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CNAS L3428

Test Report

Date: 2019-08-15
No. : DY19060320

TEST FACILITY

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CONFIDENTIAL

STUDY TITLE

Guinea Pig Maximization Sensitization Test

TEST ARTICLE NAME

FRAME

TEST ARTICLE IDENTIFICATION

CP-MD-1197

CSD NO: CL20190605262

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Test Report

Summary

The test article, FRAME, was evaluated for the potential to cause delayed dermal contact sensitization in a guinea pig maximization test. This study was conducted based on the requirements of ISO 10993-10:2010, Biological evaluation of medical devices - Part 10: Tests for irritation and skin sensitization. The test articles were extracted in 0.9% sodium chloride injection and soybean oil. Each extract was intradermally injected and occlusively patched to ten test guinea pigs (per extract). Following a recovery period, the test and control animals received a challenge patch of the appropriate test article extract, the vehicle control. All sites were scored for dermal reactions at 24 and 48 hours after patch removal.

The test article extracts showed no evidence of causing delayed dermal contact sensitization in the guinea pig. The test article was not considered a sensitizer in the guinea pig maximization test.

Authorized Signatory Approval: Tang

Jonathan Tang

1. Introduction

1.1 Purpose

The purpose of this study was to evaluate the potential of the test articles to cause delayed dermal contact sensitization in the guinea pig maximization test.

1.2 Testing Guidelines

This study was conducted based on the requirements of the International Organization for Standardization ISO 10993-10:2010, Biological evaluation of medical devices - Part 10: Tests for irritation and skin sensitization.

1.3 Dates

Test Article Received:	2019.06.28
Treatment Started:	2019.07.13
Observations Concluded:	2019.08.10

2. Identification of Test and Control Articles

The test articles provided by the sponsor were identified and handled as described below:

Table 1: Test Article

Name:	FRAME
Size:	N.A
CAS Code:	N.A
Model:	TRUEGRASSES
Lot:	N.A
Initial State:	Not Sterilized
Strength, Purity and Composition:	TRUEGRASSES
Color:	N.A
Physical Description of the Test Article:	Solid
Manufacture date:	N.A
Expiration Date:	N.A

Table 2: Negative Control Article

Name:	0.9% Sodium chloride injection (SC) Soybean oil (SO)
Purity, Composition, And Other Characteristics:	SC: Composition: 0.9% NaCl ± 5.0% of label claim, balance is water; sodium chloride CAS No.: 7647-14-5/water CAS No.: 7732-18-5 SO: Composition: CAS No.: 8001-22-7

Table 3: Ancillary Material

Name:	Freund's Complete Adjuvant (FCA) was mixed 50:50 (v/v) with the appropriate vehicle and used at Induction I. A 10% (w/w) sodium lauryl sulfate (SLS) suspension in petrolatum was used prior to Induction II. These materials were provided by the test facility.

Table 4: Reagents

Name	Brand	Lot
SC	YUYUAN	H19050908
SO	TIANYUSHAN	20181002/20190306
Freund's Adjuvant, Complete	SIGMA	SLBR3877V

3. Test System

3.1 Test System and Justification of Test System

Species: Guinea pig (Cavia porcellus)
Strain: Hartley
Source: Guangzhou baiyun district longgui xingke animal farm （广州市白云区龙归兴科动物养殖场）
Sex: Male
Age: Young adult
Acclimation Period: Minimum 5 days
Number of Animals: 30

3.2 Justification of Test System

The albino guinea pig (animal) 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. This study was referred to the quarterly positive control test report number DCA20190002, which confirmed guinea pig strain sensitivity to known sensitizer l-chloro-2, 4-dinitrobenzene (DNCB) in the STC.

4. Animal Management

4.1 Husbandry, Housing and Environment

Conditions conformed to STC Standard Operating Procedures. Animals were housed in groups in stainless steel or plastic suspended cages identified by a card indicating the animal numbers, test code, sex, animal code and date dosed.

The animal housing room is conventional system lab. The lab animal use permit No. SYXK(粤)2019-0159. The animal housing room temperature and relative humidity were monitored daily. The temperature for the room was set to 19-26℃ and the relative humidity was set to 40-70%. There were no significant temperature or relative humidity excursions that adversely affected the health of the animals.

The light cycle was controlled (12 hours light, 12 hours dark).

4.2 Food, Water and Contaminants

Food: Laboratory animal formula feed (Guinea pig), Shenyang Maohua biotechnology co. LTD（沈阳茂华生物科技有限公司）, was provided daily.

Water: The water quality met the "Sanitary standard for drinking water" (GB5749-2006)

Food and water were sterile. No contaminants present in the feed and water impacted the results of this study.

4.3 Personnel

Associates involved in this study were appropriately qualified and trained.

4.4 Veterinary Care

Standard veterinary medical care was provided in this study.

4.5 IACUC

This procedure has been approved by the STC Institutional Animal Care and Use Committee (IACUC), and is reviewed at least annually by the same committee.

4.6 Selection

Only healthy, previously unused animals were selected.

5. Method

5.1 Test and Control Article Preparation

The test articles were measured and calculated. The test article extracts and the vehicle control (extraction vehicle without the test article) were prepared fresh for each phase of testing and subjected to the extraction conditions as described in Table 5. The extracts were continuously agitated during extraction.

Table 5: Extraction

Vehicle: SC

Testing Phase	Treatment Group	Extraction Ratio	Article Amount	Volume of Vehicle	Extraction Condition
Induction I	Test	0.2g:1 mL	24.206g	121.0mL	50±2°C for 72±2 h
	Control	N. A	N. A	20mL	
Induction II	Test	0.2g:1 mL	18.723g	93.6mL	
	Control	N. A	N. A	20 mL	
Challenge	Test	0.2g:1 mL	17.878g	89.4mL	
	Control	N. A	N. A	20mL	

Vehicle: SO

Testing Phase	Treatment Group	Extraction Ratio	Article Amount	Volume of Vehicle	Extraction Condition
Induction I	Test	0.2g:1 mL	24.133g	120.7mL	50±2°C for 72±2 h
	Control	N. A	N. A	20 mL	
Induction II	Test	0.2g:1 mL	18.196g	91.0mL	
	Control	N. A	N. A	20mL	
Challenge	Test	0.2g:1 mL	18.389g	91.9mL	
	Control	N. A	N. A	20 mL	

The following table contain a description of the test and control article extracts before and after extraction and prior to dosing.

Table 6: Condition of Extracts

Vehicle: SC

Treatment group	Time Observed	Extract	Condition of Extracts		
			Color	Clarity	Particulates
Test	Before Extraction	Induction I	Colorless	Clear	None
		Induction I I	Colorless	Clear	None
		Challenge	Colorless	Clear	None
	After Extraction	Induction I	Colorless	Clear	None
		Induction I I	Colorless	Clear	None
		Challenge	Colorless	Clear	None
	Prior to Use	Induction I	Colorless	Clear	None
		Induction I I	Colorless	Clear	None
		Challenge	Colorless	Clear	None
Control	Before Extraction	Induction I	Colorless	Clear	None
		Induction I I	Colorless	Clear	None
		Challenge	Colorless	Clear	None
	After Extraction	Induction I	Colorless	Clear	None
		Induction I I	Colorless	Clear	None
		Challenge	Colorless	Clear	None
	Prior to Use	Induction I	Colorless	Clear	None
		Induction I I	Colorless	Clear	None
		Challenge	Colorless	Clear	None

Vehicle: SO

Treatment group	Time Observed	Extract	Condition of Extracts		
			Color	Clarity	Particulates
Test	Before Extraction	Induction I	Colorless	Oily	None
		Induction I I	Colorless	Oily	None
		Challenge	Colorless	Oily	None
	After Extraction	Induction I	Colorless	Oily	None
		Induction I I	Colorless	Oily	None
		Challenge	Colorless	Oily	None
	Prior to Use	Induction I	Colorless	Oily	None
		Induction I I	Colorless	Oily	None
		Challenge	Colorless	Oily	None
Control	Before Extraction	Induction I	Colorless	Oily	None
		Induction I I	Colorless	Oily	None
		Challenge	Colorless	Oily	None
	After Extraction	Induction I	Colorless	Oily	None
		Induction I I	Colorless	Oily	None
		Challenge	Colorless	Oily	None
	Prior to Use	Induction I	Colorless	Oily	None
		Induction I I	Colorless	Oily	None
		Challenge	Colorless	Oily	None

The test article extracted in SC and SO remained unchanged during the extraction process. The extracts were maintained at ambient temperature <24 hours before use for all phases. The extracts were not centrifuged, filtered, or otherwise altered prior to dosing.

5.2 Test Procedure

5.2.1 Induction I

On the first day of treatment, the animals were weighed and arbitrarily assigned to a treatment group as shown below.

Table 7: Treatment Group Assignment

Vehicle	Treatment Group	Number of Animals
SC	Test	10
	Control	5
SO	Test	10
	Control	5

The fur over the dorsoscapular region was removed with an electric clipper. The test animals were injected with the test article extract and the control animals were injected with the vehicle control. Three rows of intradermal injections (two injections per row) were given to each animal within an approximate 2 cm x 4 cm boundary of the fur clipped area as illustrated below

Cranial

4cm

2cm

a a
b b

c c

Caudal

Control Animals:

a. 0.1 mL of 50:50 (v/v) mixture of FCA and the chosen vehicle

b. 0.1 mL of vehicle

c. 0.1 mL of a 1:1 mixture of the 50:50 (v/v) vehicle/FCA mixture and the vehicle

Test Animals:

a. 0.1 mL of 50:50 (v/v) mixture of FCA and the chosen vehicle

b. 0.1 mL of test extract

c. 0.1 mL of a 1:1 mixture of the 50:50 (v/v) vehicle/FCA mixture and the test extract

5.2.2 Induction II

At 7±1 days after completion of the Induction I injection, the fur over the dorsoscapular region (same area as used during Induction I) of each animal was removed with an electric clipper. The area was treated with a 10% SLS suspension in petrolatum sufficient to coat the skin. The SLS suspension, applied to provoke a mild acute inflammation, was massaged into the skin over the injection site. The area was left uncovered.

At 24 hours (±2 hours) any remaining SLS residue was gently removed with a gauze pad. An approximate 2 cm x 4 cm section of gauze patch, saturated with 0.3 mL of freshly prepared test article extract, was then topically applied to the previously injected sites of the test animals. The control animals were similarly patched with the appropriate vehicle control. Each patch was secured with a nonreactive tape and the trunk of each animal was wrapped with an elastic bandage. At 48 hours, the bandages and patches were removed

5.2.3 Challenge

At 14±1 days after completion of Induction II, the fur was removed from the sides and flank areas with an electric clipper. Nonwoven cotton disks contained in a Hill Top Chamber® were saturated with 0.3 mL of the test article extract or vehicle control. The test extract was applied to the right flank of each animal and the control vehicle was applied to the left flank of each animal. The trunk of each animal was wrapped with an elastic bandage to maintain well-occluded sites. At 24 hours, the wraps and Hill Top Chambers were removed. Any residue remaining at the sites was removed.

5.2.4 Laboratory Observations

1. Animals were observed daily for general health.
2. Body weights were recorded at pretreatment.

3. Observations for dermal reactions were conducted at 24 and 48 hours after challenge patch removal. Dermal reactions were scored in accordance with the criteria shown below:

Table 8: Test Scoring

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

6. Evaluation

The responses from the challenge phase were compared within the test animal group and between test and control conditions. In the final analysis of data, consideration was given to the overall pattern, intensity, duration and character of reactions of the test as compared to the control conditions. The control conditions are (1) the control vehicle on the test animals, (2) the test on the control animals, and (3) the control vehicle on the control animals. Statistical manipulation of data was not applicable to this study. Grades of 1 or greater observed in the test group generally indicated sensitization, provided that grades of less than 1 were observed on the control animals. If grades of 1 or greater were noted on control animals, then the reactions of test animals that exceeded the most severe control reaction were considered to be due to sensitization.

7. Results

7.1 Clinical Observations and Body Weight Data

All animals were clinically normal throughout the study. The clinical observations and individual body weights at pretreatment are presented in Appendix 1.

7.2 Dermal Observations

No evidence of sensitization of test extracts group was observed. Individual results of dermal scoring for the challenge phase are presented in Appendix 2.

Moderate and intense dermal reactions of positive group were observed. Individual results of dermal scoring for the challenge phase are presented in Appendix 3.

8. Conclusion

The test article extracts showed no evidence of causing delayed dermal contact sensitization in the guinea pig. The test article was not considered a sensitizer in the guinea pig maximization test. Results and conclusions apply only to the test article tested. Any extrapolation of these data to other articles is the sponsor's responsibility.

9. Records

All raw data pertaining to this study and a copy of the final report are retained in designated STC archive files in accordance with STC SOPs.

10. ISO Compliance

All procedures were complanced to ISO 17025.

11. References

International Organization for Standardization (ISO) 10993-1, Biological evaluation of medical devices -Part 1: Evaluation and testing within a risk management process (2018).
International Organization for Standardization (ISO) 10993-2, Biological evaluation of medical devices -Part 2: Animal welfare requirements (2006).
International Organization for Standardization (ISO) 10993-10, Biological evaluation of medical devices -Part 10: Tests for irritation and skin sensitization (2010).
International Organization for Standardization (ISO) 10993-12, Biological evaluation of medical devices -Part 12: Sample preparation and reference materials (2012).
International Organization for Standardization/International Electrotechnical Commission (ISO/IEC) 17025, General requirements for the competence of testing and calibration laboratories (2017).

Appendix 1 - Clinical Observations and Individual Body Weight Data

SC group

Treatment Group	Animal number	Individual Observation	
		Pretreatment Body weight(g)	Clinical Observations
Test	1	372.4	Healthy
	2	303.4	Healthy
	3	336.4	Healthy
	4	328.2	Healthy
	5	352.7	Healthy
	6	320.7	Healthy
	7	386.1	Healthy
	8	329.4	Healthy
	9	300.4	Healthy
	10	312.7	Healthy
Control	1	350.9	Healthy
	2	350.1	Healthy
	3	310.9	Healthy
	4	328.9	Healthy
	5	317.2	Healthy

SO group

Treatment Group	Animal number	Individual Observation	
		Pretreatment Body weight(g)	Clinical Observations
Test	1	335.4	Healthy
	2	346.4	Healthy
	3	314.8	Healthy
	4	311.7	Healthy
	5	327.8	Healthy
	6	348.4	Healthy
	7	323.7	Healthy
	8	325.4	Healthy
	9	335.1	Healthy
	10	314.0	Healthy
Control	1	324.6	Healthy
	2	321.9	Healthy
	3	394.1	Healthy
	4	363.4	Healthy
	5	303.4	Healthy

Appendix 2 - Dermal Reactions Following Challenge Exposure

SC group

Treatment Group	Animal number	Dermal reaction			
		24 hour score		48 hour score	
		Control Site	Test Extract Site	Control Site	Test Extract Site
Test	1	0	0	0	0
	2	0	0	0	0
	3	0	0	0	0
	4	0	0	0	0
	5	0	0	0	0
	6	0	0	0	0
	7	0	0	0	0
	8	0	0	0	0
	9	0	0	0	0
	10	0	0	0	0
Control	1	0	0	0	0
	2	0	0	0	0
	3	0	0	0	0
	4	0	0	0	0
	5	0	0	0	0

SO group

Treatment Group	Animal number	Dermal reaction			
		24 hour score		48 hour score	
		Control Site	Test Extract Site	Control Site	Test Extract Site
Test	1	0	0	0	0
	2	0	0	0	0
	3	0	0	0	0
	4	0	0	0	0
	5	0	0	0	0
	6	0	0	0	0
	7	0	0	0	0
	8	0	0	0	0
	9	0	0	0	0
	10	0	0	0	0
Control	1	0	0	0	0
	2	0	0	0	0
	3	0	0	0	0
	4	0	0	0	0
	5	0	0	0	0

Appendix 3 - Periodic Positive Control Study for the Guinea Pig Maximization Test

What was tested
1 -chloro-2,4-dinitrobenzene (DNCB)

Dates
Treatment Started: 2019.02.26 under DCA20190002
Observations Concluded: 2019.03.23

Purpose
A periodic positive control study was conducted for the Guinea Pig Maximization Test to meet the following objectives: 1) confirm the methodology in ISO 10993-10, Biological Evaluation of Medical Devices - Part 10: Tests for Irritation and Skin Sensitization, 2) substantiate the potential of DNCB to cause delayed dermal contact sensitization, 3) verify proper training of the technicians performing these studies, and 4) substantiate the susceptibility of the guinea pig strain to dermal contact sensitization.

Methods
The test utilized young adult, nulliparous and not pregnant, female and male FMMU guinea pigs supplied by Southern Medical University. The weight at study initiation ranged from 300 grams to 400 grams. A 0.1% (w/w) concentration of DNCB in ethanol was intradermally injected and occlusively patched to five test guinea pigs in an attempt to induce sensitization. The ethanol vehicle was similarly injected and occlusively patched to five control guinea pigs. Following a recovery period, the test and control animals received a challenge patch of 0.1% (w/w) DNCB in ethanol and ethanol alone. All sites were scored for dermal reactions at 24 and 48 hours after patch removal. The patch sites were graded using the scale:

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

Results

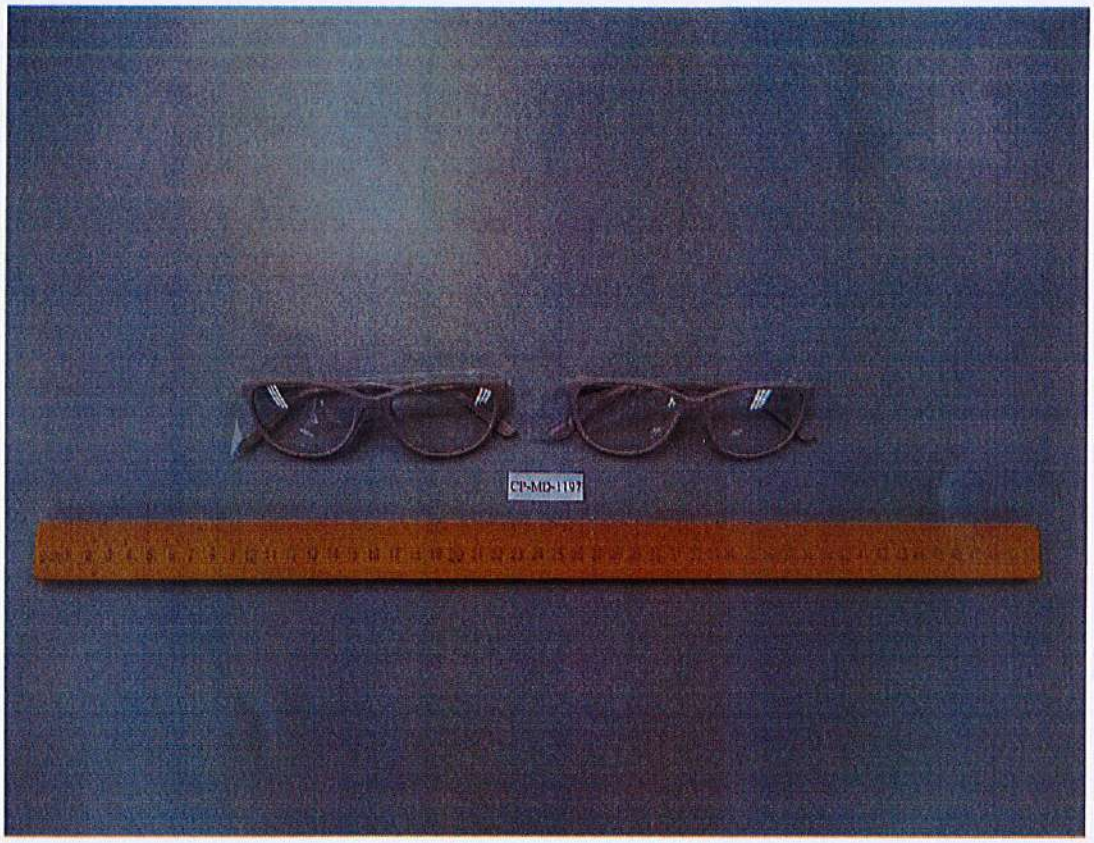
All of the ten animals demonstrated a positive sensitization response to the known sensitizer, DNCB. None of the control animals demonstrated a sensitization response. The results are shown below:

Treatment Group	Animal number	Dermal reaction				Results (+) or (-)
		24 hour score		48 hour score		
		Test	Control	Test	Control	
Test	1	2	0	2	0	+
	2	2	0	2	0	+
	3	1	0	1	0	+
	4	2	0	2	0	+
	5	1	0	1	0	+
Control	1	0	0	0	0	-
	2	0	0	0	0	-
	3	0	0	0	0	-
	4	0	0	0	0	-
	5	0	0	0	0	-

Conclusion

The known sensitizer DNCB produced evidence of causing delayed dermal contact sensitization in the guinea pig. Therefore, the following objectives were met: 1) the methodology in ISO 10993-10, Biological Evaluation of Medical Devices, Part 10: Tests for Irritation and Skin Sensitization was confirmed, 2) the potential for DNCB to cause delayed contact sensitization was substantiated, 3) proper training of the technicians performing this study design was verified and 4) the susceptibility of the guinea pig strain to sensitization was substantiated.

Appendix 4 – Photograph of Test Articles



***** END OF TEST REPORT *****

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