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CNAS L13034

Skin Sensitization Test

Guinea Pig Maximization

Final Report

Verification

Report Number: CSTBB2022010640

Article Name: Tubing

Method Standard: ISO 10993-10: 2010

Sponsor

TÜV Rheinland (Shenzhen) Co., Ltd.

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6. ISO 10993-2:2006 and ISO 10993-12:2021 are not within the scope of qualification.

Abstract

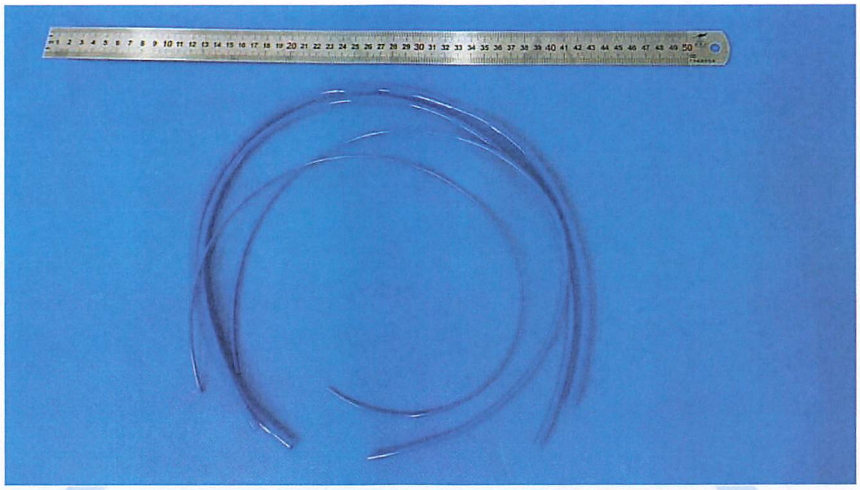
In this study, we took guinea pigs to observe the skin sensitization of the test article according to ISO 10993-10: 2010.

The test article were extracted by 0.9% Sodium Chloride Injection and Sesame Oil. The extract was mixed with Fresch's complete adjuvant into a stable emulsifier. Intradermal induction and topical induction were operated in the clipped intrascapular region of each animal. At 14d after completion of the topical induction phase, Challenge all test and control animals with the test sample at sites that were not treated during topical induction phase. The erythema and edema of the challenge site were observed to test the sensitization response of the test article. According to the Magnusson and Kligman scales, the response to erythema and edema at each application site of the skin was described and scored 24 hours and 48 hours after the challenge phase.

The results showed that the guinea pigs in the negative control group (0.9% Sodium Chloride Injection, Sesame Oil) retained a normal appearance throughout the test and showed no skin irritants. A severe skin reactions for erythema and oedema were shown in the positive control group (DNCB). While in test article group, the response of skin on testing side did not exceed that on the control side. The skin reactions for erythema and oedema were not observed in test article group. The data of each group met the acceptance criteria, and the results of this test were considered valid.

Based on the above results, it can be concluded that under the experimental conditions, the test article has no potential skin sensitization on guinea pigs in the extraction method.

Study Verification and Signature



Protocol Number	SST2112041308BB
Protocol Effective Date	2021-12-30
Technical Initiation Date	2021-12-31
Technical Completion Date	2022-01-28
Final Report Completion Date	2022-03-08

Personnel — Betty Zhuang — 2022-03-08 — Date Completed

Approved — Vicky Yi — Study Director — 2022-03-08 — Date Completed

Supervisory — Test Facility Manager — Date Completed

CCIC Huatongwei International Inspection (Suzhou) Co., Ltd.

Quality Assurance Statement and GLP Statement

Quality Assurance Statement

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

The final report was reviewed to assure that the report accurately describes the methods and standard operating procedures. The reported results accurately reflect the raw data of the nonclinical study conducted per the protocol.

Phase Inspected	Date	Study Director	Management
Experiment	2021-12-31	2021-12-31	2021-12-31
Raw Data	2022-01-28	2022-01-28	2022-01-28
Final Report	2022-03-08	2022-03-08	2022-03-08

The findings of these inspections have been reported to Management and the Study Director.

Hongjia Li
Quality Assurance

2022-03-08
Date

GLP Statement

This study was conducted in compliance with current 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 HTW, 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.

Vicky Yin
Study Director

2022-03-08
Date

1.0 Purpose

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.

2.0 Reference

Biological evaluation of medical devices-Part 10: Tests for irritation and skin sensitization (ISO 10993-10:2010)

Biological evaluation of medical devices-Part 2: Animal welfare requirements (ISO 10993-2:2006)

Biological evaluation of medical devices-Part 12: Sample preparation and reference materials (ISO 10993-12:2021)

3.0 Test and control articles

Groups	Test article	Negative Control Article(Polar)	Negative Control Article(Non-Polar)	Positive Control
Name	Tubing	0.9% Sodium Chloride Injection(SC)	Sesame Oil (SO)	2, 4-Dinitrochlorobenzene (DNCB)
Manufacturer	Beyes Dental Canada Inc.	Guangxi Yuyuan Pharmaceutical Co., Ltd	Zhejiang Tianyu yam Oil Co., Ltd	TOKYO CHEMICAL INDUSTRY CO., LTD
Size	/	500 ml	1L	25 g
Model	/	/	/	/
Lot Batch#	/	H21011707	MX20210401	H2UKD-DM
Test Article Material	Plastic	/	/	/
Physical State	Solid	Liquid	Liquid	Solid
Color	/	Colorless	Light yellow	Light yellow
Package material	/	/	/	/
Sterilized or Not	Not Sterilized	/	/	/
Concentration	/	0.9 %	/	Induction Concentration: 0.1 % Challenge Concentration: 0.05 % Dissolved in ethanol
Surface (cm^2)	Not Provided	/	/	/
Weight (g)	Not Provided	/	/	/
Storage	Room Temp.	Room Temp.	Room Temp.	Room Temp.

Condition				
The information about the test article was supplied by the sponsor wherever applicable.				

4.0 Identification of test system

4.1 Test animal

Species: Hartley Guinea Pig (Cavia Porcellus)

Number: 30 (20 Test +10 Control)

Sex: either sex

Initial body weight: 300.0~500.0 g

Health status: Healthy, not previously used in other experimental procedures. Female animals were nul liparous and not pregnant.

Animal identification: Ear tag

Cages: Plastic cage

Acclimation Period: 7 days under the same conditions as for the actual test

4.2 Justification of test system

The albino guinea pig has been used historically for sensitization studies (Magnusson and Kligman, 1 970). The guinea pig is believed to be the most sensitive animal model for this type of study. DNCB is the positive control article recommended in the test instructions. To ensure the sensitivity of the experime ntal system, the positive control article should be verified every three months.

5.0 Animal management

Animal purchase: Wuxi hengtai experimental animal breeding co. LTD SCXK (SU) 2020-0003

Bedding: Corncob Jiangsu Xietong Pharmaceutical Bio-engineering Co., Ltd.

Feed: Guinea pigs were fed with full-price pellets Jiangsu Xietong Pharmaceutical Bio-engineering 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.

6.0 Equipment and reagents

6.1 Instruments

Constant Temperature Vibrator (SHB007, calibration data: 2021/03/11), Electronic scale (SHB017, calibration data: 2021/03/11)

6.2 Reagents

Freund’s adjuvant Complete liquid (SIGMA, Lot No: SLCC3348), Sodium dodecyl sulfate (Solarbio, Lot No: 1019Y032)

7.0 Experiment design

7.1 Sample preparation

The extracts of test article will be prepared according to the following steps:

Aseptic Sampling			Extraction in sterile vessels				
	Sampling Manner	Actually sampling	Ratio	Reagent		Condition	pH
Intradermal induction phase I	Whole	158.4 cm^2	6 cm^2: 1 ml	SC	26.4 ml	50 ℃ / 72 h/ 60rpm	5.5
		158.4 cm^2		SO	26.4 ml		/
Topical induction phaseII	Whole	158.4 cm^2	6 cm^2: 1 ml	SC	26.4 ml	50 ℃ / 72 h/ 60rpm	5.5
		158.4 cm^2		SO	26.4 ml		/
Challenge phase	Whole	158.4 cm^2	6 cm^2: 1 ml	SC	26.4 ml	50 ℃ / 72 h/ 60rpm	5.5
		158.4 cm^2		SO	26.4 ml		/

Both induction and challenge phase extracts were prepared by the number of times. The state of the leaching solution did not change visually after the leaching was advanced. The control solution was prepared under the same conditions.The extraction of the test article could be stored at room temperature. for no more than 24 h. No particulates or color changes were observed in pre- and post-extraction, the color and pH of the extraction solution did not change before and after use,and the pH value was 5.5, the status of the extract was shown in the table below.

Phase	Vehicle	Time Observed	Extracts	Condition of Final Extracts		
				Color	Clear or Not	Particulates
Intradermal induction phase I	0.9% Sodium Chloride Injection(SC)	Before Extraction	Test article	Colorless	Clear	None
			Negative Control	Colorless	Clear	None
		After Extraction	Test article	Colorless	Clear	None
			Negative Control	Colorless	Clear	None
	Sesame Oil (SO)	Before Extraction	Test article	Light yellow	Clear	None
			Negative Control	Light yellow	Clear	None
		After Extraction	Test article	Light yellow	Clear	None
			Negative Control	Light yellow	Clear	None
Topical induction phaseII	0.9% Sodium Chloride Injection(SC)	Before Extraction	Test article	Colorless	Clear	None
			Negative Control	Colorless	Clear	None
		After Extraction	Test article	Colorless	Clear	None
			Negative Control	Colorless	Clear	None
	Sesame Oil (SO)	Before Extraction	Test article	Light yellow	Clear	None
			Negative Control	Light yellow	Clear	None

		After Extraction	Test article	Light yellow	Clear	None
			Negative Control	Light yellow	Clear	None
Challenge phase	0.9% Sodium Chloride Injection(SC)	Before Extraction	Test article	Colorless	Clear	None
			Negative Control	Colorless	Clear	None
		After Extraction	Test article	Colorless	Clear	None
			Negative Control	Colorless	Clear	None
	Sesame Oil (SO)	Before Extraction	Test article	Light yellow	Clear	None
			Negative Control	Light yellow	Clear	None
		After Extraction	Test article	Light yellow	Clear	None
			Negative Control	Light yellow	Clear	None

7.2 Test method

7.2.1 Intradermal induction phaseI

Make a pair of 0.1 ml intradermal injections of 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); inject the control animals with the extraction 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; inject the control animals with an emulsion of the blank liquid with adjuvant.

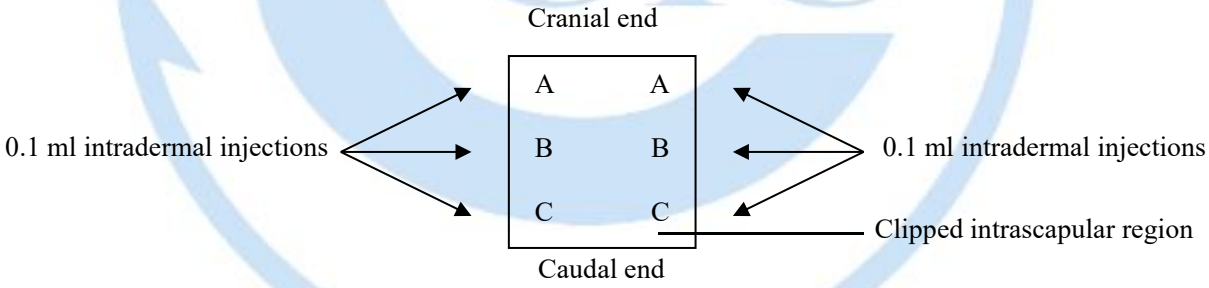


Figure 1 Location of intradermal injection sites

7.2.2 Topical induction phaseII

At (7±1) d after the intradermal induction phase, administer the 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. If the maximum concentration that can be achieved in Intradermal induction phase I does not produce irritation, pretreat the area with 10% sodium dodecyl sulfate(SDS) massaged into the skin (24±2) h before the patch is applied. 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.

7.2.3 Challenge phase

All test and control animals shall be challenged at 14 d after completion of the topical induction phase.

Absorbent gauzes (2.5 cm x 2.5 cm) were soaked respectively with test article and control article. Apply the test article extract and control article topically to two sites that were not treated during the induction stage. The patches shall be secured with an occlusive dressing. Dressings and patches shall be removed after (24±2) h.

8.0 The results observed

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. Use of natural lighting is highly recommended in order to visualize the skin reactions. Describe and grade the skin reactions for erythema and oedema according to the Magnusson and Kligman grading scale given in Table 1 for each challenge site and at each time interval.

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

9.0 Evaluation criteria

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.

10.0 Results of the test

All animals were survived and no abnormal signs were observed during the study.Individual results of dermal scoring for the challenge appear in Table 2.

11.0 Conclusion

The test article showed no evidence of causing delayed dermal contact sensitization in the guinea pig. Results and conclusions apply only to the test article tested. Any extrapolation of these data to other articles is the sponsor’s responsibility.

12.0 Compliance

US FDA Good Laboratory Practice Regulations 21 CFR 58, effective June 20, 1979, as amended 52 FR 33780, Sept. 4, 1987, and subsequent amendments

Standard operating procedure of CCIC Huatongwei International Inspection (Suzhou) Co., Ltd.

13.0 Record

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

14.0 Confidentiality agreement

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

15.0 Protocol amendment/deviations

There were no amendments or deviations that occurred during the course of this study.

Table 2 Guinea pig Sensitization Dermal Reactions

Group		No.	Pretest weight(g)	Finished weight(g)	The Challenge patch was removed 24h later		The Challenge patch was removed 48h later		Positive rate
					Erythema	Swelling	Erythema	Swelling	
SC	Test article	1	314.4	379.2	0	0	0	0	0%
		2	304.4	367.9	0	0	0	0	
		3	313.9	378.8	0	0	0	0	
		4	307.7	372.8	0	0	0	0	
		5	303.7	368.1	0	0	0	0	
		6	314.9	380.0	0	0	0	0	
		7	307.7	371.7	0	0	0	0	
		8	316.9	381.9	0	0	0	0	
		9	304.6	369.6	0	0	0	0	
		10	317.6	381.9	0	0	0	0	
	Negative Control	11	306.8	370.7	0	0	0	0	0%
		12	311.2	375.1	0	0	0	0	
		13	308.4	373.8	0	0	0	0	
		14	309.9	374.9	0	0	0	0	
		15	313.1	378.3	0	0	0	0	
SO	Test article	16	317.2	382.3	0	0	0	0	0%
		17	309.1	374.2	0	0	0	0	
		18	314.1	379.1	0	0	0	0	
		19	315.5	380.6	0	0	0	0	
		20	309.0	373.5	0	0	0	0	
		21	313.7	378.9	0	0	0	0	
		22	303.5	370.3	0	0	0	0	
		23	313.3	378.4	0	0	0	0	
		24	307.1	372.5	0	0	0	0	
		25	303.3	368.2	0	0	0	0	
	Negative Control	26	314.3	379.6	0	0	0	0	0%
		27	306.9	373.3	0	0	0	0	
		28	316.2	381.3	0	0	0	0	
		29	304.1	370.3	0	0	0	0	
		30	317.0	382.2	0	0	0	0	

Table 3 Positive control

Group	No.	Pretest weigh(g)	Finished weigh(g)	The Challenge patch was removed 24 h later		The Challenge patch was removed 48 h later		Positive rate
				Erythema	Swelling	Erythema	Swelling	
Positive Article Group	1	314.1	375.1	2	2	2	2	100%
	2	304.2	361.5	1	0	1	0	
	3	312.6	379.8	1	1	1	1	
	4	308.7	368.7	1	2	1	2	
	5	311.5	378.4	1	0	1	1	
	6	308.4	368.7	2	0	2	1	
	7	313.7	378.4	2	1	3	2	
	8	309.2	367.8	1	1	2	1	
	9	317.3	386.7	1	0	1	1	
	10	315.1	376.2	1	1	2	1	
Solution Control Group	11	307.2	365.4	0	0	0	0	0%
	12	313.5	379.1	0	0	0	0	
	13	304.9	367.6	0	0	0	0	
	14	315.1	377.5	0	0	0	0	
	15	308.7	363.4	0	0	0	0	

Note：The positive control was CSTBB21120001P1 (Finish date：2021-12-24)