TEST REPORT

STUDY REPORT NUMBER: MB-**DSA-01-01**-E-16-10-A1

(Replacement to study report number MB-DSA-01-01-E-16-10)

Study Completion Date: 09 NOV 2010

Title: DERMAL SENSITIZATION ASSAY

Sky Gel (Ultrasound Gel)

Study Sponsor	**Testing Facility**
ISD Meditech Sdn Bhd No. 12, Jalan Teras 1, Taman Industri Teras, 43300 Balakong, Selangor, Malaysia.	**Makmal Bioserasi** INBIOSIS Universiti Kebangsaan Malaysia 43600 UKM Bangi Selangor Malaysia.

Study Director : Assoc Prof Dr Md Anuar Osman

Quality Assurance Personnel : Nurdiana Ishak

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- This report contains 18 pages.
- This test report concerns only the product being tested.

Dermal Sensitization Assay

Study Director	**Signature**
Assoc. Prof. Dr. Md. Anuar Osman *DVM (Pak), M. Sc (W. Aust), Ph.D (Murdoch)*	
	Date: 9/11/10
Sponsor	**Signature**
ISD MEDITECH SDN BHD (915725-T) NO. 12, JALAN TERAS 1, TAMAN INDUSTRI TERAS 43300 BALAKONG, SELANGOR TEL 03-89616880 FAX 03-89619880 ISD Meditech Sdn Bhd	
	Date: 15/11/2010

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TEST REPORT - Dermal Sensitization Assay - Modified Buehler Method

TABLE OF CONTENTS		**PAGE**

SUMMARY

Dermal Sensitization Assay – Modified Buehler Method

Protocol reference : PR-DSA-01-01

Method used : Consumer Product Safety Commission, Title 16, Chapter II, Part 1500

Study completion date : 9 Nov 2010

Study reference number : DSA-01-01-01-01-10

Study report number : MB-DSA-01-01-E-16-10-A1
(Replacement to study report number MB-DSA-01-01-E-16-10)

Job number : E-16-10

Test material : Sky Gel (Ultrasound Gel)

Condition of use : As supplied

1. OBJECTIVE

To determine the potential of test material, Sky Gel (Ultrasound Gel) to elicit a skin sensitization reaction.

2. EXPERIMENTAL PROCEDURE

Animals: Fifteen albino guinea pigs (10 for test animals and 5 for negative control) with initial weight of 430 g to 500 g.

Acclimatization period	27 April – 3 May 2010
Induction phase	
Patch application 1	3 May 2010
Patch application 2	5 May 2010
Patch application 3	7 May 2010
Patch application 4	10 May 2010
Patch application 5	12 May 2010
Patch application 6	14 May 2010
Patch application 7	17 May 2010
Patch application 8	19 May 2010
Patch application 9	21 May 2010
Challenge phase	
Patch application (challenge)	4 June 2010
6 hours	4 June 2010
24 hours	5 June 2010
48 hours	6 June 2010
End of test	6 June 2010

TEST REPORT

1.0 SPONSOR OF TEST MATERIAL

1.1	**Name**	: ISD Meditech Sdn Bhd
1.2	**Address**	: No. 12, Jalan Teras 1, Taman Industri Teras, 43300 Balakong, Selangor, Malaysia.
1.3	**Study report number**	: MB-DSA-01-01-E-16-10-A1 *(Replacement to study report number MB-DSA-01-01-E-16-10)*
1.4	**Job number**	: E-16-10

2.0 DETAILS OF TEST MATERIAL

2.1	**Name**	: Sky Gel (Ultrasound Gel)
2.2	**Intended use of test material**	: Ultrasound screening procedure
2.3	**Test material reference**	: MD16-E0410
2.4	**Study reference number**	: DSA-01-01-01-01-10
2.5	**Lot number**	: N/A
2.6	**Date received**	: 16 April 2010
2.7	**Expiry date**	: N/A
2.8	**Appearance**	: Gel
2.9	**Colour**	: Blue
2.10	**Quantity**	: 2 packets
2.11	**Storage**	: Refrigerated
2.12	**Solubility**	: Water
2.13	**Condition of use**	: As supplied
2.14	**Dates of study**	: 3 May 2010 – 6 June 2010
2.15	**End of test**	: 6 June 2010
2.16	**pH**	: N/A

3.0 LABORATORY FACILITY

3.1	**Name**	: Makmal Bioserasi
3.2	**Address**	: INBIOSIS, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor, Malaysia.

3.5 Study timetable

3.5.1	Acclimatization period	27 April – 3 May 2010
3.5.1.1	Induction phase	
	Patch application 1	3 May 2010
	Patch application 2	5 May 2010
	Patch application 3	7 May 2010
	Patch application 4	10 May 2010
	Patch application 5	12 May 2010
	Patch application 6	14 May 2010
	Patch application 7	17 May 2010
	Patch application 8	19 May 2010
	Patch application 9	21 May 2010
3.5.1.2	Challenge phase	
	Patch application (challenge)	4 June 2010
	6 hours	4 June 2010
	24 hours	5 June 2010
	48 hours	6 June 2010
3.5.2	End of test	6 June 2010

3.6 Authorization

Approval from the Universiti Kebangsaan Malaysia's Animal Ethics Committee (UKMAEC) for Project Title: Safety assessment of cosmetics, topical traditional preparations, plastics, gloves, synthetic fibers and medical devices (Animal study).

3.7 Environment and Husbandry

3.7.1 Species and strain

Dunkin Hartley strain guinea pigs.

3.7.2 Supplier

Lab Animal Resource Unit, Faculty of Medicine, Universiti Kebangsaan Malaysia Kuala Lumpur.

3.7.3 Number of animal and sex

Fifteen guinea pigs; ten for test group (6 males and 4 non pregnant females) and five for negative control (all females).

3.7.4 Body weight at initiation of treatment

Minimum: 430 g; Maximum: 500 g.

3.7.5 Age

Adult.

3.7.6 Housing

The animals were housed in a plastic caging of internal dimension: 56 cm by 34 cm by 20 cm. Two animals were housed per cage.

3.7.6.1 Animal room temperature

20^0C to 26^0C.

4.0 TEST METHOD

4.1 Name of test: Dermal Sensitization Assay – Modified Buehler Method

Summary

The test material is topically applied to the left side of ten healthy guinea pigs for 6 hours, three times per week for a three-week induction period. Fourteen days after the last induction, a challenge dose is applied in a similar manner onto a naive site on the right side of each animal.

The skin is examined for allergic reactions, and the intensity of the reaction scored at 6, 24 and 48 hours after patch application.

A negative control group (five animals) is maintained under the same environmental condition and treated with the test material at challenge only.

Based on the results of the study, the test material is not considered as a contact sensitizer. The positive response observed in the historical positive control validation study with 1-Chloro-2, 4-Di-Nitrobenzene (DNCB) validates the test system used.

4.2 Objective

To demonstrate the potential of the device in eliciting a delayed hypersensitivity (Type IV) immunological response through contact with the skin. The reaction is primarily due to substances that leach out of a material.

4.2.1 Significance and use

In selecting a new material for human contact in medical applications, it is important to ensure that the material will not stimulate the immune system to produce an allergic reaction. The skin sensitization study also provides the use of test material extracts. The rationale for this is based on the fact that guinea pig has been shown to be the best animal model to demonstrate contact dermatitis in human. Hence the test, while not guaranteeing that a test material is non allergenic, is the most appropriate animal test commonly used today.

4.3 Materials

4.3.1 Animals

Albino guinea pigs. Ten animals were used for each test material.

4.3.2 Miscellaneous

Gauze
Non-reactive adhesive tape
Elastic bandage

4.3.3 Test material

0.5 g of test was applied on a 2.5 cm by 2.5 cm size filter paper and placed on the test site.

4.3.4 Positive control

1-Chloro-2, 4-Di-Nitrobenzene (DNCB) in ethyl alcohol and 1-Chloro-2, 4-Di-Nitrobenzene (DNCB) in acetone were used as positive control (historical data).

4.5 Scoring Method

Challenge sites were visually assessed at 6, 24 and 48 hours. Each site was graded for erythema and oedema in accordance with the description in Table 1.

Tabular listing of reactions at 6, 24 and 48 hours were recorded according to the scoring criteria (Table 1) and the results recorded in Table 6.

Table 1. Evaluation of the skin reactions and scoring criteria for cutaneous effects.

Reaction	Description	Score
Erythema (E)	Erythema and eschar formation	
	No erythema	0
	Very slight erythema (barely perceptible)	1
	Well defined erythema (pale red in colour)	2
	Moderate to severe erythema (red and area well defined)	3
	Severe erythema (beet redness to slight eschar formation- injuries in depth)	4
Oedema (O)	Oedema formation	
	No oedema	0
	Very slight oedema (barely perceptible)	1
	Slight oedema (edges of the area well defined by definite raising)	2
	Moderate oedema (raised approx 1mm)	3
	Severe oedema (raised more than 1mm and extending beyond the area of exposure)	4

4.6 Interpretation of Results

Any animal showing a reaction at 6, 24 or 48 hours of 2 or greater for erythema and oedema shall be considered sensitized.

The allergenicity of the test material was rated in accordance to Table 2.

If a significant number (more than 50 %) of animals show a reaction score of 1, the test is repeated using 10 additional animals.

Table 2. Rating of sensitization response.

% Sensitized	Grades	Classification
0-8	I	No different than control
9-28	II	Mild
29-64	III	Moderate
65-80	IV	Strong
81-100	V	Extreme

Note: If 60 % of the animals in the positive control group do not show a reaction of two or greater, the test will be repeated.

5.0 NUMBER OF TEST MATERIALS / REPLICATES

Ten pieces of 2.5 cm by 2.5 cm size filter paper were applied with 0.5 g test material and tested on the skin of 10 animals.

6.0 CONTROLS

1. Ten guinea pigs for positive control (historical data).
2. Five additional guinea pigs during the challenge phase were used as negative control.

11.0 OBSERVATIONS

11.1 Induction phase (Test Group)

Test Material : Sky Gel (Ultrasound Gel)
Study reference number : DSA-01-01-01-01-10

Table 3: Response Indices at 6, 24 and 48 hours during challenge phase.

Duration	6 hours		24 hours		48 hours	
Response indices	Erythema	Oedema	Erythema	Oedema	Erythema	Oedema
Test animals	0/10	0/10	0/10	0/10	0/10	0/10
Test of negative control animals	0/5	0/5	0/5	0/5	0/5	0/5
Test of positive control animals * Based on historical data (erythema and oedema)	10/10	0/10	10/10	0/10	10/10	0/10

Table 4: **IDENTIFICATION OF ANIMALS - TEST GROUP**

Test Animal Sequences	Animal Number	Sex	Initial (g)	Day After Challenge (g)
E16-1	g027A	Male	480	550
E16-2	g028A	Male	460	570
E16-3	g029A	Male	460	540
E16-4	g030A	Male	500	600
E16-5	g031A	Male	500	620
E16-6	g032A	Male	480	530
E16-7	g042A	Female	470	520
E16-8	g043A	Female	430	500
E16-9	g044A	Female	480	560
E16-10	g045A	Female	500	600

Table 4a: **IDENTIFICATION OF ANIMALS – POSITIVE CONTROL**

Test Material	Animal Number	Sex	Initial (g)	Day After Challenge (g)
PC-1	g1765	Female	500	620
PC-2	g1766	Female	480	600
PC-3	g1767	Female	490	650
PC-4	g1768	Female	500	640
PC-5	g1769	Female	460	700
PC-6	g1770	Female	480	630
PC-7	g1771	Female	480	600
PC-8	g1772	Female	500	680
PC-9	g1773	Male	460	560
PC-10	g1774	Male	480	680

11.2 Challenge phase (Test Group)

Table 6: **GRADING OF CUTANEOUS REACTION DURING CHALLENGE PHASE**
(For scoring please refer to Table 1)

Day		33		34		35	
Week		4		4		4	
Hour		6		24		48	
Test Guinea pig		Score		Score		Score	
Animal number	Test animal sequences	E	O	E	O	E	O
g027A	E16-1	0	0	0	0	0	0
g028A	E16-2	0	0	0	0	0	0
g029A	E16-3	0	0	0	0	0	0
g030A	E16-4	0	0	0	0	0	0
g031A	E16-5	0	0	0	0	0	0
g032A	E16-6	0	0	0	0	0	0
g042A	E16-7	0	0	0	0	0	0
g043A	E16-8	0	0	0	0	0	0
g044A	E16-9	0	0	0	0	0	0
g045A	E16-10	0	0	0	0	0	0

11.2.1 Challenge phase (Negative Control)

Table 6A: **GRADING OF CUTANEOUS REACTION DURING CHALLENGE PHASE**
(For scoring please refer to Table 1)

Day	33		34		35	
Week	4		4		4	
Hour	6		24		48	
Test Guinea pig	Score		Score		Score	
Animal number	E	O	E	O	E	O
Negative control						
g048A	0	0	0	0	0	0
g049A	0	0	0	0	0	0
g050A	0	0	0	0	0	0
g051A	0	0	0	0	0	0
g052A	0	0	0	0	0	0

11.2.2 Challenge phase (Positive Control)

Table 6B: **GRADING OF CUTANEOUS REACTION DURING CHALLENGE PHASE**
(For scoring please refer to Table 1)

Day		33		34		35	
Week		4		4		4	
Hour		6		24		48	
Test Guinea pig		Score		Score		Score	
Animal number	Test material	E	O	E	O	E	O
Positive control		**1-Chloro-2, 4-Di-Nitrobenzane (DNCB)**					
g1765	PC-1	2	0	2	0	2	0
g1766	PC-2	3	0	2	0	2	0
g1767	PC-3	2	0	2	0	2	0
g1768	PC-4	2	0	2	0	2	0
g1769	PC-5	3	0	2	0	2	0
g1770	PC-6	3	0	2	0	2	0
g1771	PC-7	2	0	2	0	2	0
g1772	PC-8	3	0	3	0	2	0
g1773	PC-9	1	0	2	0	2	0
g1774	PC-10	2	0	2	0	2	0

E: Erythema O: Oedema

15.0 VERIFICATION

I the undersigned declare that the methods, results and data contained in this report faithfully reflect the procedures used and the raw data collected during the course of this study.

Assoc. Prof Dr. Md. Anuar Osman
Study Director

9/11/10
Date

16.0 QUALITY ASSURANCE INSPECTIONS STATEMENT

This study has been inspected by the Quality Assurance Personnel, and the findings have been reported to Study Director on the following dates:

Procedures Inspected	Inspected Dates	Reported Dates
Records and Report	17 Jun 2010, 18 Jun 2010, 22 Jun 2010	18 Jun 2010

Nurdiana Ishak
Quality Assurance Personnel

9 Nov 2010
Date

12.0 RESULTS

No reaction was observed upon removal of the test material during the challenge phase. Similarly, no reaction was observed in the negative control animals. All guinea pigs (>60%) in the positive control group were sensitized (based on historical data).

Allergenicity of test material

There was no positive allergic reaction observed on the test guinea pigs during the challenge phase. None of the test guinea pigs was sensitized.

13.0 CONCLUSION

There was no skin sensitization induced by the application of the test material in the albino guinea pigs under the condition of this test.

14.0 REFERENCES

1. Guidance for Medical Gloves: A workshop manual (FDA 97-4257).
2. Consumer Product Safety Commission, Title 16, Chapter II, Part 1500.3(c)(4);
3. E.V. Buehler, PhD; Delayed contact hypersensitization in the Guinea Pig. Arch Dermatol, vol. 91 Feb 1965.

Table 5: **GRADING OF CUTANEOUS REACTION DURING INDUCTION PHASE (TEST GROUP)**
(For scoring please refer to Table 1)

Phase	Induction-week 1												Induction-week 2												Induction-week 3											
Day	1				3				5				8				10				12				15				17				19			
Patch number	1				2				3				4				5				6				7				8				9			
	Score				Score				Score				Score				Score				Score				Score				Score				Score			
Hours of patch	0		6		0		6		0		6		0		6		0		6		0		6		0		6		0		6		0		6	
Animal number	E	O	E	O	E	O	E	O	E	O	E	O	E	O	E	O	E	O	E	O	E	O	E	O	E	O	E	O	E	O	E	O	E	O	E	O
g027A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
g028A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
g029A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
g030A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	1	0	0	0	1	0
g031A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	1	0	0	0	1	0
g032A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	1	0	0	0	1	0
g042A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
g043A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0
g044A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
g045A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

11.1.1 Induction phase (Positive Control)

Table 5A: **GRADING OF CUTANEOUS REACTION DURING INDUCTION PHASE (POSITIVE CONTROL - DNCB)**
(For scoring please refer to Table 1)

Phase	Induction-week 1												Induction-week 2												Induction-week 3											
Day	1				3				5				8				10				12				15				17				19			
Patch number	1				2				3				4				5				6				7				8				9			
	Score				Score				Score				Score				Score				Score				Score				Score				Score			
Hours of patch	0		6		0		6		0		6		0		6		0		6		0		6		0		6		0		6		0		6	
Animal number	E	O	E	O	E	O	E	O	E	O	E	O	E	O	E	O	E	O	E	O	E	O	E	O	E	O	E	O	E	O	E	O	E	O	E	O
g1765	0	0	2	0	0	0	2	0	0	0	3	0	1	0	2	0	1	0	2	0	1	0	2	0	2	0	3	0	1	0	2	0	1	0	2	0
g1766	0	0	2	0	1	0	2	0	0	0	3	0	1	0	3	0	2	0	2	0	1	0	2	0	1	0	2	0	1	0	3	0	1	0	2	0
g1767	0	0	1	0	0	0	1	0	1	0	2	0	0	0	2	0	1	0	2	0	2	0	3	0	1	0	1	0	1	0	2	0	2	1	3	1
g1768	0	0	2	0	1	0	1	0	1	0	2	0	0	0	2	0	2	0	3	0	2	0	3	0	1	0	2	0	1	0	2	0	1	1	3	1
g1769	0	0	2	0	0	0	1	0	1	0	2	0	1	0	2	0	2	0	3	0	1	0	2	0	2	0	2	0	2	0	2	0	1	0	3	0
g1770	0	0	3	0	0	0	1	0	0	0	2	0	1	0	2	0	2	0	3	0	1	0	2	0	2	0	2	0	2	0	2	0	1	0	2	0
g1771	0	0	1	0	1	0	2	0	1	0	3	0	1	0	2	0	1	0	3	0	2	0	3	0	2	0	2	0	2	0	2	0	1	0	3	0
g1772	0	0	2	0	1	0	2	0	1	0	2	0	0	0	3	0	2	0	3	0	1	0	2	0	1	0	2	0	2	0	2	0	1	1	3	0
g1773	0	0	2	0	1	0	3	0	1	0	3	0	1	0	3	0	2	0	3	0	2	0	3	0	1	0	2	0	2	0	2	0	1	0	3	0
g1774	0	0	2	0	0	0	1	0	1	0	3	0	0	0	3	0	2	0	2	0	1	0	2	0	1	0	2	0	2	0	3	0	1	0	2	0

E: Erythema O: Oedema

7.0 QUALITY ASSURANCE

The final report was audited in agreement with the raw data records and for compliance with the Standard Operating Procedures of Makmal Bioserasi. Dates of inspections and audits performed during the study and the dates of reporting of the inspection and audit findings to the Study Director are presented in the Quality Assurance Statement.

8.0 DEVIATIONS FROM APPROVED PROTOCOL

None.

9.0 RECORDS TO BE MAINTAINED

A copy of this signed report together with all raw data generated at the laboratory are retained in Makmal Bioserasi archive.

10.0 DATES OF TEST PROCEDURES

Acclimatization period	27 April – 3 May 2010
<u>Induction phase</u>	
Patch application 1	3 May 2010
Patch application 2	5 May 2010
Patch application 3	7 May 2010
Patch application 4	10 May 2010
Patch application 5	12 May 2010
Patch application 6	14 May 2010
Patch application 7	17 May 2010
Patch application 8	19 May 2010
Patch application 9	21 May 2010
<u>Challenge phase</u>	
Patch application (challenge)	4 June 2010
6 hours	4 June 2010
24 hours	5 June 2010
48 hours	6 June 2010
End of test	6 June 2010

4.4 Description of Test Procedure

The basic procedure involves three phases, i.e. the induction phase, rest phase and challenge phase. An initial induction phase involves repeated insult to the skin with the test material followed by a rest period to allow for completion of immunological events permitting development of sensitization. A primary challenge involves re-exposure with the test material at the highest non-irritating dose level followed by an additional rechallenge in the event of ambiguous results.

4.4.1 Preparation and selection of animals.

4.4.1.1 On the day before initiation, the fur of a group of ten guinea pigs was removed by clipping the dorsal area and flanks. After clipping and prior to initiation, animals were weighed and the skin was examined for abnormalities. Only animals without pre-existing skin lesion on the test sites were selected. Animals were reclipped prior to each procedure.

4.4.2 Procedure for test using test material.

4.4.2.1 Induction phase

The induction phase consists of 9 six-hour induction patch applications of test material on the skin of guinea pigs, three times per week, for a period of three-weeks as described below. For each patch application, 0.5 g test material was applied on a 2.5 cm by 2.5 cm filter paper and placed on the left side of each animal. Test sites were occluded with non-reactive adhesive tape and wrapped with an elastic bandage to secure the tape. After a 6-hour exposure period, upon removal of the test material, observations were made on the local reactions (erythema & oedema) and graded according to the scoring system described in Table 1.

4.4.2.2 Rest phase

During the rest period of fourteen days, the guinea pigs were given food and shelter the usual way but were not subjected to any test application procedure.

4.4.2.3 Challenge phase

Fourteen days after the last induction dose, a challenge dose was applied to a naive site on the right side of each animal using the application procedure described above (4.4.2.1). These sites were evaluated for sensitization responses (erythema & oedema) at 6, 24 and 48 hours according to the scoring system described in Table 1.

4.4.3 Procedure for test for negative control.

Additional five guinea pigs were treated with the test material as described in 4.4.2.3 at challenge only. These animals constitute the "naive" group.

4.4.4 Procedure for test for positive control (historical data).

4.4.4.1 The positive control consist of ten guinea pigs that were selected and prepared as in 4.4.1 and tested with positive control material (DNCB) and subjected to patch application procedure similar to that of test material as described in 4.4.2. A 2.5 cm by 2.5 cm filter paper, soaked in DNCB in ethyl alcohol was used in place of the test material during the induction phase and a 2.5 cm by 2.5 cm filter paper, soaked in DNCB in acetone, was used in place of the test material during the challenge phase.

4.4.5 Animals were examined twice daily and their state of health were recorded in the raw data. Individual animal body weight was recorded prior to initiation and again on the day after challenge.

3.7.6.2 Photoperiod
Twelve hours light/dark cycle.

3.7.6.3 Acclimatization period
Six days.

3.7.7 Diet
Gold Coin brand animal feed, fresh vegetables.

3.7.8 Water
Pre-filtered tap water *ad libitum* continuously supplied in water dispensing bottles.

3.8 Pre-Treatment Procedures

3.8.1 Check for ill health
On arrival and just before the beginning of treatment to ensure only healthy animals were used in the study. Guinea pigs showing cutaneous lesions that would interfere with the study were discarded.

3.8.2 Body weight
All animals were weighed just before treatment.

3.8.3 Acclimatization period
At least 5 days between arrival of animals and the start of treatment.

3.8.4 Selection and allocation of animal
Guinea pigs were selected randomly prior to acclimatization.

3.8.5 Identification

Animals	:	Individually numbered (permanent marker pen) on the forehead.
Cage	:	Labeled with animal number, sex and job number.
Animal	:	Each guinea pig was marked with a color code (permanent marker pen) on the forehead and given a sequential animal number assigned to a job number which constitute a unique identification system.

3.8.6 Preparation of the skin
The shoulder region of each guinea pig was clipped free of hair exposing a 5 inch by 3 inch area at the anterior dorsal region within 24 hours of initiating treatment. After clipping and prior to initiation of study, animals were weighed and the skin of each guinea pig was examined for any abnormalities and ill health. Only animals without pre-existing skin lesions on the test sites were selected.

3.3 Project staff

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Treatment
The test material was topically applied to the left side of ten healthy guinea pigs for 6 hours, three times a week for a three-week induction period. Fourteen days after the last induction, a challenge dose was applied in a similar manner onto a naive site on the right side of each guinea pig. The skin was examined for allergic reaction and the intensity of reaction was scored at 6, 24 and 48 hours.

3. OBSERVATIONS

Challenge sites were visually assessed at 6, 24 and 48 hours. Evaluations of erythematous and oedematous lesions were conducted according to the classification established by the **Consumer Product Safety Commission, Title 16, Chapter II, Part 1500.**

Response Indices at 6, 24 and 48 hours during challenge phase.

Duration	**6 hours**		**24 hours**		**48 hours**	
Response indices	Erythema	Oedema	Erythema	Oedema	Erythema	Oedema
Test animals	0/10	0/10	0/10	0/10	0/10	0/10
Test of negative control animals	0/5	0/5	0/5	0/5	0/5	0/5
Test of positive control animals * Based on historical data (erythema and oedema)	10/10	0/10	10/10	0/10	10/10	0/10

4. RESULT

There was no positive allergic reaction observed during the challenge phase in animals treated with the test material and negative control.

5. CONCLUSION

There was no skin sensitization induced by the application of the test material on the albino guinea pigs under the condition of this test.