

ORIGINAL

TSCA NON-CONFIDENTIAL BUSINESS INFORMATION

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May 14, 2010

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Office of Pollution Prevention and Toxics
U.S. Environmental Protection Agency
Attn: TSCA Section 8(e) Coordinator
Ariel Rios Building
1200 Pennsylvania Avenue, NW
Washington, DC 20004



8EHQ-0510-17782B

Re: TSCA Section 8(e) Notification of Substantial Risk:
Supplemental Submission

Dear TSCA Section 8(e) Coordinator:

In accordance with the provisions of Section 8(e) of the Toxic Substances and Control Act (TSCA), as interpreted in the TSCA Section 8(e) Policy Statement and Guidance, Fed. Reg. 33129 (June 3, 2003) and other Agency guidance, Dow Corning submits the following information to supplement our initial TSCA Section 8(e) notification of December 22, 2009, (Document ID: 8EHQ-10-17782). The preliminary findings reported in the initial notification were confirmed in the final analysis and are reflected in the attached final report. Dow Corning has not made a determination at this time that any significant risk of injury to human health or the environment is presented by the findings within the subject study.

Chemical Substances

1185-55-3 Methyltrimethoxysilane



DCN: 89100000219

Study Titles

Methyltrimethoxysilane: Contact Hypersensitivity in Albino guinea Pigs, Buehler Test

Summary

The initial submission was based on preliminary findings that methyltrimethoxysilane may cause skin sensitization in guinea pig.

Dow Corning has not made a determination at this time that any significant risk of injury to human health or the environment is presented by the findings within the subject studies. Dow Corning Corporation will notify EPA of any further relevant

information that may be developed concerning these materials. Attached is the final report (Dow Corning Report Number: 2010-I0000-61874). If you have any questions concerning this submission, please contact me at (989) 496-8046, kathy.plotzke@dowcorning.com, or at the address provided herein.

Sincerely,



Kathleen P. Plotzke, Ph.D.
Director, Health and Environmental Sciences

REPORT

METHYLTRIMETHOXYSILANE

Contact Hypersensitivity in Albino Guinea Pigs, Buehler Test

Study Director:

Test Facility:

Sponsor: **ReachCentrum SPRL**
for account of the Members of the Reconsile Consortium
Avenue E. van Nieuwenhuysse 6
1160 Brussels / Belgium

Study Identification:

Version: *Final*

Study Completion Date: 29-Dec-2009

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Report

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SPONSOR'S REPORT APPROVAL SIGNATURE

The following signature indicates that this report was reviewed and approved by

ReachCentrum SPRL
For account of the Members of the Reconcile Consortium
Avenue E. van Nieuwenhuyse 6
1160 Brussels / Belgium

Sponsor Representative:

Martina Jovanović
Date: 23 Dec 09

Report

Methyltrimethoxysilane

**GOOD LABORATORY PRACTICE
STATEMENT OF COMPLIANCE**

Laboratories Study:

Test Item: Methyltrimethoxysilane
Study Director:
Study Title: Contact Hypersensitivity in Albino Guinea Pigs,
Buchler Test

All data collected on the first two days of acclimatization before the study plan was signed are excluded from this Statement

The stability of the test item dilutions under the test conditions is unknown. The formulation trials were performed before the study initiation date. Therefore, they are excluded from this statement.

This study has been performed in compliance with the Swiss Ordinance relating to Good Laboratory Practice adopted May 18th, 2005 [SR 813.112.1]. This Ordinance is based on the OECD Principles of Good Laboratory Practice, as revised in 1997 and adopted November 26th, 1997 by decision of the OECD Council [C (97)186/Final].

These principles are consistent with Good Laboratory Practice regulations specified by regulatory authorities throughout the European Community, the United States (EPA and FDA) and Japan (MHLW, MAFF and METI).

Study Director:


Date: 23 Dec - 2009

Sponsor Representative:


Date: 23 Dec 09

Report

Methyltrimethoxysilane

QUALITY ASSURANCE GLP STATEMENT

Laboratories Study:

Test Item: Methyltrimethoxysilane
 Study Director:
 Study Title: Contact Hypersensitivity in Albino Guinea Pigs, Buehler Test

The general facilities and activities are inspected periodically and the results are reported to the responsible person and the management.

Study procedures were periodically inspected. The study plan and this report were audited by the Quality Assurance. The dates are given below.

Dates and Types of QA Inspections		Dates of Reports to the Study Director and Test Facility Management
16-Jul-2009	Study Plan	16-Jul-2009
07-Sep-2009	Process Based (Test System, Test Item, Raw Data, Body Weight)	07-Sep-2009
26-Nov-2009	Report	26-Nov-2009

This statement also confirms that this final report reflects the raw data.

Quality Assurance:

(For)

S. Dan Douglas
 Date: 29-Dec-2009

Report

Methyltrimethoxysilane

PREFACE

General Information

Title: Methyltrimethoxysilane: Contact Hypersensitivity in Albino Guinea Pigs, Buehler Test

Sponsor: ReachCentrum SPRL
For account of the Members of the Reconcile Consortium
Avenue E. van Nieuwenhuysse 6
1160 Brussels / Belgium

Sponsor Representative:

Test Facility:

QA:

Responsibilities

Study Director:
Laboratory/Technical Coordinator:

Quality Assurance:
Head of QA:

Schedule

Experimental Starting Date: 15-Jul-2009
Experimental Completion Date: 01-Oct-2009
Delivery of the Animals: 15-Jul-2009
Pretest Start: 16-Jul-2009
Acclimatization (main study): 15-Jul-2009 to 21-Jul-2009

Report

Methyltrimethoxysilane

Administration / Treatment Start (main study): 22-Jul-2009 (induction)
19-Aug-2009 (challenge)
02-Sep-2009 (re-challenge)

Termination: 01-Oct-2009

Data Requirements / Test Guidelines

This study followed the procedures indicated by the following internationally accepted guidelines and recommendations:

- Commission Regulation (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), B.6. Skin sensitisation (Official Journal No L 142, 31/05/2008 p. 0202-0209).
- OECD Guidelines for Testing of Chemicals, Number 406 "Skin Sensitization", adopted by the Council on July 17, 1992 (reported Paris, April 29, 1993).

Animal Welfare

This study was performed in an AAALAC-approved laboratory in accordance with the Swiss Animal Protection Law under license no. 61.

Classification Guidelines

Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labeling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006. According to the cited classification criteria "*For a non-adjuvant guinea pig test method a response of at least 15% of the animals is considered positive*".

Archiving

will retain the study plan, study plan amendment, raw data, a sample of the test item and the final report of the present study for at least ten years. Any remaining test item dosing mixtures will be discarded. Residual test item will be returned to the Sponsor. No data will be discarded without the Sponsor's written consent.

Report

Methyltrimethoxysilane

1 SUMMARY

The purpose of this skin sensitizing study was to assess the ability of the test item, Methyltrimethoxysilane, to induce delayed contact hypersensitivity when applied topically to albino guinea pigs. A modified Buehler method (Ritz and Buehler, 1980) was used. The study was conducted according to OECD Guidelines for Testing of Chemicals, Number 406 "Skin Sensitization".

Twenty male animals of the test group were treated topically with Methyltrimethoxysilane at 50% in PEG 300 once per week for a 3-week induction phase. Ten animals in the control group were treated in the same way as the test animals, but with the vehicle (PEG 300) only. Two weeks after the final induction application the control and test animals were challenged with the test item at 25% in PEG 300 and PEG 300 alone. Fourteen days after challenge, a re-challenge was performed with the same concentration of 25%, as well as a lower concentration of 15%, using the same test group and with a new, naive control group II.

Challenge - Primary Sensitization Results (Incidence Tables)

The incidence of positive skin reactions after topical challenge with the test item at 25% in PEG 300 is summarized as follows:

Erythema Score	Test Group 20 animals		Control Group I 10 animals	
	25%		25%	
	24 hrs	48 hrs	24 hrs	48 hrs
0	1	11	0	2
1	17	9	10	8
2	2	0	0	0
3	0	0	0	0
4	0	0	0	0
No. with grades \geq 1	19	9	10	8
No. tested	20	20	10	10
Incidence*	19/20 (95%)	9/20 (45%)	10/10 (100%)	8/10 (80%)
Severity**	1.05	0.45	1.0	0.8

*Number of animals showing a response of grade 1 or greater at either 24- or 48-hour reading out of the total animals.

**Total sum of 24- and 48-hour response readings divided by the number of animals exposed (maximum of 4).

The right flank of both control and test groups was treated with PEG 300 alone and all animals were devoid of any local signs at the observation time.

Report

Methyltrimethoxysilane

Re-Challenge - Primary Sensitization Results (Incidence Tables)

The incidence of positive skin reactions after topical re-challenge with the test item at 25% and 15% in PEG 300 is summarized as follows:

- Re-challenge at 25% in PEG 300:

Erythema Score	Test Group 20 animals		Control Group II 10 animals	
	25%		25%	
	24 hrs	48 hrs	24 hrs	48 hrs
0	14	16	10	10
1	6	4	0	0
2	0	0	0	0
3	0	0	0	0
4	0	0	0	0
No. with grades \geq 1	6	4	0	0
No. tested	20	20	10	10
Incidence*	6/20(30%)	4/20 (20%)	0/10 (0%)	0/10 (0%)
Severity**	0.3	0.2	0.0	0.0

- Re-challenge at 15% in PEG 300:

Erythema Score	Test Group 20 animals		Control Group II 10 animals	
	15%		15%	
	24 hrs	48 hrs	24 hrs	48 hrs
0	20	20	10	10
1	0	0	0	0
2	0	0	0	0
3	0	0	0	0
4	0	0	0	0
No. with grades \geq 1	0	0	0	0
No. tested	20	20	10	10
Incidence*	0/20(0%)	0/20(0%)	0/10 (0%)	0/10 (0%)
Severity**	0.0	0.0	0.0	0.0

*Number of animals showing a response of grade 1 or greater at either 24- or 48-hour reading out of the total animals.

**Total sum of 24- and 48-hour response readings divided by the number of animals exposed (maximum of 4).

Report

Methyltrimethoxysilane

2 DISCUSSION AND CONCLUSION

Considering that Methyltrimethoxysilane at 25% in PEG 300 was non-irritating in the two prescreen experiments and also in the new control group II, the skin reactions observed in the test group in the first and second challenge when treated at 25% in PEG 300 would indicate the test item sensitization potential. Additionally, knowing that the sensitization reaction is dose-dependent and local skin reactions were observed at the concentration of 25% in PEG 300 while no local skin reaction were observed at the test item concentration of 15% in PEG 300, the reactions are likely to be skin sensitization when applied at 25% rather than irritation. The presence of skin reactions of grade 1 in 30% and 20% of the test animals after 24 and 48hr, respectively in the second challenge and absence of any evidence of irritation in controls II demonstrated the persistency of the skin reactions in the sensitized test animals. Equivocal first challenge data followed by positive re-challenge data, render the entire study positive.

Based on the findings observed in a non- adjuvant sensitization test in guinea pigs, Methyltrimethoxysilane is classified and labelled as a skin sensitizer.

Report

Methyltrimethoxysilane

3 PURPOSE

The purpose of this skin sensitization study was to assess the ability of the test article, Methyltrimethoxysilane, to induce delayed contact hypersensitivity when applied topically to the skin of guinea pigs. A modified Buehler method (Ritz and Buehler, 1980) was used.

This study should provide a rational basis to assess the skin sensitizing potential of the test item.

The sensitivity and reliability of the experimental technique employed was assessed in a recent study by use of ALPHA-HEXYLCINNAMALDEHYDE, which is recommended by the OECD 406 Guidelines and is known to have moderate skin sensitization properties in the guinea pig strain. The results from this study are included in the report (See Appendix I on p. 41).

4 MATERIALS AND METHODS

4.1 Test System

Animals:	Albino Dunkin Hartley Guinea Pig, CRL:(HA)BR, SPF
Rationale:	Skin reactions in the guinea pig are classically used for determining the potential of test items to induce delayed contact hypersensitivity. No valid non-animal model (<i>in-vitro</i>) is available at present for the test of contact sensitization.
Breeder:	Charles River Deutschland GmbH Stolzenseeweg 32-36 88353 Kisslegg / Germany
Number of Animals for Main Study / Irritation Screening:	20 test males and 20 control males / 6 males Animals of either sex are acceptable for use according to Commission Regulation (EC) No 440/2008, B.6 and OECD 406.
Age at Delivery / Acclimatization Start:	4-6 weeks
Body Weight at Delivery / Acclimatization Start:	Test and control animals: 284 - 365 g Animals used for irritation screening: 311 - 365 g
Identification:	By unique cage number and individual animal number.
Randomization:	Selected by hand at time of delivery. No computer generated randomization program.
Acclimatization:	Seven days under laboratory conditions after health examination. Only animals without any visible signs of illness were used for the study. One day for the animals used in the irritation screening for induction and challenge. Only animals without any visible signs of illness were used for the study.

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Methyltrimethoxysilane

4.2 Allocation

	Number of Animals per Group	Animal Numbers
1 Irritation Screening for Induction and Challenge ¹⁾	3	2498 - 2500
2 Control Group I ^{2), 3)}	10	2501 - 2510
3 Test Group ^{2), 3), 5)}	20	2511 - 2530
4 Irritation Screening for Re-Challenge ⁴⁾	3	2531 - 2533
5 Control Group II ⁵⁾	10	2534 - 2543

¹⁾ Concentrations at the 1st irritation screening: 100% (undiluted), 75%, 50% and 25%, formulated in PEG 300

²⁾ At induction, the test group was treated with the test item at 50% in PEG 300 and the control group with PEG 300 only.

³⁾ At challenge, both test and control group animals were treated with the test item at 25% in PEG 300 and PEG 300.

⁴⁾ Concentrations at the 2nd irritation screening: 25%, 15%, 10% and 5%, formulated in PEG 300.

⁵⁾ At re-challenge, both test and control group animals were treated with the test item at 25% and 15% in PEG 300.

4.3 Husbandry

Room Number:

Conditions:

Standard Laboratory Conditions: Air-conditioned with ranges for room temperature 22 ± 3 °C, relative humidity 30-70% and approximately 10-15 air changes per hour. Room temperature and humidity were monitored continuously and values outside of these ranges occasionally occurred, usually following room cleaning. These transient variations are considered not to have any influence on the study and, therefore, these data are not reported but are retained at

The animals were provided with an automatically controlled light cycle of 12 hours light / 12 hours dark. Music was played during the daytime light period.

Accommodation:

Individually in Makrolon type-4 cages with standard softwood bedding ('Lignocel' J. Rettenmaier&Söhne GmbH&CoKG, 73494 Rosenberg / Germany, imported by Provimi Kliba AG, 4303 Kaiseraugst / Switzerland).

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Methyltrimethoxysilane

Diet: Pelleted standard Provimi Kliba 3418, batch nos. 27/09 and 44/09 guinea pig breeding / maintenance diet, containing Vitamin C (Provimi Kliba AG, 4303 Kaiseraugst / Switzerland), *ad libitum*. Results of analyses for contaminants are archived at

Water: Community tap water from Füllinsdorf, *ad libitum*. Results of bacteriological, chemical and contaminant analyses are archived at

4.4 Test Item and Vehicle

4.4.1 Test Item

The following information was provided by the sponsor
 Characterization of Methyltrimethoxysilane (Lot Number 0005552461).
 Methyltrimethoxysilane was characterized according to EPA (TSCA) Good Laboratory Practices 40 CFR Part 792:

Identification: Methyltrimethoxysilane
 supplied as Dow Corning® Z-6070 Silane

Source: Dow Corning Corporation, Auburn, Michigan 48611

Description: Colourless Liquid

Batch Number: 0005552461

CAS Number: 1185-55-3

Purity: 96.4 ± 0.2 area %

Stability of Test Item: Stable under storage conditions.

Expiry Date: 01 March, 2012

Storage Conditions: At room temperature (range of 20 ± 5 °C, provided by light protected. Kept away from sources of ignition, from water, moisture or humid air and from oxidizing agents

Safety Precautions: Routine hygienic procedures were used to ensure the health and safety of the personnel.

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4.4.2 Vehicle

The vehicle, polyethylene glycol 300 (PEG 300) was selected based on preliminary solubility testing which was performed before the study initiation date. Therefore, the formulation trials were excluded from the statement of compliance. The test item prepared at 75% (w/w) in PEG 300 was described as a colourless suspension.

The following information was provided by

Identification:	Polyethylene glycol 300 (PEG 300)
Description:	Colorless viscous liquid
Lot Number:	S60502-099
Source:	Sigma-Aldrich Chemie GmbH 89555 Steinheim / Germany
Stability of the Vehicle:	Stable under storage conditions
Expiry Date:	30-Apr-2014
Storage Conditions:	At room temperature (range of 20 ± 5 °C), light protected.
Safety Precautions:	Routine hygienic procedures were used to ensure the health and safety of the personnel.

4.5 Preparation of Dose Formulations

The test item and vehicle were placed into a glass beaker on a tared Mettler balance and weight/weight dilutions were prepared. A magnetic stirrer was used to ensure homogeneous distribution of the test item preparations. The preparations were made immediately prior to each dosing.

Homogeneity of the test item in the vehicle was maintained during administration using a magnetic stirrer. The test item formulations (in PEG 300) were applied within the first 30 minutes following their preparation.

Dose levels were in terms of material as supplied by the Sponsor.

4.6 Selection of Concentration of Test Item for Definitive Study

A number of factors contributed to the selection of the concentrations of test item including irritancy, slope of dose response curve and experience with similar test items. Irritation screenings were conducted to determine the minimal irritating concentration for the induction period and the highest non-irritating concentration for the challenge and re-challenge periods. The results of these screenings are described below.

The concentrations of the test item required for the induction, challenge and re-challenge were agreed between the Sponsor Representative and Study Director after the irritation screenings had been completed.

4.6.1 Epidermal Induction

For the epidermal induction phase, the concentration selected should produce some irritation but not adversely affect the animals. In the first irritation screening, the test article was applied to three animals at 100% (undiluted) or 75%, 50% and 25% formulated in PEG 300. Moderate skin reactions (grade 2) were observed at the 24- and 48-hour reading with the neat test item applied topically. Treatment with the test item at 75% in PEG 300 resulted in slight skin reactions (grade 1), but with scaling at the application sites in the three animals. The test item at 50% in PEG 300 produced slight skin irritation (grade 1), but without scaling; this concentration was selected for the epidermal induction period.

4.6.2 Epidermal Challenge

For challenge and re-challenge phases, the concentration selected should be the maximum tested non-irritant concentration. As mild-to-moderate local skin reactions were observed after application of the neat test item and preparations of 75% or 50% in PEG 300 in the first irritation screening, these concentrations could not be selected for the challenge procedure. Therefore, a second irritation screening was performed in which the test article was applied to three additional animals at concentrations of 25%, 15%, 10% and 5% in PEG 300. No local skin reactions were observed at any of the four concentrations tested. Therefore, test item concentrations of 25% and 15% in PEG 300 were chosen for the challenge and re-challenge periods.

4.7 Treatment Method

Patching method:

The animal's fur was shaved with a fine clipper blade just prior to the exposure. Closed patches were applied to the animals as follows:

0.5 mL of the test item or a freshly prepared test item dilution in a 25 mm Hill Top Chamber (approximately 4.9 cm² skin exposure).

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Methyltrimethoxysilane

The 25 mm Hill Top Chamber (skin application area of approximately 4.9 cm²) was firmly secured by an elastic plaster wrapped around the trunk of the animal and secured with impervious adhesive tape. The occlusive dressing was left in place for six hours (\pm 15 minutes).

An identical patching method was used for the irritation screen, induction, challenge and re-challenge phases.

4.8 Rationale

Dermal administration has historically been used as the route chosen to assess delayed contact hypersensitivity.

4.9 Determination of Skin Reactions

4.9.1 Observation and Scoring

In order to evaluate the skin reactions following the irritation screenings, challenge and re-challenge, the trunk skin of each animal where the testing patches had been applied was depilated approximately 21 hours after the patches had been removed, using an approved depilatory cream (VEET Cream, Reckitt & Colman AG, 4123 Allschwil / Switzerland). The depilation was performed to clean the stratum corneum to facilitate the reading of the possible skin reaction. The depilatory cream was placed on the patch sites and surrounding areas, and left on for up to 3-5 minutes. It was then thoroughly washed off with a stream of warm, running water. The animals were then dried with a disposable towel, and returned to their cages.

The grading method used for the irritation screenings, induction and challenges was identical. It was performed 24 ± 2 hours after removal of the patches for the irritation screening, induction and challenge and repeated 24 ± 2 hours later (48-hour grades) for the irritation screening and the challenge.

The scoring was performed by visual assessment of erythema and oedema. They were assessed as follows:

0	=	no visible change
1	=	discrete or patchy erythema
2	=	moderate and confluent erythema
3	=	intense erythema and swelling

If observed, any other gross lesions not covered by this scoring system were described.

Grading of all animals was done by positioning each animal under true-light (Philips Master TLS HE 28W/840).

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For evaluation, two parameters were used: the incidence index and the severity index, for both test and control animals. The incidence index is an expression of the number of animals showing a response of grade 1 or greater at the 24- or 48-hour readings out of the total animals in the group. The severity index, designed to describe the intensity of the reaction, is calculated from the total sum of 24- and 48 hour response readings divided by the number of animals exposed.

5 STUDY CONDUCT – TREATMENT PROCEDURE

5.1 Diagrammatic Study Plan

Acclimatization	Study Day						
-6	1	8	15	22	29	37	43
IS(1)	I	I	I		C(1)	IS(2)	C(2)

IS = Irritation screenings (1 and 2) to determine the minimal irritating concentration used in the induction period and the highest non-irritating concentration used for the challenge and re-challenge.

I = Induction (control and test group)

C = Challenge (control and test group)

5.2 Irritation Screening for Induction and Challenge

- Performed during the acclimatization period of the main animals

This investigation identified the test item concentration required for the induction and challenge phase of the main study.

The test item concentrations described below were selected during a preliminary solubility testing which was performed before the study initiation date. Therefore, the formulation trials are excluded from the statement of GLP compliance.

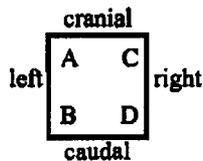
The concentrations of test item and patch positions to assess skin irritation are given below. Up to 4 different concentrations were used on each animal.

Naive animals were treated with test item, undiluted and diluted in PEG 300, for an approximate 6-hour period.

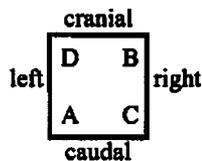
Test Item: Methyltrimethoxysilane

Vehicle: PEG 300

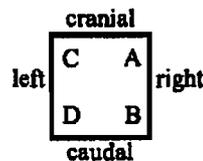
Formulation: weight/weight



Animal no. 2498



Animal no. 2499



Animal no. 2500

Concentrations

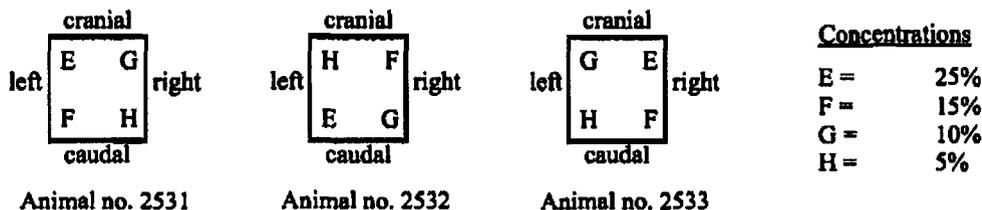
A =	100%
B =	75%
C =	50%
D =	25%

Report

Methyltrimethoxysilane

The allocation of the different test sites (A, B, C, D) on the animals was alternated in order to minimize site to site variation in responsiveness.

In addition, a second irritation screening was performed to support the results observed in the first screening after application of the test item at 25% and to determine the test item concentrations used in the re-challenge. Three additional animals were treated in the same manner with the test item formulated in PEG 300 as follows:



5.3 Induction

- Performed on Test Days 1, 8 and 15

Each animal received one patch on left shoulder per week which remained in place for approximately 6 hours. The patches were applied once per week over a period of 3 weeks. The repeated application was performed at the same site. Based on the results of the screening, the test item was applied at 50% in PEG 300 for the induction phase and the control animals were treated in the same way as the test animals but with the vehicle (PEG 300) only.

After the last induction exposure the animals were left untreated for 2 weeks before the challenge.

Any gross skin reactions were recorded without depilation.

5.4 Challenge and Re-Challenge

- Performed on Test Days 29 and 43

Control and test animals were challenged 2 weeks (14 days) after the last induction exposure. The test item was applied at 25% (highest tested non-irritating concentration) in PEG 300 in a similar manner used for the epidermal induction. The vehicle PEG 300 was also applied.

The re-challenge was performed 14 days after challenge with the same concentration of 25%, as well as a lower concentration of 15%, using the same test group and with a new, naive control group II.

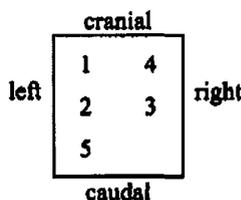
The test item and vehicle sites used are described below.

The occlusive exposure period was 6 hours on a naive skin site.

Report

Methyltrimethoxysilane

6 FORMAT FOR INDUCTION, CHALLENGE AND RE-CHALLENGE PATCH APPLICATION



- 1 = Induction (test group with the test item at 50% and the control group with the vehicle)
- 2 = Challenge (control and test groups with the test item at 25%)
- 3 = Challenge (control and test groups with the vehicle)
- 4 = Re-challenge (control and test groups with test item at 15% selected in the 2nd irritation screening)
- 5 = Re-challenge (control and test groups with test item at 25% selected in the 2nd irritation screening)

6.1 Observations

The following observations were recorded as follows:

Viability / Mortality:	Daily from delivery of the animals to the termination of the test.
Clinical Signs / Grading of Skin Response:	Daily from delivery of the animals to the termination of test. Skin responses were graded during the irritation screening, induction and challenge periods.
Body Weights:	At delivery/acclimatization start, at the end of the irritation screening, at test day 1 (day of treatment) and at the termination of the study.

6.2 Pathology

6.2.1 Necropsy

No necropsies were performed on the animals of the control and test group sacrificed at termination of their observation period or on the animals of the irritation screening sacrificed on test day 2.

The animals were euthanized by intraperitoneal injection of pentobarbitone at a dose of at least 2.0 mL/kg body weight (equivalent to 324 mg sodium pentobarbitone/kg body weight) and discarded.

6.3 Statistical Analysis

Descriptive statistics (means and standard deviations) were calculated for body weights. No inferential statistics were used.

6.4 Data Compilation

The following data were carried out on data sheets and transcribed in the report: Mortality/viability, clinical signs, skin reactions.

The following data were recorded on-line: body weights.

The RCC Tox Computer System (RCC-Tox-Lims, version 7.0) has been validated with respect to data collection, storage and retrievability.

7 RESULTS OF THE MAIN STUDY

7.1 Viability / Mortality / Macroscopic Findings

There were no deaths during the course of the study and no necropsies were performed.

7.2 Clinical Signs (Systemic)

No signs of systemic toxicity were observed in any of the animals.

7.3 Skin Reaction after Induction

(See Individual Tables on p. 35)

No skin effect (grade 0) was observed in the control group after treatment with PEG 300 during the three weeks of induction.

Discrete/patchy erythema (grade 1) was observed in twelve (60%), sixteen (80%) and all (100%) of twenty test animals after treatment with the test item at 50% in PEG 300 during the three weeks of induction, respectively.

7.4 Skin Reaction after Challenge and Re-Challenge

(See Individual Tables on p. 36)

After the challenge, no skin reactions were observed in the control and test animals after treatment with the vehicle (PEG 300); results are not shown.

After challenge, 95% of the test animals showed discrete/patchy erythema (85%; grade 1) to confluent/moderate erythema (10%; grade 2) at 24 hours after treatment with Methyltrimethoxysilane applied at 25% in PEG 300. With the same treatment, all 10 control animals showed discrete/patchy erythema (grade 1) at 24 hours. At 48 hours, the skin reactions faded slightly in both groups with 80% of the control animals showing grade 1 and 45% of the test animals showing grade 1. No grade 2 responses were observed in either group. Due to uncertainty over interpretation of the challenge reactions caused by the effects seen in the control animals, a re-challenge was performed two weeks after the first challenge.

After re-challenge, no skin reactions were observed in the test animals at either 24 or 48 hours after treatment with the test item at 15% in PEG 300. However, discrete/patchy erythema (grade 1) was observed in six (30%) and four (20%) of twenty test animals after treatment with the test item at 25% in PEG 300 at the 24- or 48-hour readings, respectively. No skin reactions were observed in the control animals at either 24 or 48 hours after treatment with the test item at 15% or 25% in PEG 300.

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7.5 Body Weights

(See Summary Tables on p. 29 and Individual Table on p. 39)

The body weight of the animals was within the range commonly recorded for animals of this strain and age.

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8 DISCUSSION AND CONCLUSION

Considering that Methyltrimethoxysilane at 25% in PEG 300 was non-irritating in the two prescreen experiments and also in the new control group II, the skin reactions observed in the test group in the first and second challenge when treated at 25% in PEG 300 would indicate the test item sensitization potential. Additionally, knowing that the sensitization reaction is dose-dependent and local skin reactions were observed at the concentration of 25% in PEG 300 while no local skin reaction were observed at the test item concentration of 15% in PEG 300, the reactions are likely to be skin sensitization when applied at 25% rather than irritation. The presence of skin reactions of grade 1 in 30% and 20% of the test animals after 24 and 48hr, respectively in the second challenge and absence of any evidence of irritation in controls II demonstrated the persistency of the skin reactions in the sensitized test animals. Equivocal first challenge data followed by positive re-challenge data, render the entire study positive.

Based on the findings observed in a non- adjuvant sensitization test in guinea pigs, Methyltrimethoxysilane is classified and labelled as a skin sensitizer.

9 REFERENCE

1. Ritz, H.L. and Buehler, E.V. Current Concepts Cutaneous Toxicity, ed. Drill, V.A. and Lazar, T. (Academic Press, 1980) pp. 25-40: Planning, Conduct and Interpretation of Guinea Pig Sensitization Patch Tests.

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10 SUMMARY TABLES

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**BODY WEIGHTS (GRAM) SUMMARY
MALES**

ACCLI./PRETEST		GROUP 1 IRRITATION SCREENING I	GROUP 2 CONTROL GROUP	GROUP 3 TEST GROUP
DAY	1	335	335	324
WEEK	1	22.5	16.0	23.4
	MEAN	311	302	284
	ST. DEV.	355	351	365
	MINIMUM	3	10	20
	MAXIMUM			
	N			

		GROUP 4 IRRITATION SCREENING II	GROUP 5 CONTROL GROUP II
	MEAN	---	---
	ST. DEV.	---	---
	MINIMUM	---	---
	MAXIMUM	---	---
	N	0	0

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**BODY WEIGHTS (GRAM) SUMMARY
MALES**

TREATMENT		GROUP 1 IRRITATION SCREENING I	GROUP 2 CONTROL GROUP	GROUP 3 TEST GROUP
DAY	1	391	399	378
WEEK	1	42.5	29.8	35.6
	MEAN	345	348	325
	ST. DEV.	429	439	450
	MINIMUM	3	10	20
	MAXIMUM			
	N			
		GROUP 4 IRRITATION SCREENING II	GROUP 5 CONTROL GROUP II	
	MEAN	---	---	
	ST. DEV.	---	---	
	MINIMUM	---	---	
	MAXIMUM	---	---	
	N	0	0	
		GROUP 1 IRRITATION SCREENING I	GROUP 2 CONTROL GROUP	GROUP 3 TEST GROUP
DAY	36	---	569	---
WEEK	6	---	61.1	---
	MEAN	---	498	---
	ST. DEV.	---	683	---
	MINIMUM	---	10	---
	MAXIMUM	---		---
	N	0		0
		GROUP 4 IRRITATION SCREENING II	GROUP 5 CONTROL GROUP II	
	MEAN	339	523	
	ST. DEV.	24.3	17.7	
	MINIMUM	317	488	
	MAXIMUM	365	550	
	N	3	10	
		GROUP 1 IRRITATION SCREENING I	GROUP 2 CONTROL GROUP	GROUP 3 TEST GROUP
DAY	43	---	---	---
WEEK	7	---	---	---
	MEAN	---	---	---
	ST. DEV.	---	---	---
	MINIMUM	---	---	---
	MAXIMUM	---	---	---
	N	0	0	0
		GROUP 4 IRRITATION SCREENING II	GROUP 5 CONTROL GROUP II	
	MEAN	---	588	
	ST. DEV.	---	31.3	
	MINIMUM	---	534	
	MAXIMUM	---	636	
	N	0	10	

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BODY WEIGHTS (GRAM) SUMMARY
MALES

TREATMENT		GROUP 1 IRRITATION SCREENING I	GROUP 2 CONTROL GROUP	GROUP 3 TEST GROUP
DAY	72	---	---	783
WEEK	11	---	---	70.0
	MEAN	---	---	655
	ST. DEV.	---	---	903
	MINIMUM	---	---	20
	MAXIMUM	---	---	
	N	0	0	

	GROUP 4 IRRITATION SCREENING II	GROUP 5 CONTROL GROUP II
MEAN	640	773
ST. DEV.	6.7	48.8
MINIMUM	634	713
MAXIMUM	647	853
N	3	10

Methyltrimethoxysilane

11 INDIVIDUAL TABLES

Report

Methyltrimethoxysilane

Skin Reactions during Irritation Screening for Induction and Challenge**Irritation Screening I****Test Item:** Methyltrimethoxysilane**Vehicle:** PEG 300**Animal Number:** 2498 / Male

Test Item Concentration	Skin reactions after		Test Item Concentration	Skin reactions after	
	24 Hours	48 Hours		24 Hours	48 Hours
A = 100%	2*	2*	C = 50%	1	1
B = 75%	1*	1*	D = 25%	0	0

Animal Number: 2499 / Male

Test Item Concentration	Skin reactions after		Test Item Concentration	Skin reactions after	
	24 Hours	48 Hours		24 Hours	48 Hours
D = 25%	0	0	B = 75%	1*	1*
A = 100%	2*	2*	C = 50%	1	1

Animal Number: 2500 / Male

Test Item Concentration	Skin reactions after		Test Item Concentration	Skin reactions after	
	24 Hours	48 Hours		24 Hours	48 Hours
C = 50%	1	1	A = 100%	2*	2*
D = 25%	0	0	B = 75%	1*	1*

* = with scaling

Three hours prior to the 24-hour reading both flanks were depilated.

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Skin Reactions during Irritation Screening for Induction and Challenge (continued)

Irritation Screening II

Test Item: Methyltrimethoxysilane

Vehicle: PEG 300

Animal Number: 2531 / Male

Test Item Concentration	Skin reactions after		Test Item Concentration	Skin reactions after	
	24 Hours	48 Hours		24 Hours	48 Hours
E = 25%	0	0	G = 10%	0	0
F = 15%	0	0	H = 5%	0	0

Animal Number: 2532 / Male

Test Item Concentration	Skin reactions after		Test Item Concentration	Skin reactions after	
	24 Hours	48 Hours		24 Hours	48 Hours
H = 5%	0	0	F = 15%	0	0
E = 25%	0	0	G = 10%	0	0

Animal Number: 2533 / Male

Test Item Concentration	Skin reactions after		Test Item Concentration	Skin reactions after	
	24 Hours	48 Hours		24 Hours	48 Hours
G = 10%	0	0	E = 25%	0	0
H = 5%	0	0	F = 15%	0	0

Three hours prior to the 24-hour reading both flanks were depilated.

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Methyltrimethoxysilane

Skin Reactions in the Three-Week Induction

Test Item: Methyltrimethoxysilane
Test Item Concentration: 50%
Vehicle: PEG 300

Induction Week 1 / Application on Test Day 1 / Test Group

Animal Number (Males):	2511	2512	2513	2514	2515	2516	2517	2518	2519	2520
Skin Reaction:	1	1	1	1	0	0	1	0	1	0

Animal Number (Males):	2521	2522	2523	2524	2525	2526	2527	2528	2529	2530
Skin Reaction:	1	1	1	0	1	1	0	1	0	0

Induction Week 2 / Application on Test Day 8 / Test Group

Animal Number (Males):	2511	2512	2513	2514	2515	2516	2517	2518	2519	2520
Skin Reaction:	1	1	1	1	1	1	1	0	1	0

Animal Number (Males):	2521	2522	2523	2524	2525	2526	2527	2528	2529	2530
Skin Reaction:	1	1	1	1	1	1	0	1	0	1

Induction Week 3 / Application on Test Day 15 / Test Group

Animal Number (Males):	2511	2512	2513	2514	2515	2516	2517	2518	2519	2520
Skin Reaction:	1	1	1	1	1	1	1	1	1	1

Animal Number (Males):	2521	2522	2523	2524	2525	2526	2527	2528	2529	2530
Skin Reaction:	1	1	1	1	1	1	1	1	1	1

The control group was treated with PEG 300 during the 3-week induction and all control animals were devoid of any skin reaction at the observation time.

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Skin Reactions after Challenge**Test Item:** Methyltrimethoxysilane**Test Item Concentration:** 25%**Vehicle:** PEG 300**Control Group 1**

Animal No.	Skin Reactions after (\pm 2 Hours)	
	24 Hours	48 Hours
2501	1	0
2502	1	1
2503	1	1
2504	1	1
2505	1	1

Animal No.	Skin Reactions after (\pm 2 Hours)	
	24 Hours	48 Hours
2506	1	1
2507	1	1
2508	1	1
2509	1	1
2510	1	0

Test Group

Animal No.	Skin Reactions after (\pm 2 Hours)	
	24 Hours	48 Hours
2511	1	0
2512	1	0
2513	1	0
2514	1	0
2515	1	1
2516	1	0
2517	0	0
2518	1	0
2519	1	1
2520	1	1

Animal No.	Skin Reactions after (\pm 2 Hours)	
	24 Hours	48 Hours
2521	1	1
2522	1	0
2523	1	0
2524	1	1
2525	1	1
2526	1	0
2527	2	1
2528	2	1
2529	1	1
2530	1	0

Three hours prior to the 24-hour reading, the test item-treated flank was depilated.

The right flank of both control and test groups was treated with PEG 300 alone and all animals were devoid of any local signs at the observation time.

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Methyltrimethoxysilane

Skin Reactions after Re-challenge

Test Item: Methyltrimethoxysilane
Test Item Concentration: 25%
Vehicle: PEG 300

Control Group II

Animal No.	Skin Reactions after (\pm 2 Hours)	
	24 Hours	48 Hours
2534	0	0
2535	0	0
2536	0	0
2537	0	0
2538	0	0

Animal No.	Skin Reactions after (\pm 2 Hours)	
	24 Hours	48 Hours
2539	0	0
2540	0	0
2541	0	0
2542	0	0
2543	0	0

Test Group

Animal No.	Skin Reactions after (\pm 2 Hours)	
	24 Hours	48 Hours
2511	0	0
2513	0	0
2514	0	0
2514	0	0
2515	0	0
2516	0	0
2517	0	0
2518	0	0
2519	0	0
2520	0	0

Animal No.	Skin Reactions after (\pm 2 Hours)	
	24 Hours	48 Hours
2521	1	1
2522	0	0
2523	0	0
2524	0	0
2525	1	1
2526	1	0
2527	1	1
2528	0	0
2529	1	0
2530	1	1

Three hours prior to the 24-hour reading, the test item-treated flank was depilated.

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Skin Reactions after Re-challenge (continued)

Test Item: Methyltrimethoxysilane
 Test Item Concentration: 15%
 Vehicle: PEG 300

Control Group II

Animal No.	Skin Reactions after (\pm 2 Hours)	
	24 Hours	48 Hours
2534	0	0
2535	0	0
2536	0	0
2537	0	0
2538	0	0

Animal No.	Skin Reactions after (\pm 2 Hours)	
	24 Hours	48 Hours
2539	0	0
2540	0	0
2541	0	0
2542	0	0
2543	0	0

Test Group

Animal No.	Skin Reactions after (\pm 2 Hours)	
	24 Hours	48 Hours
2511	0	0
2513	0	0
2514	0	0
2514	0	0
2515	0	0
2516	0	0
2517	0	0
2518	0	0
2519	0	0
2520	0	0

Animal No.	Skin Reactions after (\pm 2 Hours)	
	24 Hours	48 Hours
2521	0	0
2522	0	0
2523	0	0
2524	0	0
2525	0	0
2526	0	0
2527	0	0
2528	0	0
2529	0	0
2530	0	0

Three hours prior to the 24-hour reading, the test item-treated flank was depilated.

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**BODY WEIGHTS (GRAM) INDIVIDUAL
MALES**

	ACCLI./PRETEST	TREATMENT			
DAYS	1	1	36	43	72
WEEKS	1	1	6	7	11
ANIMAL					

GROUP 1 (IRRITATION SCREENING I)

2498	311	345	---	---	---
2499	341	399	---	---	---
2500	355	429	---	---	---

GROUP 2 (CONTROL GROUP)

2501	334	407	550	---	---
2502	348	439	683	---	---
2503	302	356	544	---	---
2504	351	422	651	---	---
2505	335	403	580	---	---
2506	327	379	512	---	---
2507	350	426	508	---	---
2508	318	348	498	---	---
2509	350	413	598	---	---
2510	334	394	567	---	---

GROUP 3 (TEST GROUP)

2511	310	351	---	---	735
2512	284	325	---	---	655
2513	361	424	---	---	847
2514	345	423	---	---	709
2515	321	368	---	---	903
2516	298	343	---	---	683
2517	324	325	---	---	812
2518	330	403	---	---	763
2519	340	411	---	---	868
2520	365	450	---	---	895
2521	312	355	---	---	708
2522	304	343	---	---	810
2523	297	351	---	---	753
2524	296	351	---	---	709
2525	334	402	---	---	826
2526	339	396	---	---	770
2527	334	402	---	---	837
2528	341	392	---	---	807
2529	298	355	---	---	759
2530	351	384	---	---	816

GROUP 4 (IRRITATION SCREENING II)

2531	---	---	365	---	639
2532	---	---	317	---	634
2533	---	---	336	---	647

GROUP 5 (CONTROL GROUP II)

2534	---	---	546	636	853
2535	---	---	522	604	740
2536	---	---	516	561	743
2537	---	---	525	552	786
2538	---	---	550	614	843
2539	---	---	488	534	713
2540	---	---	524	602	745
2541	---	---	509	606	809
2542	---	---	519	579	773
2543	---	---	528	588	724

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APPENDIX I – RESULTS OF POSITIVE CONTROL

Report

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POSITIVE CONTROL

ALPHA-HEXYLCINNAMALDEHYDE

Contact Hypersensitivity in Albino Guinea Pigs, Buehler Test

Study Director:

Test Facility:

Study Identification:

Study Performed: 28-May-2009 to 17-Jul-2009

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1 SUMMARY

1.1 General

The purpose of this skin sensitizing study was to assess the possible allergenic potential of ALPHA-HEXYLCINNAMALDEHYDE when administered topically to albino Dunkin Hartley guinea pigs.

For this purpose the "Buehler Test" modified by Ritz, H.L. and Buehler, E.V. (1980) was used. Twenty male animals of the test group were treated topically with ALPHA-HEXYLCINNAMALDEHYDE at 25% in PEG 300 once a week for a 3-week induction phase. Two weeks after the final induction application the animals were challenged with the same test item concentration of 5% and 1% in PEG 300 as used for induction.

The ten animals of the control group were not treated during the induction. They were treated once at challenge with ALPHA-HEXYLCINNAMALDEHYDE at 5% and 1% in PEG 300.

Due to equivocal findings after the first challenge with a rapid fading of the skin reactions at the 24 hours when the test group was treated with the test item at 5% in PEG 300, a second challenge was performed two weeks later by repeating the first challenge procedure at 5% in PEG 300.

1.2 Results

None of the control animals were observed with skin reactions after the challenge treatment with the highest tested non-irritating concentration of ALPHA-HEXYLCINNAMALDEHYDE at 5% and 1% in PEG 300.

Fifty percent of the test animals were observed with discrete/patchy erythema at the 24-hour observation after the first challenge treatment with the highest tested non-irritating concentration of ALPHA-HEXYLCINNAMALDEHYDE at 5% in PEG 300. All the skin reactions faded at the 48 hours. No skin effect was observed at the 24- and 48-hour readings after the challenge treatment with ALPHA-HEXYLCINNAMALDEHYDE at 1% in PEG 300.

Discrete/patchy erythema was observed in 75% or 50% of the test animals at the 24- or 48-hour observation, respectively after the second challenge treatment with ALPHA-HEXYLCINNAMALDEHYDE at 5% in PEG 300.

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First Challenge - Primary Sensitization Results (Incidence Tables)

The highest tested non-irritating concentrations of ALPHA-HEXYLCINNAMALDEHYDE used for challenge were 5% and 1% in PEG 300. The incidence of positive erythema reactions after topical challenge is described as follows:

Challenge with ALPHA-HEXYLCINNAMALDEHYDE, 5% in PEG 300

Erythema Score	Test Group 20 animals		Control Group 10 animals	
	24 hrs	48 hrs	24 hrs	48 hrs
0	10	20	10	10
1	10	0	0	0
2	0	0	0	0
3	0	0	0	0
No. with grades \geq 1	0	0	0	0
No. tested	20	20	10	10
Incidence*	10/20		0/10	
Severity**	0.5		0	

*Number of animals showing a response of grade 1 or greater at either 24- or 48-hour reading out of the total animals.

**Total sum of 24- and 48-hour response readings divided by the number of animals exposed (maximum of 3).

Challenge with ALPHA-HEXYLCINNAMALDEHYDE, 1% in PEG 300

Erythema Score	Test Group 20 animals		Control Group 10 animals	
	24 hrs	48 hrs	24 hrs	48 hrs
0	20	20	10	10
1	0	0	0	0
2	0	0	0	0
3	0	0	0	0
No. with grades \geq 1	0	0	0	0
No. tested	20	20	10	10
Incidence*	0/20		0/10	
Severity**	0		0	

*Number of animals showing a response of grade 1 or greater at either 24- or 48-hour reading out of the total animals.

**Total sum of 24- and 48-hour response readings divided by the number of animals exposed (maximum of 3).

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Second Challenge - Primary Sensitization Results (Incidence Tables)

Challenge with ALPHA-HEXYLCINNAMALDEHYDE, 5% in PEG 300

Erythema Score	Test Group 20 animals	
	24 hrs	48 hrs
0	5	10
1	15	10
2	0	0
3	0	0
No. with grades \geq 1	0	0
No. tested	20	20
Incidence*	15/20	
Severity**	0.5 - 0.75	

* Number of animals showing a response of grade 1 or greater at either 24- or 48-hour reading out of the total animals.

** Total sum of 24- and 48-hour response readings divided by the number of animals exposed (maximum of 3).

2 SCHEDULE

Experimental Starting Date:	28-May-2009
Delivery of Animals:	28-May-2009
Pretest Start:	28-May-2009 and 18-Jun-2009
Acclimatization (main study):	28-May-2009 to 01-Jun-2009
Administration/Treatment (main study):	02-Jun-2009
Termination:	17-Jul-2009
Experimental Completion Date:	17-Jul-2009

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3 TEST SYSTEM

Animals:	Albino Dunkin Hartley Guinea Pig, CRL:(HA), SPF
Rationale:	Skin reactions in the guinea pig are classically used for determining the potential of test items to induce delayed contact hypersensitivity. No valid non-animal model (in-vitro) is available at present for the test of contact sensitization.
Breeder:	Charles River Deutschland GmbH Stolzenseeweg 32-36 88353 Kisslegg / Germany
Number of Animals for Main Study / Irritation Screen:	30 males / 6 males First and second challenge: 20 test animals 10 control animals Irritation Screen: 6 animals
Age at Delivery / Acclimatization Start:	5 to 6 weeks
Body Weight at Delivery / Acclimatization Start:	Test and control animals: 260 to 371 g Animals used for irritation screens: 344 to 370 g
Identification:	By unique cage number and individual animal number.
Randomization:	Randomly selected by hand at time of delivery. No computer randomization.
Acclimatization:	Under laboratory conditions after health examination. Six days for the control and test group. However, contrary to the test group the control group remained untreated during the 3 induction weeks. One day for the animals used in the irritation screen for induction and challenge. Only animals without any visible signs of illness were used for the study.

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4 TEST ITEM

Identification: ALPHA-HEXYLCINNAMALDEHYDE
Description: Yellow to yellow-green liquid
Lot Number: 04012JE
Source: Aldrich Chemicals Company, inc.
3050 Spruce Street
Saint Louis, Missouri 63105 / USA
Purity: 99.0%
Stability of Test Item: Stable under storage conditions.
Expiry Date: 24-Oct-2012
Stability of Test Item Dilution: Stable in PEG 300 and in a 1:1 (v/v) mixture of FCA/physiological saline for at least 2 hours at room temperature; was determined at Harlan Laboratories Ltd., under a non-GLP study.
Storage Conditions: At room temperature (range of 20 ± 5 °C, provided by Harlan Laboratories Ltd.), light protected.
Safety Precautions: Routine hygienic procedures were used to ensure the health and safety of the personnel.

5 VEHICLE

Identification: Polyethylene glycol 300 (PEG 300)
Description: Colorless viscous liquid
Lot Number: 541673-128
Source: Sigma-Aldrich Chemie GmbH
9471 Buchs / Switzerland
Stability of the Vehicle: Stable under storage conditions
Expiry Date: 31-Jan-2014
Storage Conditions: At room temperature (range of 20 ± 5 °C), light protected.
Safety Precautions: Routine hygienic procedures were used to ensure the health and safety of the personnel.

6 CONCLUSION

The initial challenge indicated that there were some low grade reactions after treatment with the test item at 5% in PEG 300. However, the nature of the reactions (fading at the later time point) suggested that they might be due to skin irritation, despite any evidence of irritation in controls. Rechallenge under identical conditions on a new skin site demonstrated that the previous skin reactions observed were reproducible with a higher incidence of reactions when compared to the first challenge. The absence of a response in the controls previously treated in the first challenge further confirmed the allergic nature of the reactions.

The control and test animals treated with the test item at 1% in PEG 300 were devoid of any skin reactions at the first challenge.

Based on the above mentioned findings in a non-adjuvant sensitization test in guinea pigs and in accordance to Commission Directive 2001/59/EC, ALPHA-HEXYLCINNAMALDEHYDE is classified and labelled as a skin sensitizer.

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7 TABLES OF RESULTS**Skin Reactions during Irritation Screen for Induction****Irritation Screen I****Test Item: ALPHA-HEXYLCINNAMALDEHYDE****Vehicle: PEG 300****Animal Number: 1755 / M**

Test Item Concentration	Skin Reactions after		Test Item Concentration	Skin Reactions after	
	24 Hours	48 Hours		24 Hours	48 Hours
A = 50%	2	2	C = 15%	1	1
B = 25%	2	1	D = 5%	1	1

Animal Number: 1756 / M

Test Item Concentration	Skin Reactions after		Test Item Concentration	Skin Reactions after	
	24 Hours	48 Hours		24 Hours	48 Hours
D = 5%	1	1	B = 25%	2	1
A = 50%	2	2	C = 15%	1	1

Animal Number: 1757 / M

Test Item Concentration	Skin Reactions after		Test Item Concentration	Skin Reactions after	
	24 Hours	48 Hours		24 Hours	48 Hours
C = 15%	1	1	A = 50%	2	2
D = 5%	0	0	B = 25%	2	1

Three hours prior to the 24-hour reading both flanks were depilated.

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Skin Reactions during Irritation Screen for Challenge**Irritation Screen II****Test Item: ALPHA-HEXYLCINNAMALDEHYDE****Vehicle: PEG 300****Animal Number: 1788 / Male**

Test Item Concentration	Skin Reactions after		Test Item Concentration	Skin Reactions after	
	24 Hours	48 Hours		24 Hours	48 Hours
E = 5%	0	0	G = 0.5%	0	0
F = 1%	0	0	H = 0.1%	0	0

Animal Number: 1789 / Male

Test Item Concentration	Skin Reactions after		Test Item Concentration	Skin Reactions after	
	24 Hours	48 Hours		24 Hours	48 Hours
H = 0.1%	0	0	F = 1%	0	0
E = 5%	0	0	G = 0.5%	0	0

Animal Number: 1790 / Male

Test Item Concentration	Skin Reactions after		Test Item Concentration	Skin Reactions after	
	24 Hours	48 Hours		24 Hours	48 Hours
G = 0.5%	0	0	E = 5%	0	0
H = 0.1%	0	0	F = 1%	0	0

Three hours prior to the 24-hour reading both flanks were depilated.

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Skin Reactions in the Three-Week Induction

Test Item: **ALPHA-HEXYLCINNAMALDEHYDE**
 Test Item Concentration: **25%**
 Vehicle: **PEG 300**

Induction Week 1 / Application on Test Day 1 / Test Group

Animal Number (Males):	1768	1769	1770	1771	1772	1773	1774	1775	1776	1777
Skin Reaction:	1	2	1	1	2	2	2	1	2	2

Animal Number (Males):	1778	1779	1780	1781	1782	1783	1784	1785	1786	1787
Skin Reaction:	2	2	2	2	2	1	2	2	2	2

Induction Week 2 / Application on Test Day 8

Animal Number (Males):	1768	1769	1770	1771	1772	1773	1774	1775	1776	1777
Skin Reaction:	2	2	2	2	2	2	2	2	2	2

Animal Number (Males):	1778	1779	1780	1781	1782	1783	1784	1785	1786	1787
Skin Reaction:	2	2	2	2	2	2	2	2	2	2

Induction Week 3 / Application on Test Day 15

Animal Number (Males):	1768	1769	1770	1771	1772	1773	1774	1775	1776	1777
Skin Reaction:	2	2	2	2	2	2	2	2	2	2

Animal Number (Males):	1778	1779	1780	1781	1782	1783	1784	1785	1786	1787
Skin Reaction:	2	2	2	3	2	2	2	2	3	2

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Skin Reactions in the First Challenge**Table 1: Control Group treated with 5% ALPHA-HEXYLCINNAMALDEHYDE in PEG 300 (Left Flank)**

Animal No. (Males)	Skin Reactions after (\pm 2 Hours)		Animal No. (Males)	Skin Reactions after (\pm 2 Hours)	
	24 Hours	48 Hours		24 Hours	48 Hours
1758	0	0	1763	0	0
1759	0	0	1764	0	0
1760	0	0	1765	0	0
1761	0	0	1766	0	0
1762	0	0	1767	0	0

Table 2: Control Group treated with 1% ALPHA-HEXYLCINNAMALDEHYDE in PEG 300 (Right Flank)

Animal No. (Males)	Skin Reactions after (\pm 2 Hours)		Animal No. (Males)	Skin Reactions after (\pm 2 Hours)	
	24 Hours	48 Hours		24 Hours	48 Hours
1758	0	0	1763	0	0
1759	0	0	1764	0	0
1760	0	0	1765	0	0
1761	0	0	1766	0	0
1762	0	0	1767	0	0

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Skin Reactions in the First Challenge (continued)**Table 3: Test Group treated with 5% ALPHA-HEXYLCINNAMALDEHYDE in PEG 300 (Left Flank)**

Animal No. (Males)	Skin Reactions after (\pm 2 Hours)		Animal No. (Males)	Skin Reactions after (\pm 2 Hours)	
	24 Hours	48 Hours		24 Hours	48 Hours
1768	0	0	1778	1	0
1769	0	0	1779	1	0
1770	1	0	1780	0	0
1771	0	0	1781	1	0
1772	0	0	1782	0	0
1773	1	0	1783	1	0
1774	1	0	1784	1	0
1775	0	0	1785	1	0
1776	0	0	1786	1	0
1777	0	0	1787	0	0

Table 4: Test Group treated with 1% ALPHA-HEXYLCINNAMALDEHYDE in PEG 300 (Right Flank)

Animal No. (Males)	Skin Reactions after (\pm 2 Hours)		Animal No. (Males)	Skin Reactions after (\pm 2 Hours)	
	24 Hours	48 Hours		24 Hours	48 Hours
1768	0	0	1778	0	0
1769	0	0	1779	0	0
1770	0	0	1780	0	0
1771	0	0	1781	0	0
1772	0	0	1782	0	0
1773	0	0	1783	0	0
1774	0	0	1784	0	0
1775	0	0	1785	0	0
1776	0	0	1786	0	0
1777	0	0	1787	0	0

Three hours prior to the 24-hour reading, the test item-treated flank was depilated.

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Skin Reactions in the Second Challenge
**Table 5: Test Group treated with 5% ALPHA-HEXYLCINNAMALDEHYDE
in PEG 300 (Right Shoulder)**

Animal No. (Males)	Skin Reactions after (\pm 2 Hours)		Animal No. (Males)	Skin Reactions after (\pm 2 Hours)	
	24 Hours	48 Hours		24 Hours	48 Hours
1768	1	1	1778	1	1
1769	1	1	1779	1	0
1770	1	1	1780	1	0
1771	0	0	1781	1	1
1772	1	1	1782	1	1
1773	1	0	1783	1	0
1774	1	1	1784	1	0
1775	0	0	1785	1	1
1776	0	0	1786	1	1
1777	0	0	1787	0	0

Three hours prior to the 24-hour reading, the test item-treated flank was depilated.

APPENDIX II – STUDY PLAN AND AMENDMENT

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STUDY PLAN

METHYLTRIMETHOXYSILANE

Contact Hypersensitivity in Albino Guinea Pigs, Buehler Test

Study Director:

Test Facility:

Sponsor: **ReachCentrum SPRL**
for account of the Members of the Reconsile Consortium
Avenue E. van Nieuwenhuysse 6
1160 Brussels/ Belgium

Study Identification:

Version: **Final**

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SIGNATURES

Study Director:

Date: 17 Jul 2009

Management:

Date: 17 July 2009

Sponsor Representative:

Date: 16 July 2009

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PREFACE

General Information

Study Title: Methyltrimethoxysilane: Contact Hypersensitivity in Albino Guinea Pigs, Buehler Test

Sponsor: ReachCentrum SPRL
For account of the Members of the Reconcile Consortium
Avenue E. van Nieuwenhuysse 6
1160 Brussels/ Belgium

Sponsor Representative:

Health and Environmental Sciences (HES)

Test Facility:

QA:

Responsibilities

Study Director:

Laboratory/Technical Coordinator:

Quality Assurance:

Head of QA:

Schedule

Anticipated Experimental Starting Date: 15-Jul-2009

Anticipated Experimental Completion Date: 19-Aug-2009 (depending on termination)

Pretest Start: 16-Jul-2009

Acclimatization (main study): 15-Jul-2009 to 21-Jul-2009

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Administration / Treatment Start (main study):	22-Jul-2009	
Termination (Anticipated):	21-Aug-2009	
Anticipated Date of audited Draft Report:	18-Sep-2009	
Anticipated Date of Final report:	07-Oct-2009	

Good Laboratory Practice

This study will be performed in compliance with the Swiss Ordinance relating to Good Laboratory Practice adopted May 18th, 2005 [SR 813.112.1]. This Ordinance is based on the OECD Principles of Good Laboratory Practice, as revised in 1997 and adopted November 26th, 1997 by decision of the OECD Council [C (97)186/Final].

These principles are compatible with Good Laboratory Practice regulations specified by regulatory authorities throughout the European Community, the United States (EPA and FDA), and Japan (MHLW, MAFF and METI).

Data Requirements / Test Guidelines

This study will follow the procedures indicated by the following internationally accepted guidelines and recommendations:

- Commission Regulation (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), B.6. Skin sensitisation (Official Journal No L 142, 31/05/2008 p. 0202-0209).
- OECD Guidelines for Testing of Chemicals, Number 406 "Skin Sensitization", adopted by the Council on July 17, 1992 (reported Paris, April 29, 1993).

Animal Welfare

This study will be performed in an AAALAC-accredited laboratory in accordance with the Swiss Animal Protection Law under license no. 61.

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Classification Guidelines

Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labeling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006. According to the cited classification criteria "*For a non-adjuvant guinea pig test method a response of at least 15% of the animals is considered positive*".

Amendment and Deviation Procedures

Amendments (planned changes) to the study plan will be issued and signed by the Study Director and will become effective at the time of Study Director signature. The Sponsor will receive the original amendment, which must be signed and returned to _____ and one copy. The amendment will be distributed (see Distribution) and added to all copies of the study plan.

Deviations (unplanned changes) to the study plan will be documented and acknowledged by the Study Director and maintained with the raw data. The report will reflect any deviations. The Sponsor will be promptly informed of any relevant deviations from the study plan.

Archiving

_____ will retain the study plan, raw data, a sample of the test item and the final report of the present study for at least ten years. No data will be discarded without the Sponsor's written consent.

1 PURPOSE

The purpose of this skin sensitization study will be to determine if Methyltrimethoxysilane, under the conditions described in this study plan, causes an increased reaction in the skin of guinea pigs at challenge when compared to appropriate controls.

This study should provide a rational basis to assess the sensitizing potential of the test item in man.

The sensitivity and reliability of the experimental technique employed was assessed at least twice a year by use of substances such as alpha-hexylcinnamaldehyde or 2 mercaptobenzothiazole which are recommended by the Commission Regulation (EC) No 440/2008, B.6 or OECD 406 Guidelines and are known to have moderate skin sensitization properties in the guinea pig strain. The results from the most recent test run will be included in the report as Appendix.

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2 MATERIALS AND METHODS

General Remark

Details of the materials and methods that are not specified in the subsequent sections of this study plan are contained in the appropriate standard operating procedures.

2.1 Test System

Animals:	Albino Dunkin Hartley Guinea Pig, CRL:(HA)BR, SPF
Rationale:	Skin reactions in the guinea pig are classically used to determine the potential of test items to induce delayed contact hypersensitivity. No valid non-animal (in vitro) model is available at present for the testing of contact sensitization.
Breeder:	Charles River Deutschland GmbH Stolzenseeweg 32-36 88353 Kisslegg / Germany
Number of animals for Main Study / Irritation Screen (at least):	30 animals / 3 animals If necessary, 10 additional animals for a rechallenge. Animals of either sex are acceptable for use according to Commission Regulation (EC) No 440/2008, B.6 and OECD 406. Primary challenge: 20 test- and 10 control animals Rechallenge: 10 additional untreated control animals (optional) At least 3 animals for the irritation screen
Age at Beginning of Acclimatization Period:	Approximately 4 – 6 weeks
Body Weight at Beginning of Acclimatization Period:	300 – 400 g whenever possible
Identification:	By unique cage number and corresponding individual animal number.
Randomization:	Selected by hand at time of delivery. No computer generated randomization program.
Acclimatization:	Under laboratory conditions after health examination. Only animals without any visible signs of illness will be used for the study. Approximately one week for test and control animals. The acclimatization period of the animals used in the irritation

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screen required for induction and challenge, if possible, depends upon the schedule of the study.

2.2 Allocation

	Number of Animals per Group	Animal Numbers
1 Irritation Screen for Induction and Challenge	3	2498 - 2500
2 Control Group	10	2501 - 2510
3 Test Group	20	2511 - 2530

2.3 Husbandry

Room Number:	Will be specified in the raw data and the report (Föllinsdorf).
Conditions:	Standard laboratory conditions. The animal room will be air-conditioned with 10-15 air changes per hour. The air will be continuously monitored for temperature and relative humidity. The ranges for room temperature and relative humidity will be 22 ± 3 °C and 30-70 %, respectively, although the upper range for humidity may be exceeded during room cleaning. The animals will be provided with an automatically controlled light cycle of 12 hours light and 12 hours dark. Music will be played during the daytime light period.
Accommodation:	Individually in Makrolon type-4 cages with standard softwood bedding ('Lignocel' J. Rettenmaier&Söhne GmbH&CoKG, 73494 Rosenberg / Germany, imported by Provimi Kliba AG, 4303 Kaiseraugst / Switzerland).
Diet:	Pelleted standard Provimi Kliba 3418 guinea pig breeding/ maintenance diet, containing Vitamin C (Provimi Kliba AG, 4303 Kaiseraugst / Switzerland), ad libitum. Results of analyses for contaminants will be archived at

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Water: Community tap-water, from Füllinsdorf ad libitum. Results of bacteriological, chemical and contaminant analyses will be archived at

2.4 Test Item and Vehicle

2.4.1 Test Item

The following information was provided by the sponsor and can be found in (Characterization of Methyltrimethoxysilane (Lot Number 0005552461)). Methyltrimethoxysilane was characterized according to EPA (TSCA) Good Laboratory Practices 40 CFR Part 792:

Identification:	Methyltrimethoxysilane supplied as Dow Corning® Z-6070 Silane
Source:	Dow Corning Corporation, Auburn, Michigan 48611
Description:	Colourless Liquid
Batch Number:	0005552461
CAS Number:	1185-55-3
Purity:	96.4 ± 0.2 area %
Stability of Test Item:	Stable under storage conditions.
Expiry Date:	01 March, 2012
Storage Conditions:	At room temperature (range of 20 ± 5 °C, provided , light protected. Keep away from sources of ignition, from water, moisture or humid air and from oxidizing agents
Safety Precautions:	Routine hygienic procedures will be used to ensure the health and safety of the personnel.

2.4.2 Vehicle

The following information was provided by

Identification:	Polyethylene glycol 300 (PEG 300)
Description:	Colorless viscous liquid
Lot Number:	Will be added in the raw data and report
Source:	Sigma-Aldrich Chemie GmbH, 89555 Steinheim / Germany
Stability of the Vehicle:	Stable under storage conditions

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Expiry Date:	Will be added in the raw data and report
Storage Conditions:	At room temperature (range of 20 ± 5 °C), light protected.
Safety Precautions:	Routine hygienic procedures will be used to ensure the health and safety of the personnel.

The vehicle was selected based on preliminary solubility testing which was performed before the study initiation date. Therefore, the formulation trials will be excluded from the statement of compliance. PEG 300 was a suitable vehicle to be used for the study.

2.5 Preparation of Dose Formulations

The test item and vehicle will be placed into a glass beaker on a tared Mettler balance and a weight/weight dilution will be prepared. A homogenizer will be used to ensure homogeneous distribution of the test item preparation. The preparations will be made immediately prior to each dosing.

Homogeneity of the test item in the vehicle will be maintained during administration using a suitable homogenizer when possible.

Dose levels are in terms of material as supplied unless otherwise stated by the sponsor.

2.6 Test Item Administration

A number of factors contribute to the selection of the concentrations of test item including irritancy, slope of dose response curve and experience with similar test items. Selection will be based on the following criteria:

Epidermal Induction

Concentration that will produce some irritation but not adversely affect the animals (determined at the irritation screen).

Epidermal Challenge

Concentration that will be the maximum tested non-irritant concentration. Selection of this concentration may depend on a number of factors and exact criteria do not always apply. If no irritation is observed the highest technically possible concentration will be applied.

2.7 Rationale

Dermal administration has historically been used as the route chosen to assess delayed contact hypersensitivity.

2.8 Observations

The following observations will be recorded as follows:

Viability / Mortality:	Daily from delivery of the animals to the termination of the test.
Clinical Signs / Grading of Skin Response:	Daily from delivery of the animals to the termination of test. Skin responses will be graded during the irritation screen, induction and challenge periods.
Body Weights:	At delivery/acclimatization start, at the end of the irritation screen, at test day 1 (day of treatment) and at the termination of the study.

2.9 Pathology

2.9.1 Necropsy

No necropsy will be performed on all surviving animals. The control and test animals will be euthanized at the end of the test period by intraperitoneal injection of pentobarbitone at a dose of at least 2.0 mL/kg body weight (324 mg sodium pentobarbitone/kg body weight) and discarded. The pretest animals will be euthanized as described above at the treatment start (if possible) in the main study.

Animals which die spontaneously during the observation period will be necropsied as soon as they are found dead and any abnormalities recorded.

No organs or tissues will be retained.

Note: All animals that exhibit signs of undue stress or discomfort as judged by the study director and the technical coordinator will be sacrificed immediately for ethical reasons. The sponsor will be informed immediately. The reason for sacrifice will be included in the final report.

2.10 Treatment Method

The animal's fur will be shaved with a fine clipper blade or equivalent just prior to the exposure. Closed patches are applied to the animals as follows:

0.5 mL of the test item or a freshly prepared test item dilution in a 25 mm Hill Top Chamber.

Note: If possible, the volume of test item will be delivered to the designated patch appliance with a push button fixed volume or adjustable pipette. Extremely viscous materials may be applied using a 1 mL tuberculin syringe with the needle removed. If the test item

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preparation is described as a paste it will be applied with a spatula in a 25 mm Hill Top Chamber (test item amount not determined).

The 25 mm Hill Top Chamber will be firmly secured by an elastic plaster wrapped around the trunk of the animal and secured with impervious adhesive tape. The occlusive dressing will be left in place for six hours (\pm 15 minutes).

Identical patching method is used for the irritation screen, induction, challenge and rechallenge.

2.10.1 Irritation Screen for Induction and Challenge

- Performed during the acclimatization period of the main animals

This investigation will identify the test item concentration required for the induction and challenge phase of the main study.

The test item concentrations described below were selected during a preliminary solubility testing which was performed before the study initiation date. Therefore, the formulation trials will be excluded from the statement of GLP compliance.

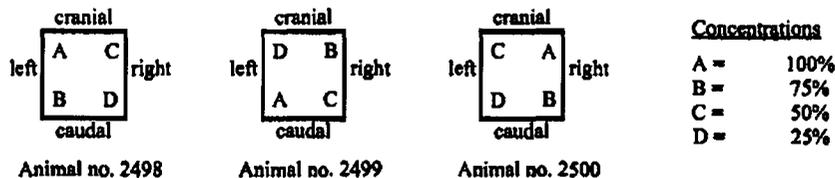
The concentrations of test item and patch positions to be used to treat 3 guinea pigs are given below. Up to 4 different concentrations will be used on each animal.

- Treating naive animals with test item/vehicle on patches for an approximate 6-hour period.

Test item: Methyltrimethoxysilane

Vehicle: PEG 300

Formulation: weight/weight



The allocation of the different test sites (A, B, C, D) on the animals will be alternated in order to minimize site to site variation in responsiveness.

If the highest non-irritating concentration cannot be determined after the first irritation screen, at least one additional irritation screen with additional animals may be performed at the discretion of the study director. All information concerning the additional animals will be included in the report.

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2.10.2 Induction

Each animal will receive one patch per week which will remain in place for approximately 6 hours each. The patches will be applied over a period of 3 weeks. The repeated application will be performed at the same site. In case of severe skin reactions a new, naive application site will be used for the following induction. The interval between each exposure will be a week.

After the last induction exposure the animals will be left untreated for approximately 2 weeks before the primary challenge.

Any gross skin reactions will also be recorded without depilation.

The control animals will be treated with the vehicle alone if the test item has to be diluted with the vehicle through the main study. Otherwise, they will be remained untreated (just covered with the 25 mm Hill Top Chamber and elastic plaster).

2.10.3 Challenge

Control animals and animals previously exposed during the induction period (test group) will be challenged approximately 2 weeks (12-16 days) after the last induction exposure. The highest non-irritating concentration of test item will be applied on the flank of the control and test group in a similar manner used for the epidermal induction. The vehicle will be applied too if test item dilution has to be done in the main study.

The animals will be exposed for approximately 6 hours on a naive skin site.

For a single challenge the fur will be removed from the left posterior quadrant of the side and back of the animal.

For a rechallenge the entire right side will be used as well.

2.10.4 Rechallenge

If results from the first challenge are equivocal the test-, control- and/or untreated control group animals will be rechallenged two weeks after the primary challenge.

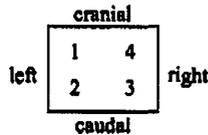
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2.10.5 Formats for Induction, Challenge and Rechallenge Patch Application

- 1 = Induction (test group with the test item and the control group with the vehicle (optional))
- 2 = Primary Challenge (control and test group with the test item)
- 3 = Primary Challenge (optional, control and test group with the vehicle)
- 4 = Rechallenge (will depend on the primary challenge results)

2.11 Determination of Skin Reactions**2.11.1 Observation and Scoring**

The test item skin area of the animals used for the irritation screen and challenge will be depilated approximately 21 hours after the patches have been removed, using an approved depilatory cream (VEET Cream, Reckitt & Colman AG, 4123 Allschwil / Switzerland). The depilation will be performed to clean the stratum corneum from the possible staining produced by the test item and to facilitate the reading of the possible skin reaction. The depilatory cream will be placed on the patch sites and surrounding areas, and left on for up to 3-5 minutes. It will be then thoroughly washed off with a stream of warm, running water. The animals will be then dried with a disposable towel, and returned to their cages.

The grading method used for the irritation screen, induction and challenges is identical. It will be performed 24 ± 2 hours after removal of the patches for the irritation screen, induction and challenge and repeated 24 ± 2 hours later (48-hour grades) for the irritation screen and the challenge.

The scoring system will be performed by visual assessment of erythema and oedema. They will be assessed as follows:

- 0 = no visible change
- 1 = discrete or patchy erythema
- 2 = moderate and confluent erythema
- 3 = intense erythema and swelling

Any other gross lesions not covered by this scoring system will be described.

Grading of all animals will be done by positioning each animal under true-light (Philips Master TLS HE 28W/840 or FL-LPE Osram D16 FH 28W/840 EP).

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For evaluation, two parameters are used: the incidence index and the severity index, for both test and control animals. The incidence index is an expression of the number of animals showing a response of grade 1 or greater at either 24- or 48-hour reading out of the total animals in the group, while the severity index is calculated from the total sum of 24- and 48 hour response readings divided by the number of animals exposed.

2.12 Statistical Analysis

Descriptive statistics (means and standard deviations) will be calculated for body weights. No inferential statistics will be used.

2.13 Data Compilation

The following data will be carried out on data sheets and transcribed in the report: Mortality/viability, clinical signs, skin reactions.

The following data will be recorded on-line: body weights.

The following data will be compiled into the RCC Tox Computer System during recording: macroscopic findings (if death occurs).

The RCC Tox Computer System (RCC-Tox-Lims, version 7.0) has been validated with respect to data collection, storage and retrievability.

If technical problems arise during the on-line recording/immediate compilation of data during observation or additional registration of unplanned data, recording will be carried out on data sheets. These data will be transcribed later for compilation and analysis.

3 REFERENCES

1. Ritz, H.L. and Buehler, E.V. Current Concepts Cutaneous Toxicity, ed. Drill, V.A. and Lazar, T. (Academic Press, 1980) pp. 25-40: Planning, Conduct and Interpretation of Guinea Pig Sensitization Patch Tests.

4 REPORTING

A GLP-compliant draft report will be submitted to the sponsor for scientific review. Following receipt of the sponsor's comments, a QA-audited final report will be issued.

5 DISTRIBUTION

This study plan will be distributed as follows:

Sponsor

Sponsor Representative (responsible for distribution to the Sponsor company): 1 x (PDF)

Harlan Laboratories Ltd.

Study File:	1 x (original)
Study Director:	1 x (PDF)
Deputy Study Director:	1 x (PDF)
Laboratory Technical Coordinator:	1 x (PDF)
Logistics:	1 x (PDF)
QA:	1 x (PDF)

FIRST AMENDMENT TO STUDY PLAN

METHYLTRIMETHOXYSILANE

Contact Hypersensitivity in Albino Guinea Pigs, Buehler Test

Study Director:

Test Facility:

Sponsor: **ReachCentrum SPRL**
for account of the Members of the Reconsile Consortium
Avenue E. van Nieuwenhuysse 6
1160 Brussels/ Belgium

Study Identification:

Version: **Final**

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SIGNATURES

Study Director:

G. Haulk
Date: 31 August 2009

Sponsor Representative:

Marina Joravonic
Date: 31 August 2009

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First Amendment to Study Plan

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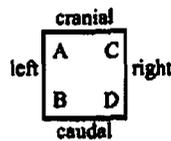
CONCERNING**2.10.1 Irritation Screen for Induction and Challenge****PRESENT**

...

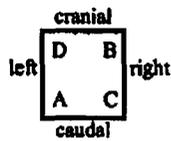
Test item: Methyltrimethoxysilane

Vehicle: PEG 300

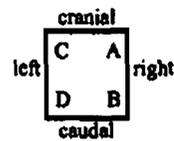
Formulation: weight/weight



Animal no. 2498



Animal no. 2499



Animal no. 2500

Concentrations

A =	100%
B =	75%
C =	50%
D =	25%

The allocation of the different test sites (A, B, C, D) on the animals will be alternated in order to minimize site to site variation in responsiveness.

If the highest non-irritating concentration cannot be determined after the first irritation screen, at least one additional irritation screen with additional animals may be performed at the discretion of the study director. All information concerning the additional animals will be included in the report.

NEW

...

Test item: Methyltrimethoxysilane

Vehicle: PEG 300

Formulation: weight/weight

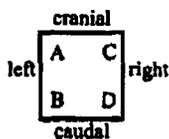
Report

Methyltrimethoxysilane

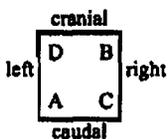
Methyltrimethoxysilane

First Amendment to Study Plan

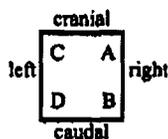
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Animal no. 2498



Animal no. 2499



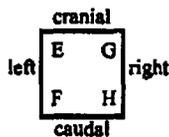
Animal no. 2500

Concentrations

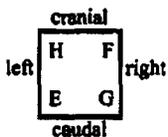
A =	100%
B =	75%
C =	50%
D =	25%

The allocation of the different test sites (A, B, C, D) on the animals will be alternated in order to minimize site to site variation in responsiveness.

If the highest non-irritating concentration cannot be determined after the first irritation screen or if the results of the first challenge are equivocal, at least one additional irritation screen with additional animals will be performed after discussion with the Sponsor and the Study Director. The proposal of the irritation screen will be to test three additional animals with the test item concentrations of in PEG 300 as follows:



Animal no. 2531



Animal no. 2532

E =	25%
F =	15%
G =	10%
H =	5%

All information concerning the additional animals will be included in the report.

REASON FOR THE ALTERATION

Both the control and test animals were observed with local skin reactions at the challenge procedure after application of the test item at 25% in PEG 300. This concentration was previously selected through the first irritation screen. Therefore, a second irritation screen will be performed by applying the same concentration (for comparison) as well as three lower concentrations. Thereafter, two concentrations will be selected and used for the second challenge procedure which will be performed two weeks after the first challenge. The same test animals and a new control group (control group II) will be treated using the same procedure as in the first challenge. The original control group (control group I) will remain untreated and may be sacrificed before the termination of the study.

Report

Methyltrimethoxysilane

Methyltrimethoxysilane

First Amendment to Study Plan

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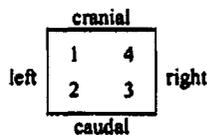
PAGE

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CONCERNING

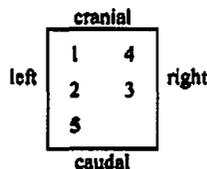
2.10.5 Formats for Induction, Challenge and Rechallenge Patch Application

PRESENT



- 1 = Induction (test group with the test item and the control group with the vehicle (optional))
- 2 = Primary Challenge (control and test group with the test item)
- 3 = Primary Challenge (optional, control and test group with the vehicle)
- 4 = Rechallenge (will depend on the primary challenge results)

NEW



- 1 = Induction (test group with the test item and the control group with the vehicle (optional))
- 2 = Primary Challenge (control and test group with the test item)
- 3 = Primary Challenge (control and test group with the vehicle)
- 4 = Rechallenge (control and test group with test item 1st concentration selected in the 2nd irritation screen)
- 5 = Rechallenge (control and test item with 2nd concentration selected in the 2nd irritation screen)

Report

Methyltrimethoxysilane

Methyltrimethoxysilane

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REASON FOR THE ALTERATION

Equivocal local skin reactions were observed after the first challenge. Therefore, a second challenge will be performed by including two test item concentrations to determine the possible allergenic potential of the test item as well as a possible concentration dependence.

Report

Methyltrimethoxysilane

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Methyltrimethoxysilane

DISTRIBUTION

This study plan amendment will be distributed as follows:

Sponsor

Sponsor Representative (responsible for distribution to the Sponsor company): 1 x (copy)

Harlan Laboratories Ltd.

Study File: 1 x (original)
Study Director: 1 x (copy)
Deputy Study Director: 1 x (copy)
Laboratory Technical Coordinator: 1 x (copy)
Logistics: 1 x (copy)
QA: 1 x (copy)

Report

Methyltrimethoxysilane

APPENDIX III – GLP CERTIFICATE

Report

Methyltrimethoxysilane

The Swiss GLP Monitoring Authorities



Schweizerische Eidgenossenschaft
Confédération suisse
Confederazione Svizzera
Confederaziun svizra
Swiss Confederation

Federal Department of Home Affairs DHA
Federal Office of Public Health FOPH

Federal Department of the Environment,
Transport, Energy and Communications DETEC
Federal Office for the Environment FOEN

swissmedic
Swiss Agency for Therapeutic Products

Statement of GLP Compliance

According to Article 14 paragraph 3 Ordinance on Good Laboratory Practice [OGLP, SR 813.112.1]

The notification authority for chemicals confirms that the following test facility was inspected with respect to the compliance with the Swiss Ordinance on Good Laboratory Practice, adopted on 18th May 2006 [OGLP, SR 813.112.1]. This Ordinance is based on the OECD Principles of Good Laboratory Practice, as revised in 1997 and adopted on 26th November 1997 by decision of the OECD Council [C(97)186/Final].

Unequivocal name and address
of the test facility:

Areas of expertise according to
article 3 paragraph 1 letter d OGLP:

- 1/ Physical-chemical testing,
- 2/ Toxicity studies,
- 4/ Environmental toxicity studies on aquatic and terrestrial organisms,
- 5/ Studies on behaviour in water, soil and air; bioaccumulation,
- 6/ Reactive studies,
- 7/ Studies on effects on mesocosms and natural ecosystems,
- 8/ Analytical and clinical chemistry testing,
- 9/ Other studies (safety pharmacology and animal metabolism).

Inspection authority: Federal Office for the Environment (FOEN) / Federal Office of Public Health (FOPH) / Swiss Agency for Therapeutic Products (Swissmedic)

Date of inspection: 05th to 09th and 26th to 30th November 2007

Date of decision: 30th April 2008

Based on the above mentioned decision it can be confirmed that the above mentioned test facility is able to conduct studies according to the aforementioned areas of expertise in compliance with the principles of GLP. The above mentioned test facility is listed in the register and GLP list according to the Article 14 OGLP and is inspected on a regular basis according to Article 6 paragraph 2 OGLP.

Swiss Federal Office of Public Health
Consumer protection directorate
Notification authority for chemicals
CH-3003 Bern



Dr. Deg Kappes

Bern, 12th November 2008, The Head, Dr. Deg Kappes.

The notification authority for chemicals is the notification and decision authority for the good laboratory practice (GLP) for the FOEN, the FOPH and Swissmedic.

Swiss Federal Office of Public Health, Consumer protection directorate, Notification authority for chemicals, CH-3003 Bern.

www.ch.admin.ch, Phone: +41 (0)31 322 73 00, Fax: +41 (0)31 322 94 00

UPS Internet Shipping: View/Print Label

1. **Print the label(s):** Select the Print button on the print dialog box that appears. Note: If your browser does not support this function select Print from the File menu to print the label.
2. **Fold the printed label at the solid line below.** Place the label in a UPS Shipping Pouch. If you do not have a pouch, affix the folded label using clear plastic shipping tape over the entire label.

3. GETTING YOUR SHIPMENT TO UPS

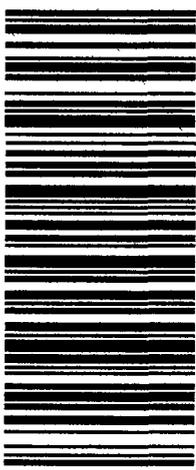
Customers without a Daily Pickup

- Schedule a same day or future day Pickup to have a UPS driver pickup all of your Internet Shipping packages.
- Hand the package to any UPS driver in your area.
- Take your package to any location of The UPS Store®, UPS Drop Box, UPS Customer Center, UPS Alliances (Office Depot® or Staples®) or Authorized Shipping Outlet near you. Items sent via UPS Return ServicesSM (including via Ground) are also accepted at Drop Boxes.
- To find the location nearest you, please visit the 'Find Locations' Quick link at ups.com.

Customers with a Daily Pickup

- Your driver will pickup your shipment(s) as usual.

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<p style="text-align: right;">0.0 LBS LTR</p> <p style="text-align: right;">1 OF 1</p> <p>CONNIE LAFRAMBOISE 989-496-5393 DOW CORNING 2200 WEST SALZBURG AUBURN MI 48611</p> <p>SHIP TO: CBIC - DOCUMENT CONTROL OFFICE (202) 564-8999 EPA - OPPT DOCUMENT CONTROL OFFICER EPA EAST, MAIL: (7407), ROOM: 6248 1201 CONSTITUTION AVENUE, NW WASHINGTON DC 20460-0006</p>	<p style="font-size: 2em;">MD 201 9-80</p> 	<p style="font-size: 2em;">UPS 2ND DAY AIR A.M. 2A</p> <p>TRACKING #: 1Z 464 696 18 9200 8269</p> 	<p style="text-align: center;">BILLING: P/P</p> <p>Reference #1: Methyltrimethoxysilane</p> <p style="text-align: center;"><small>UIS 12.0.26. WXPTE60 03.0A 04/2010</small></p>  <p style="text-align: right;"><small>TM</small></p>
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