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Sanitation & Environment Technology Institute, Soochow University, Final Report

Report Number: SDWH- M201801675-3

Skin Sensitization Test of Liquid silicone rubber Using ISO 10993-10:2010 Test Methods Guinea Pig Maximization Test Sesame oil Extract

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SUPPLEMENTARY EXPLANATION

1. Please apply for rechecking within 15 days of receiving the report if there are any objections.
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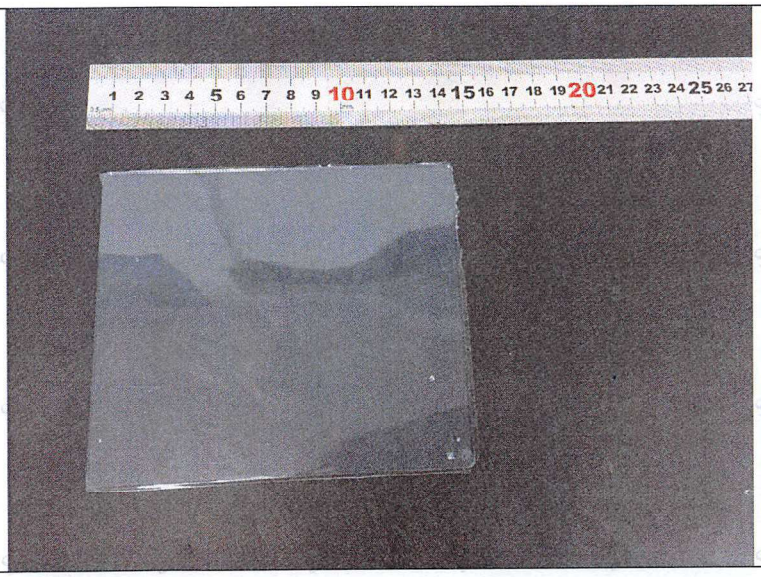
STUDY VERIFICATION AND SIGNATURE

Test Article	
Test Article Receipt:	2018- 06-05
Protocol No:	SDWH- PROTOCOL- GLP- M201801675-3
Protocol Effective Date:	2018- 06-19
Technical Initiation Date:	2018- 06-19
Technical Completion Date:	2018-07-19
Final Report Completion Date:	2018-07-25

Edited by : Jiang Xiaoan 2018-07-25 Date

苏州大学卫生与环境技术研究所 检验检测专用章 (2)

Checked by : Zhang Yan 2018-07-25
Study Director Date

Approved by : Wang Liyi 2018-07-25
Authorized signatory Date

Sanitation & Environment Technology Institute, Soochow University

QUALITY ASSURANCE STATEMENT

This study was conducted in compliance with U.S. Food and Drug Administration regulations set forth in 21 CFR, Part 58.

The sections of the regulations not performed by or under the direction of SDWH, exempt from this Good Laboratory Practice Statement, included characterization and stability of the test article and its mixture with carriers, 21 CFR, Part 58.105 and 58.113.

The Quality Assurance Unit conducted inspections on the following dates. The findings were reported to the Study Director and to SDWH's Management.

INSPECTIONS	DATE OF INSPECTION	DATE REPORTED STUDY DIRECTOR	DATE REPORTED MANAGEMENT
EXPERIMENTAL PROCEDURE	2018-06-26	2018-06-26	2018-07-25
RAW DATA	2018-07-25	2018-07-25	2018-07-25
FINAL REPORT	2018-07-25	2018-07-25	2018-07-25

Quality Assurance Unit : Zhou Ying 2018.7.25

QA Date

1.0 Study Summary

The extract of the test article Liquid silicone rubber (extraction in Sesame oil) was evaluated for its potential to induce skin sensitization in the Guinea Pig Maximization Test.
The test article extract was intradermally injected and applied topically for induction to ten guinea pigs. Five control animals were treated accordingly but with the solvent alone. The topical challenge with the test article extract elicited no skin reaction in the test and in the control animals. The skin sensitization rate was determined with 0%.
As defined by the scoring system of Magnusson and Kligman the test article extract showed no signification evidence of causing skin sensitization in the guinea pig under the conditions of this study.

2.0Purpose

The test was designed to evaluate the potential of a test article to cause skin sensitization. The test is used as a procedure for screening of contact allergens in guinea pigs and extrapolating the results to humans, but it does not establish the actual risk of sensitization.

3.0Reference

Biological evaluation of medical devices Part 10: Tests for irritation and skin sensitization (ISO 10993-10:2010)
Biological evaluation of medical devices-Part 12: Sample preparation and reference materials (ISO 10993-12:2012)
Biological evaluation of medical devices-Part 2: Animal welfare requirements (ISO 10993-2:2006)

4.0Compliance

Good Laboratory Practice Regulations, 21 CFR, Part 58
ISO/IEC 17025:2005 General requirements for the competence of testing and calibration laboratories (CNAS-CL01 Accreditation Criteria for the competence of testing and calibration laboratories)
China National Accreditation Service for Conformity Assessment
Laboratory Accreditation Certificate No.CNAS L2954
Accreditation Criteria for the competence of Inspection Body (Certification and Accreditation Admin istration of the People's Republic of China CMA 180015144061)

5.0Identification of test and control articles

5.1 Test article
Test article name: Liquid silicone rubber
Test article initial state: Not Sterilized

CAS Code: Not supplied by sponsor (N/S)
Size: N/S
Lot/ Batch: N/S
Test Article Material: Silicone rubber
Packaging Material: N/S
Physical State: Solid
Color: see the image
Density: N/S
Stability: N/S
Solubility: N/S
Storage Condition: Room Temperature
Other: Applicable to Model: MCS-3010-XY, LGS-9520-XY,PRS--9651-XY
The information about the test article was supplied by the sponsor wherever applicable.
The Sponsor was responsible for all test article characterization data as specified in the GLP regulations.

5.2 Control article

5.2.1 Negative Control

Article Name: Sesame oil (SO).
Manufacturer: Ji'an luyuanxiangliao. Co. Ltd
Size: 25kg
Lot/ Batch#: 20180523
Physical State: Oily liquid
Color: Pale yellow
Storage Condition: Room Temperature

5.2.2Positive Control

Article Name: 2, 4-Dinitrochlorobenzene (DNCB)
Manufacturer: Xiya Reagent^{R}
Size: 100g
Lot/ Batch#: W5656
Induction Concentration: 0.5%
Challenge Concentration: 0.1%
Solvent: Sesame oil
Date prepared: 2018-06-19
Physical State: Liquid
Color: light yellow
Storage Condition: Room Temperature

6.0Identification of test system

Species: Hartley Guinea Pig (Cavia Porcellus)
Number: 15 (10 Test +5 Negative Control)
Sex: males
Initial body weight: 300～500g

Health status: Healthy, not previously used in other experimental procedures
Housing: Animals were housed in groups in cages identified by a card indicating the lab number, test code and first treatment date, etc
Animal identification: Stain with picric acid
Cages: Plastic cage
Acclimation Period: 7 days under the same conditions as for the actual test

7.0Animal Care and Maintenance

Animal purchase: Suzhou Experimental Animal Sci-tech Co., Ltd. <Permit Code: SCXK (SU) 2015-0007>
Bedding: Corncob, Suzhou shuangshi laboratory animal feed science Co.,Ltd
Feed: Guinea Pig Diet, Suzhou Experimental Animal Sci-tech Co., Ltd.
Water: Drinking water met the Standards for Drinking Water Quality GB 5749-2006
Animal room temperature: 18-26℃
Animal room relative humidity: 30%-70%
Lights: 12 hours light/dark cycle, full-spectrum lighting
Personnel: Associates involved were appropriately qualified and trained
Selection: Only healthy, previously unused animals were selected
There were no known contaminants present in the feed, water, or bedding expected to interfere with the test data

8.0Justification of the test system

The albino guinea pig has been used historically for sensitization studies (Magnusson and Kligman, 1970). The guinea pig is believed to be the most sensitive animal model for this type of study .The susceptibility of the guinea pig to a known sensitizing agent, 2, 4-Dinitrochlorobenzene (DNCB) has been substantiated at SDWH.

9.0Route of administration

The test article was extracted and administered in vivo through a medium compatible with the test system. Dermal application corresponds to the likely route of human exposure.

10.0Experiment design

10.1 Sample and Control Preparation

Intradermal induction phase Ⅰ:

Aseptic Sampling		Aseptic Agitation Extraction In Inert Container			Final Extract	
Sampling Manner	Actually sampling	Ratio	sesame oil	Condition	pH	Clear or Not

Random (Remove the protective films)	Surface area 60cm^2	3cm^2:1ml	20.0ml	50℃,72h	5.5	Clear

Topical induction phase Ⅱ:

Aseptic Sampling		Aseptic Agitation Extraction In Inert Container			Final Extract	
Sampling Manner	Actually sampling	Ratio	sesame oil	Condition	pH	Clear or Not
Random (Remove the protective films)	Surface area 60cm^2	3cm^2:1ml	20.0ml	50℃,72h	5.5	Clear

Challenge phase:

Aseptic Sampling		Aseptic Agitation Extraction In Inert Container			Final Extract	
Sampling Manner	Actually sampling	Ratio	sesame oil	Condition	pH	Clear or Not
Random (Remove the protective films)	Surface area 60cm^2	3cm^2:1ml	20.0ml	50℃,72h	5.5	Clear

There is no change in the extraction solvent (pre- and post-extraction). The extract was stored at 4℃ and tested within 24h after extraction without the process of pH value adjustment, filtering, centrifugation, dilution, etc.

The vehicle (without the test article) was similarly prepared to serve as the control.

10.2 Equipment

Constant Temperature Vibrator (SDWH217), Calibration Expire (2019-05-15)
Autoclave (SDWH2097), Calibration Expire (2018-11-12)
Steel Straight Scale (SDWH463) ,Calibration Expire（2018-09-10）
Electronic scale (SDWH442) ,Calibration Expire（2019-05-15）

10.3 Reagents

Freund's Adjuvant,Complete liquid
Manufacturer: SIGMA
Lot No: SLBV0593
Sodium dodecyl sulfate (SDS)
Manufacturer: Sinopharm Chemical Reagent Co.Ltd
Lot No: 20150113
Concentration: 10%
Solvent: Distilled water
Date prepared: 2018-03-01

10.4 Intradermal induction phase Ⅰ

A pair of 0.1ml intradermal injections was made for each of the following, into each animal, at the injection sites (A, B and C) as shown in Figure 1 in the clipped intrascapular region.

Site A: A 50:50 (volume ratio) stable emulsion of Freund's complete adjuvant mixed with the chosen solvent.

Site B: The test sample (undiluted extract); the control animals were injected with the solvent alone.

Site C: The test sample at the concentration used at site B, emulsified in a 50:50 volume ratio stable emulsion of Freund's complete adjuvant and the solvent (50%); the control animals were injected with an emulsion of the blank liquid with adjuvant.

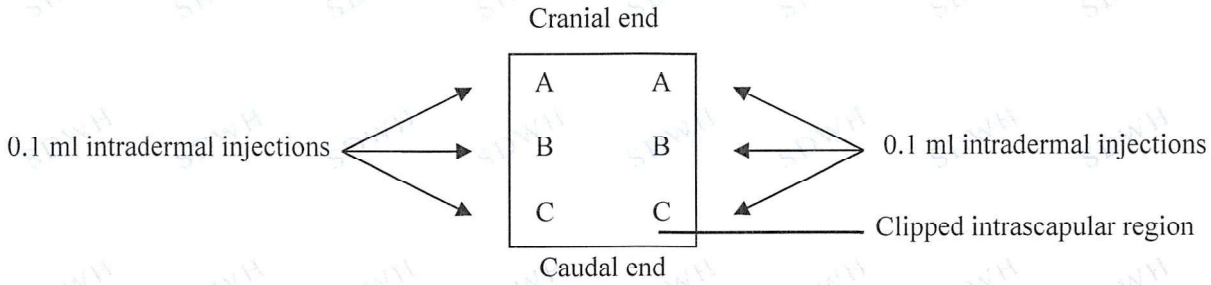


Figure 1 Location of intradermal injection sites

10.5 Topical induction phase II

The maximum concentration that can be achieved in Intradermal induction phase I did not produce irritation, animals are pretreated with 10% sodium dodecyl sulfate 24(±2) hours before the topical induction application.

At 7 d after completion of the intradermal induction phase, administer 0.5ml test article extract by topical application to the intrascapular region of each animal, using a patch of area approximately 8cm^2 (absorbent gauze), so as to cover the intradermal injection sites. Secure the patches with an occlusive dressing. Remove the dressings and patches after (48±2) h.

Treat the control animals similarly, using the blank liquid alone.

10.6 Challenge phase

At 14 d after completion of the topical induction phase, challenge all test and control animals with the test sample. Administer 0.5ml test article extract by topical application to sites that were not treated during the induction stage, using absorbent gauze (2.5cm×2.5cm) soaked in the test article extract. Secure with an occlusive dressing. Remove the dressings and patches after (24±2) h.

10.7 Observation of animal

Observe the appearance of the challenge skin sites of the test and control animals (24±2) h and (48±2)h after removal of the dressings. Full-spectrum lighting was used to visualize the skin reactions. Describe and grade the skin reactions for erythema and oedema according to the Magnusson and Kligman grading given in Table 1 for each challenge site and at each time interval.

10.8 Evaluation of results

Magnusson and Kligman grades of 1 or greater in the test group generally indicate sensitization, provided grades of less than 1 are seen in control animals.
If grades of 1 or greater are noted in control animals, then the reactions of test animals which exceed the most severe reaction in control animals are presumed to be due to sensitization.
If the response is equivocal, rechallenge is recommended to confirm the results from the first challenge.
The outcome of the test is presented as the frequency of positive challenge results in test and control animals.

Table 1 Magnusson and Kligman scale

Patch test reaction	Grading scale
No visible change	0
Discrete or patchy erythema	1
Moderate and confluent erythema	2
Intense erythema and/or swelling	3

10.9 Results

Individual results of dermal scoring for the challenge appear in Table 2.

10.10 Conclusion

Under the conditions of this study, the test article Liquid silicone rubber extract showed no significant evidence of causing skin sensitization in the guinea pig.

11.0 Record Storage

All raw data pertaining to this study and a copy of the final report are to be retained in designated SDWH archive.

12.0 Confidentiality Agreement

Statements of confidentiality were as agreed upon prior to study initiation.

13.0 Deviation statement

There were no deviations from the approved study protocol which were judged to have any impact on the validity of the data.

Table 2 Guinea pig Sensitization Dermal Reactions

Group	Animal Number	24±2h before phase II patch application		24±2 h following Challenge phase		48±2 h following Challenge phase		Positive rate after challenge phase
		Left	Right	Test sites	Control sites	Test sites	Control sites	
Test Group	1	0	0	0	0	0	0	0%
	2	0	0	0	0	0	0	
	3	0	0	0	0	0	0	
	4	0	0	0	0	0	0	
	5	0	0	0	0	0	0	
	6	0	0	0	0	0	0	
	7	0	0	0	0	0	0	
	8	0	0	0	0	0	0	
	9	0	0	0	0	0	0	
	10	0	0	0	0	0	0	
Negative control	11	0	0	0	0	0	0	—
	12	0	0	0	0	0	0	
	13	0	0	0	0	0	0	
	14	0	0	0	0	0	0	
	15	0	0	0	0	0	0	

Table 3 Weigh change and Clinical observation

Group	Animal Number	Weight (g)		Clinical observation except dermal reactions
		Before injection	After experiment	
Test Group	1	325	398	Normal
	2	302	363	Normal
	3	346	429	Normal
	4	358	448	Normal
	5	306	369	Normal
	6	317	387	Normal
	7	310	376	Normal
	8	305	366	Normal
	9	333	411	Normal
	10	307	370	Normal
Negative control	11	324	396	Normal
	12	320	391	Normal
	13	315	383	Normal
	14	356	443	Normal
	15	339	419	Normal

Table 4 Guinea pig Sensitization Dermal Reactions of Positive Group

Group	Animal Number	24±2h before phase II patch application		24±2 h following Challenge phase		48±2 h following Challenge phase		Positive rate after challenge phase
		Left	Right	Test sites	Control sites	Test sites	Control sites	
Positive Group	1	3	3	2	0	2	0	100%
	2	3	3	2	0	2	0	
	3	3	3	2	0	1	0	
	4	3	3	2	0	2	0	
	5	3	3	1	0	1	0	
Negative control	6	0	0	0	0	0	0	—
	7	0	0	0	0	0	0	
	8	0	0	0	0	0	0	
	9	0	0	0	0	0	0	
	10	0	0	0	0	0	0	

Note: The data of positive control come from SDWH-M201801772-2(Completed Date: 2018-07-12)

Table 5 Weigh change and Clinical observation of Positive Group

Group	Animal Number	Weight (g)		Clinical observation except dermal reactions
		Before injection	After experiment	
Positive Group	1	351	434	Normal
	2	315	386	Normal
	3	317	385	Normal
	4	318	387	Normal
	5	307	371	Normal
Negative control	6	327	403	Normal
	7	334	411	Normal
	8	317	383	Normal
	9	353	436	Normal
	10	318	389	Normal

Note: The data of positive control come from SDWH-M201801772-2(Completed Date: 2018-07-12)