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TESTING
CNAS L2954

Sanitation & Environment Technology Institute, Soochow University, Final Report

Report Number: SDWH-M201403679

Skin Sensitization Test of Electrode Gel G607 using ISO 10993-10:2010 Test Methods Guinea Pig Maximization Test 0.9% Sodium Chloride Injection Extract

Sponsor

GMDASZ Manufacturing Co.,Ltd.

Sanitation & Environment Technology Institute, Soochow University
Tel: 0512-65880038 Fax: 0512-65880034 Email:sudaweihuan@163.com PC: 215123
Add: No.199 Ren'ai Road, Suzhou Industrial Park, China http://yxbfzb.suda.edu.cn

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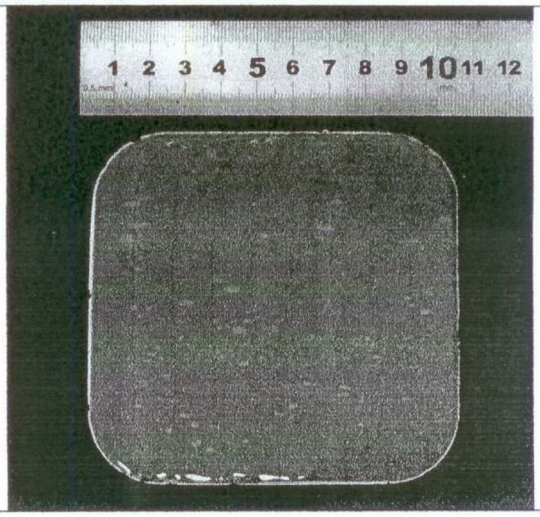
SUPPLEMENTARY EXPLANATION

1. Please apply for rechecking within 15 days of receiving the report if there are any objections.
2. Any erasure or without special testing seal renders the report null and void.
3. The report is only valid when signed by the persons who edited, checked and approved it.
4. The result relate only to the articles tested.
5. The report shall not be reproduced except in full without the written approval of the institute.

STUDY VERIFICATION AND SIGNATURE

Test Article	
Test Article Receipt:	2014-11-11
Protocol No:	SDWH-PROTOCOL-GLP-M201403679
Protocol Effective Date:	2014-11-14
Technical Initiation Date:	2014-11-14
Technical Completion Date:	2014-12-11
Final Report Completion Date:	2014-12-17

Edited by : Zhang Yan 2014.12.17
Date

Checked by :
Study Director 2014.12.17
Date

Approved by :
Authorized signatory 2014-12-18
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	2014-12-11	2014-12-11	2014-12-11
RAW DATA	2014-12-17	2014-12-17	2014-12-17
FINAL REPORT	2014-12-17	2014-12-17	2014-12-17

Quality Assurance Unit : He Qiuping

2014-12-17
Date

1.0 Study Summary

The test article was extracted by 0.9% Sodium Chloride Injection to evaluate the potential for skin sensitization.
The extract of the test article was intradermally injected and occlusively patched to ten guinea pigs in an attempt to induce sensitization. Following a recovery period, the original ten test and five control animals received a challenge by absorbent gauze patch (2.5cm×2.5cm) containing test article extract. All sites were scored at 24±2h and 48±2h after patch removal.
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 the laboratories (Quality and Technical Bureau of Jiangsu Province Metrology Accreditation Certificate CMA 2013100106S)

5.0Identification of test and control articles

5.1 Test article name: Electrode Gel G607
Test article initial state: Not Supplied by Sponsor(N/S)
CAS/Code# : N/S
Size: 10×10cm
Lot/ Batch#: 20141107

Test article materials: Hydrogel
Package materials: PE Bag
Physical State: Solid
Color: Colorless
Density: 1.1g/cm^3
Stability: Stable
Solubility: N/S
Storage Condition: Room Temperature
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

5.2.1Negative Control

Article Name: 0.9% Sodium Chloride Injection (SC)
Manufacturer: Anhui Double-Crane Pharmaceutical Co., Ltd.
Size: 500ml
Lot/ Batch#: 140811 XT
Physical State: Liquid
Color: Colourless
Storage Condition: Room Temperature

5.2.2Positive Control

Article Name: 2, 4-Dinitrochlorobenzene (DNCB)
Manufacturer: Xiya Reagent^R
Size: 100g
Lot/ Batch#: W5656
Concentration: 0.5%
Solvent: 0.9% Sodium Chloride Injection
Date prepared: 2014-10-27
Physical State: Liquid
Color: 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: 318～358g
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: Wuxi Huishan Jiangnan Experimental Animal Center; Permit Code: SCXK (SU) 2009-0005
Bedding: Corncob, Suzhou shuangshi laboratory animal feed science Co.,Ltd
Feed: Guinea Pig Diet, Wuxi Huishan Jiangnan Experimental Animal Center
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 exposed to the test system through a solvent compatible with the test system. This was the optimal route of administration available in this test system.

10.0Experiment design

10.1 Sample and Control Preparation

The sample preparation as following table:

Aseptic Sampling			Aseptic Agitation Extraction In Inert Container		Final Extract	
Ratio	Sampling Manner	Actually sampling	SC	Condition	pH	Clear or Not
$3cm^2$:1ml	Random (Remove the protective films) add additional volume of extraction vehicle that the test article absorbs(12.5ml/g, provided by sponsor)	Surface area $60cm^2$	20ml	37℃,72h	6.0	Clear

Test within 24h after extraction, stored at 4℃.
The vehicle (without the test article) was similarly prepared to serve as the control.

10.2 Equipment

Constant Temperature Vibrator（SDWH-217）,Calibration Expire（2015-11-18）
Cylindrical pressure steam sterilizer (SDWH-030), Calibration Expire (2015-05-27)
Electronic balance (SDWH-056) ,Calibration Expire（2015-03-20）
Steel Straight Scale (SDWH-463) ,Calibration Expire（2015-10-14）
Electronic scale (SDWH-442) ,Calibration Expire（2015-10-22）

10.3 Reagents

Freund's Adjuvant,Complete liquid
Manufacturer: SIGMA
Lot No: SLBJ2845V
Sodium dodecyl sulfate (SDS)
Concentration: 10%
Solvent: Distilled water
Date prepared: 2014-10-27
Manufacturer: Sinopharm Chemical Reagent Co.Ltd
Lot No: F20090922

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%) was injected into the control animals with an emulsion of the blank liquid with adjuvant.

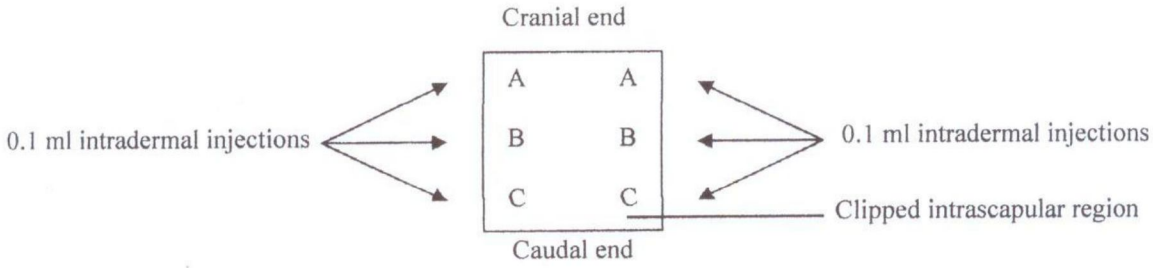


Figure 1 Location of intradermal injection sites

10.5 Topical induction phase Ⅱ

The maximum concentration that can be achieved in Intradermal induction phase Ⅰ did not produce irritation, pretreated the area with 10 % sodium dodecyl sulfate massaged into the skin (24±2) h

before the patch was applied.
At 7 d after completion of the intradermal induction phase, administer the test sample by topical application to the intrascapular region of each animal, using a patch of area approximately 8 cm^2 (absorbent gauze), so as to cover the intradermal injection sites. Use the concentration selected in Intradermal induction phase I for site B. 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 the test sample and a blank by topical application to sites that were not treated during the induction stage, using absorbent gauze (2.5cm×2.5cm) soaked in the test sample at the concentration selected in the intradermal induction phase I for site C. 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 Electrode Gel G607 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.

Table 2 Guinea pig Sensitization Dermal Reactions

Group	Animal Number	24±2h before phase II patch application		Hours following Challenge phase		Positive rate after challenge phase
		Left	Right	24±2 h	48±2 h	
Test Group	1	0	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	
Negative control	11	0	0	0	0	—
	12	0	0	0	0	
	13	0	0	0	0	
	14	0	0	0	0	
	15	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	392	Normal
	2	353	445	Normal
	3	328	405	Normal
	4	357	414	Normal
	5	321	381	Normal
	6	345	398	Normal
	7	358	436	Normal
	8	329	425	Normal
	9	333	405	Normal
	10	318	394	Normal
Negative control	11	337	407	Normal
	12	353	426	Normal
	13	349	420	Normal
	14	332	382	Normal
	15	328	427	Normal

Table 4 Guinea pig Sensitization Dermal Reactions of Positive Group

Group	Animal Number	24±2h before phase II		Hours following Challenge phase		Positive rate after challenge phase
		Left	Right	24±2 h	48±2 h	
Positive Group	1	3	3	2	2	100%
	2	3	3	2	2	
	3	3	3	3	3	
	4	3	3	3	2	
	5	3	3	2	2	
Negative control	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	

Note: The data of positive control come from SDWH-M201403668 (Completed Date: 2014-11-20)

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	325	395	Normal
	2	339	408	Normal
	3	310	378	Normal
	4	305	369	Normal
	5	323	393	Normal
Negative control	6	318	407	Normal
	7	344	425	Normal
	8	309	390	Normal
	9	321	398	Normal
	10	333	421	Normal

Note: The data of positive control come from SDWH-M201403668 (Completed Date: 2014-11-20)