regression (5)) significant increase in the revertant count in at least one strain of bacteria.

Phenobarbitone/β-naphthoflavone was used to induce higher enzyme levels in the rat liver S9. The S9-mix used in each experiment of this study was shown to be sterile. The test material formulations showed no evidence of contamination when tested for sterility during the preliminary toxicity test. The use of approximate half-log dilutions instead of twofold dilutions was agreed with the JMOL in January 1995.

[REMARKS] "Referential matters" – (fill in the view etc of the Study Director on the test results)

10. OTHERS

	Name	Safepharm Laboratories Ltd.		
	Address	Shardlow Business Park, Shardlow, Derbyshire, DE72 2GD		Tel No: 0044 1332 792896 Fax No: 0044 1332 799018
Study Director	Title	Senior Genetic Toxicologist	Name	P W Thompson HNC
	Years of experience: 18			
Study number	432/068			
Test dates	1. Each experiment date:	Experiment 1: From: 19 February 2004 To: 22 February 2004 Experiment 2: From: 02 March 2004 To: 05 March 2004		
	2. Test Period:	From: 09 January 2004 To: 30 MAR 2004		

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S-Face IS-1009P: REVERSE MUTATION ASSAY "AMES TEST"
USING *SALMONELLA TYPHIMURIUM* AND *ESCHERICHIA COLI*

Appendix 2 History Profile of Vehicle and Positive Control Values

COMBINED VEHICLE AND UNTREATED CONTROL VALUES 2002

Strain	TA100		TA1535		WP2uvrA⁻		TA102		TA98		TA1537		WP2uvrA⁻pKM101	
S9-Mix	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Mean	91	100	20	17	23	27	322	349	22	35	12	17	169	215
SD	16.6	18.0	5.5	4.3	4.8	5.3	47.7	34.6	5.2	6.8	4	4.7	42.8	50.2
Min	61	68	8	8	10	14	183	255	11	13	4	5	92	131
Max	160	162	38	37	47	45	414	449	44	66	29	38	234	277
Values	939	742	912	718	685	521	329	210	946	749	918	718	25	11

POSITIVE CONTROL VALUES 2002

Strain	TA100		TA1535		WP2uvrA⁻		TA102		TA98		TA1537		WP2uvrA⁻pKM101	
S9-Mix	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Mean	460	1880	347	310	621	866	1126	858	136	239	1953	453	1384	2608
SD	118.2	594.3	204.0	119.7	235.5	350.5	289.4	169.5	38.2	74.7	803.1	145.9	333.7	1109.6
Min	235	499	80	91	185	210	686	568	66	91	486	140	960	186
Max	952	3397	1385	810	1295	3406	2456	2009	323	507	4622	1365	2073	3900
Values	190	190	188	186	169	168	117	118	192	192	188	184	14	14

COMBINED VEHICLE AND UNTREATED CONTROL VALUES 2003

Strain	TA100		TA1535		WP2uvrA⁻		TA102		TA98		TA1537	
S9-Mix	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Mean	98	106	20	15	23	28	331	365	20	30	10	14
SD	20.7	23.4	8.5	3.9	5.6	7	38.2	29	5.1	7.1	3.9	5
Min	61	64	8	7	11	13	233	264	10	15	3	3
Max	165	191	45	39	44	57	400	422	52	53	26	32
Values	882	735	838	690	686	539	273	170	877	721	838	678

POSITIVE CONTROL VALUES 2003

Strain	TA100		TA1535		WP2uvrA⁻		TA102		TA98		TA1537	
S9-Mix	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Mean	429	1529	297	305	649	656	1068	911	174	249	1273	370
SD	108.1	493.1	156.8	82.6	175.7	264.4	230.0	183.8	64.6	72.2	623.1	109.9
Min	197	485	98	162	273	136	698	559	64	87	262	148
Max	849	3662	1099	625	1198	1373	1886	1887	410	465	3704	759
Values	160	160	156	156	150	150	102	100	162	162	155	155

SD = Standard deviation
Min = Minimum value
Max = Maximum value

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Appendix 3 Statement of GLP Compliance in Accordance with Directive 88/320/EEC

THE DEPARTMENT OF HEALTH OF THE GOVERNMENT OF THE UNITED KINGDOM

GOOD LABORATORY PRACTICE

STATEMENT OF COMPLIANCE
IN ACCORDANCE WITH DIRECTIVE 88/320 EEC

LABORATORY
SafePharm Limited
Shardlow Business Park,
London Road,
Shardlow,
Derbyshire,
DE72 2GD

TEST TYPE
Analytical/Clinical
Chemistry
Environmental tox.
Environmental fate
Mutagenicity
Phys./Chem. tests
Toxicology

DATE OF INSPECTION

2nd December 2002

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

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

Roger G. Alexander
13/2/03

Dr. Roger G. Alexander
Head, UK GLP Monitoring Authority

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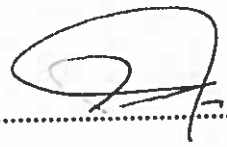
SAFEPHARM LABORATORIES LIMITED

S-Face IS-1009P

SPL PROJECT NUMBER: 432/068

I verify that this is an exact copy of the original report which is located in the Archives of SafePharm Laboratories Limited, Derby, UK.

P W Thompson HNC
Study Director ..... 

DATE ..... 31 MAR 2004 .....

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ACCORDING TO THE G.L.P.
DESCRIBED in the directive 2004/9/EC
AND C(81)30 council decision O.E.C.D.
BY G.I.P.C. 10/23/98

Phycher
Bio développement

Assessment of contact phototoxicity on the albino guinea-pig

Final report: PTC-PH-06/0283
SPL project: 0432/0091

TEST ITEM
S-Face IS-1009P
Batch n° 509103

❑ **STUDY MONITOR**	:	**SAFEPHARM Laboratories** **Mr A. POOLES** **P.O. Box 45** **Derby DE1 2BT** **England**
❑ **SPONSOR**	:	**Sakamoto Yakuhin Kogyo Co. Ltd** **1-20, Rinkaicho** **Izumiotsu-city** **OSAKA 595-01075** **Japan**
❑ **AUTHOR**	:	**Mr F. RICHEUX**
❑ **TEST FACILITY**	:	**PHYCHER Bio-Développement** **18, Chemin Lou Triball** **ZI de Toctoucau** **F-33611 CESTAS Cedex**

Report of 11 pages

Cestas, 06 November 2006

Siège social : 18, chemin de Lou Triball ZI de Toctoucau 33611 CESTAS Cedex - ✆ 05 57 97 16 80 - Fax 05 57 97 16 81
info@phycher.com - www.phycher.com
S.A.R.L. AU CAPITAL DE 60 979,61 Euros - APE 7312 - SIRET 402 924 088 000 39

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QUALITY ASSURANCE ATTESTATION

I, the undersigned Bernard Benech, Quality Assurance unit of PHYCHER *Bio développement*, attest that the study **PTC-PH-06/0283** was submitted to the inspection of Quality Assurance.

The routine inspections of the toxicity tests performed within the laboratory of PHYCHER *Bio développement*, are carried out as a control process of different major technical phases concerning at least one similar test. The frequency is once a term or more. Dates of technical phases inspections relating to this study type are given below:

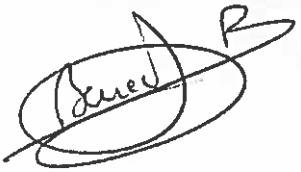
Date of inspection	: 28 August 2006 (PTC-PH-06/0283) *Treatment 30 minutes before irradiation*
Date of reporting to Study Director	: 29 August 2006
Date of reporting to Management	: 29 August 2006

This report has been audited by PHYCHER *Bio développement* Quality Assurance unit. It is considered to be an accurate account of the data generated and the application of the operating procedures in use within the laboratory:

	Draft report	Final report
Date of audit report inspection	: 17 October 2006	06 November 2006
Date of reporting to Study Director	: 17 October 2006	06 November 2006
Date of reporting to Management	: 17 October 2006	06 November 2006

Date: 06 November 2006

Bernard BENECH
Quality Assurance Manager



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G.L.P. COMPLIANCE STATEMENT

I, the undersigned, François Richeux, Study director, certify that the study **PTC-PH-06/0283** was performed on the premises of the Laboratory PHYCHER *Bio développement*.

I, certify that the objectives laid down in the technical protocol were achieved and no undesirable event occurred to affect the quality or the integrity of the study. I consider the data generated to be valid. This report fully and accurately reflects the operating procedures used and data generated.

The entire work was devised and performed in compliance with the principles of the Good Laboratory Practice (G.L.P.), as defined in *the directive 2004/9/EC, the O.E.C.D. ruling relative to the mutual acceptance of data in the evaluation of chemical substances (C(81) 30 (final) Appendix 2 - May 12th, 1981; C (97) 186, November 26th, 1997)*, and in the French decree *n° 98-1312 dated December 31st, 1998 of the Journal Officiel de la République Française (see certificate of G.L.P. in appendix).*

Date: 06 November 2006

Dr. François Richeux
Study director
Expert Toxicologist (PhD, ERT)

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SUMMARY AND CONCLUSION OF THE STUDY

In order to assess the phototoxic potential of **S-Face IS-1009P** a phototoxicty test was performed in 10 female albino Dunkin-Hartley guinea pigs in accordance with *C. Montastier, Ph Michelon: Predictive studies of the safety of cosmetics. Preprints of the XIVth IFSCC Congress, Barcelona 1986, Vol. 1 p 91-102. M. Jeanmougin: Photodermatose et Photoprotection, Produits Roche S.A., Neuilly-sur-Seine, Ed. Deltacour Paris.*

The test item **S-Face IS-1009P** was applied, as supplied at the dose of 0.5 mL, on the right flank of 10 guinea-pigs 30 minutes before irradiation at the Maximum Non Erythemateous Dose. The irradiation doses have been respectively 7000 mJ/cm² for the UV-A and 150 mJ/cm² for the UV-B.
Cutaneous reactions, i.e. erythema and oedema formation were evaluated 24 and 48 hours after the irradiation.

No macroscopic cutaneous reaction was attributable to photoirritation as compared with the reactions noticed on the references sites (8-Methoxypsoralen: positive reference and item alone: negative reference).

In conclusion, in view of these results, under these experimental conditions, we are able to conclude that the test item **S-Face IS-1009P is no phototoxic.**

Date: 06 November 2006

Scientific Direction PHYCHER *Bio développement*

Dr. Christian TOUSSAINT
Pharmacologist
(Hdr)

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TEST REPORT

1 - TEST ITEM

- Sponsor's identification: **S-Face IS-1009P**
- Container : plastic flask (n=1)
- Quantity : 128.37 g (container + contents)
- Batch n° : 509103
- CAS n° : 83138-62-9
- Composition: Polyglyceryl-10 Nonaisostearate
- Date received: 16 August 2006
- Form : liquid
- Colour : pale yellow
- Storage : Room temperature
- Purity : >99%
- Expiry date: -

It was identified under the code number: **PH-06/0283.**

The test item was considered as 100% for the study.
Informations concerning the identity, purity and stability of the test item are the responsibility of the Sponsor. An information data sheet concerning the item was provided by the study monitor.

2 - METHODOLOGY

The experimentation phase was carried out between 28 August 2006 and 30 August 2006, in *PHYCHER Bio Développement* – 18 chemin de Lou Tribail – 33611 Cestas Cedex according to the experimental protocol *Ref. PTC-version September 1999,* established in accordance with:
C. Montastier, Ph Michelon: Predictive studies of the safety of cosmetics. Preprints of the XIVth IFSCC Congress, Barcelona 1986, Vol. 1 p 91-102. M. Jeanmougin: Photodermatose et Photoprotection, Produits Roche S.A., Neuilly-sur-Seine, Ed. Deltacour Paris.

2.1 - Principle

The test is carried out to evaluate the risk of cutaneous reactions on the guinea-pig further to application of the item, followed by exposure to ultraviolet radiation.
This is an early detection research. The results obtained provide an estimate of the phototoxic potential. Extrapolation to humans implies to usual reservations, given that the model animal-based.

2.2 - Animals

Ten female albino guinea-pigs of Dunkin-Hartley strain were supplied by Charles RIVER (F-69592 L'Arbresle). They were exposed to the test item after a 5-day minimal acclimatisation period. At the beginning of the test, the animals weighed between 346 g and 424 g and were 7-week old.

Identification of animals: N° C5887 to N° C5896.

The environmental conditions were:

Temperature:	between 21 °C and 24 °C
Relative humidity:	between 40% and 70%

2.3 - Operating method

2.3.1 - Dose

The test item was applied as supplied at a dose of 0.5 mL over the whole right-hand flank of each animal.
Thirty minutes after the treatment, the animals were subjected to ultraviolet radiations (UV-B first and then UV-A).
Animals were returned to their cages after radiation.

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2.3.2 - Sources of radiations

The animals were irradiated with the irradiation source VLX 3W (Biotronic, Vilbert Lourmat) at the Maximal Non Erythemateous Dose (M.N.E.D) 7000 J/cm^2 for UV-A and 150 mJ/cm^2 for the UV-B.

2.3.3 - Treatment

The back of each animal was divided into 6 sections of approximatively 1 cm^2 (3 on either side of the spine) and was treated as follows:

1	2
3	4
5	6

1. Radiation only, in order to verify the M.N.E.D. at UV-B radiation
2. Test item with UV-B radiation
3. Positive reference (8-MOP = 8-Methoxypsoralen) with UV-A radiation
4. Test item without radiation
5. Radiation only, in order to verify the M.N.E.D. at UV-A radiation
6. Test item with UV-A radiation

3 - RESULTS

A macroscopic evaluation of the cutaneous reactions (erythema and oedema) was conducted 24 and 48 hours after the irradiation.

No macroscopic cutaneous reaction was attributable to photoirritation as compared with the reactions noticed on the reference sites (8-Methoxypsoralen: positive reference and item alone: negative reference).

The individual results after macroscopic examinations at 24 and 48 hours, the cumulated total and the mean values were recorded in the *tables 1 and 2* (pages 8 and 9).

4 - CONCLUSION

The results obtained, under these experimental conditions, enable to conclude that the test item **S-Face IS-1009P is no phototoxic.**

5 - ARCHIVES

All the original data related and the final report related to the study will be stored for a period of 10 years in the company under the reference **PTC-PH-06/0283**. After this period, the sponsor's instructions will be applied.

6 - PROTOCOL ADHERENCE

No deviation occurred during this study.

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TABLE OF COTATIONS

TEST ITEM: S-Face IS-1009P

Application: topical application of the test item

Application date (D0): 28 August 2006 Animals: 10

Table 1

MACROSCOPIC EXAMINATIONS
ERYTHEMA AND OEDEMA

Reading at 24 hours

Irradiations / Animals	UV-B		Test item + UV-B		8-MOP + UV-A		Test item		UV-A		Test item + UV-A	
GUINEA PIG N°	E	O	E	O	E	O	E	O	E	O	E	O
C5887	0	0	0	0	2	2	0	0	0	0	0	0
C5888	0	0	0	0	2	2	0	0	0	0	0	0
C5889	0	0	0	0	2	2	0	0	0	0	0	0
C5890	0	0	0	0	2	2	0	0	0	0	0	0
C5891	0	0	0	0	2	2	0	0	0	0	0	0
C5892	0	0	0	0	2	2	0	0	0	0	0	0
C5893	0	0	0	0	2	2	0	0	0	0	0	0
C5894	0	0	0	0	2	2	0	0	0	0	0	0
C5895	0	0	0	0	2	2	0	0	0	0	0	0
C5896	0	0	0	0	2	2	0	0	0	0	0	0
Cumulated total	0		0		40		0		0		0	
Average	0		0		4		0		0		0	

E: Erythema O: Oedema UV-A : ultraviolet A UV-B : ultraviolet B

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TABLE OF COTATIONS

TEST ITEM: S-Face IS-1009P

Application: topical application of the test item

Application date (D0): 28 August 2006 Animals: 10

Table 2

MACROSCOPIC EXAMINATIONS
ERYTHEMA AND OEDEMA

Reading at 48 hours

Irradiations / Animals	UV-B		Test item + UV-B		8-MOP + UV-A		Test item		UV-A		Test item + UV-A	
GUINEA PIG N°	E	O	E	O	E	O	E	O	E	O	E	O
C5887	0	0	0	0	2	2	0	0	0	0	0	0
C5888	0	0	0	0	2	2	0	0	0	0	0	0
C5889	0	0	0	0	2	2	0	0	0	0	0	0
C5890	0	0	0	0	2	2	0	0	0	0	0	0
C5891	0	0	0	0	2	2	0	0	0	0	0	0
C5892	0	0	0	0	2	2	0	0	0	0	0	0
C5893	0	0	0	0	2	2	0	0	0	0	0	0
C5894	0	0	0	0	2	2	0	0	0	0	0	0
C5895	0	0	0	0	2	2	0	0	0	0	0	0
C5896	0	0	0	0	2	2	0	0	0	0	0	0
Cumulated total	0		0		40		0		0		0	
Average	0		0		4.0		0		0		0	

E: Erythema O: Oedema UV-A : ultraviolet A UV-B : ultraviolet B

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APPENDIX

Certificate of G.L.P. (1 page)

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Liberté • Égalité • Fraternité
RÉPUBLIQUE FRANÇAISE

GROUPE INTERMINISTERIEL DES PRODUITS CHIMIQUES

Paris, le 13 DEC. 2005

OBJET : Evaluation de la conformité aux B.P.L. selon la directive 88/320/C.E.E.

Consécutivement à votre engagement vis à vis du GIPC et du COFRAC et en application du décret n° 81-278 du 25 mars 1981 portant création d'un groupe interministériel des produits chimiques, modifié notamment par le décret 90-206 du 7 mars 1990 et par le décret n° 98-1312 du 31 décembre 1998 concernant les bonnes pratiques de laboratoires, je vous confirme que le GIPC, au vu des résultats du contrôle exercé par le Comité français d'accréditation (COFRAC) - Section Laboratoires a décidé pour votre installation du statut suivant :

respect des principes de B.P.L.
Domaines de reconnaissance :
☐ 2 - **études de toxicité**
☐ 3 - **études de mutagénicité**

Date d'inspection : 3 octobre 2005

☐ **inspection de contrôle périodique (i.p)**
☐ **inspection d'extension (i.e)**

Date de décision du G.I.P.C. : 9 décembre 2005
Date de prise d'effet : 3 octobre 2005
Année de première conformité : 1998
Durée de validité : 18 mois

Le Président, p. o.

Pierre CREYSSEL
Conseiller d'Etat h.

PHYCHER BIO Developpement
ZI de Toctoucau
18, chemin de Lou Tribail
33611 CESTAS Cedex

Secrétariat général du GIPC - DGE- Sinap - 12, rue Villiot - 75572 Paris cedex 12
Téléphone : 01 53 44 96 10 – Télécopie : 01 53 44 91 72