ATTESTATION REPORT

Date: 2018-08-03
No.: T01392

Applicant	:	Hong Kong Cheung Wo Biotechnology Group Limited Rm 1003, 10/F Olympia Plaza, 255 King's Road, North Point, Hong Kong
Description of Sample(s)	:	Product: 氧離子殺菌王 Model No.: N/A Brand name: N/A Quantity submitted: 1 piece
Date Sample(s) Received	:	2018-05-28
Date Tested	:	2018-06-15 to 2018-07-15
Investigation Requested	:	ISO 10993-10 Biological evaluation of medical devices – Part 10: Tests for irritation and skin sensitization.
Conclusions	:	Refer to 8. Conclusion.
Remark(s)	:	None.

Lee Mei Yu, Amy
Certification Officer
For and on behalf of
The Hong Kong Certification Centre Ltd

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Summary

The test article, 氧離子殺菌王, 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, Biological evaluation of medical devices - Part 10: Tests for irritation and skin sensitization. The test articles were extracted in 0.9% sodium chloride and. 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.

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Statement of GLP Compliance

There were no deviations to the provisions of the FDA Good Laboratory Practice (GLP) Regulations (21 CFR, Part 58) noted during the course of the study.

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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 10993-10, Biological evaluation of medical devices - Part 10: Tests for irritation and skin sensitization.

1.3 Dates

Test Article Received:	2018.05.28
Treatment Started:	2018.06.15
Observations Concluded:	2018.07.15

1.4 GLP Compliance

The study initiated by protocol signature on 2018.5.7 was conducted in accordance with the provisions of the FDA Good Laboratory Practice (GLP) Regulations, 21 CFR 58. A Statement of Quality Assurance Activities was issued with this report.

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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:	氧離子殺菌王
Size:	N. A
Model:	N. A
Lot:	N. A
Manufacture Date:	N. A
Expired Date	N. A
Strength, Purity and Composition:	N. A
Physical Description of the Test Article:	Liquid
Storage Conditions:	Ambient Temperature

Table 2: Negative Control Article

Name:	SODIUM CHLORIDE INJECTION(SC)
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

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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
SODIUM CHLORIDE INJECTION	KELUN	Ke Lun L218011405
Freund’s Adjuvant, Complete	SIGMA	SLBR3877V

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3. Test System

3.1 Test System and Justification of Test System

Species:	Guinea pig (Cavia porcellus)
Strain:	Hartley
Source:	Guang Zhou City Bai Yun District Long Gui Xing Ke Animal farm (廣州市白雲區龍歸興科動物養殖場)
Sex:	male
Age:	Young adult
Acclimation Period:	Minimum 5 days
Number of Animals:	15

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. The susceptibility of the guinea pig strain to a known sensitizing agent, l-chloro-2,4-dinitrobenzene (DNCB), would be substantiated at STC with this method in this study.

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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(粵)2016-0159. The animal housing room temperature and relative humidity were monitored daily. The temperature for the room was set to 18-26℃ 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), Wuhan wanqian jiaxing biotechnology co. LTD（武漢市萬千佳興生物科技有限公司）, 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.

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5. Method

5.1 Test and Control Article Preparation

The test article was liquid without extraction, used directly. And the negative control were used saline directly.

5.2 Test Procedure

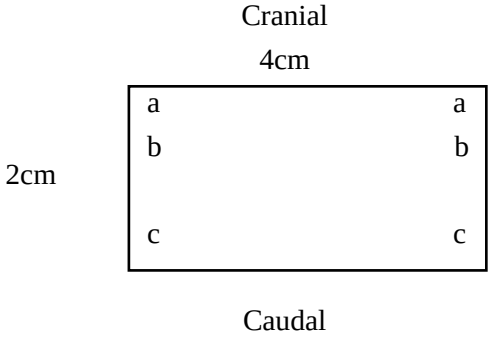
5.2.1 Induction I

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

Table 6: Treatment Group Assignment

Vehicle	Treatment Group	Number of Animals
Saline	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



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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 6 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 Liquid Band-Aid. 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 13 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 TopChamber® were saturated with 0.3 mL of the test article extract. The test extract was applied to the right flank of each animal. The trunk of each animal was wrapped with an elastic bandage to maintain

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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 7: 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

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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 condition. The control condition is the test 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.

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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. Moderate and intense dermal reactions of positive group were observed. Individual results of dermal scoring for the challenge phase are presented in Appendix 2.

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.

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11. References

Code of Federal Regulations (CFR), Title 21, Part 58, Good Laboratory Practice for Nonclinical Laboratory Studies.

International Organization for Standardization (ISO) 10993-1, Biological evaluation of medical devices -Part 1: Evaluation and testing within a risk management process (2009/Technical Corrigendum 1 2010).

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).

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Appendix 1 - Clinical Observations and Individual Body Weight Data

Treatment Group	Animal number	Individual Observation	
		Pretreatment Body weight(g)	Clinical Observations
Test	1	336	Healthy, Yong adult
	2	302	Healthy, Yong adult
	3	384	Healthy, Yong adult
	4	394	Healthy, Yong adult
	5	301	Healthy, Yong adult
	6	347	Healthy, Yong adult
	7	366	Healthy, Yong adult
	8	324	Healthy, Yong adult
	9	336	Healthy, Yong adult
	10	310	Healthy, Yong adult
Control	1	317	Healthy, Yong adult
	2	374	Healthy, Yong adult
	3	348	Healthy, Yong adult
	4	360	Healthy, Yong adult
	5	368	Healthy, Yong adult

SC group

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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

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Appendix 3 – Photograph of Test Articles

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Appendix 4 - Periodic Positive Control Study for the Guinea Pig Maximization Test

What was tested

1 -chloro-2,4-dinitrobenzene (DNCB)

Dates

Treatment Started: Oct 31, 2017 under DCA000296

Observations Concluded: Nov 25,2017

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 Hartley guinea pig strain to dermal contact sensitization.

Methods

The test utilized young adult, nulliparous and not pregnant, female and male Hartley albino guinea pigs supplied by Hua Du Xin Hua. The weight at study initiation ranged from 300 grams to 450 grams. A 0.1% (w/w) concentration of DNCB in ethanol was intradermally injected and occlusively patched to ten 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 0.01% (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

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Results

All of the ten test 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	1	3	3	+
	2	3	3	+
	3	3	3	+
	4	3	3	+
	5	3	3	+

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Control	1	0	0	-
	2	0	0	-
	3	0	0	-
	4	0	0	-
	5	0	0	-

Conclusion

The known sensitizer DNCB produced evidence of causing delayed dermal contact sensitization in the Hartley strain of 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 Hartley guinea pig strain to sensitization was substantiated.

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Statement of Quality Assurance Activities

Phase Inspected	Date Inspected	Study Director Notification Date	Management Notification Date
Dosing	2018.5.18	2018.5.18	2018.5.18
Study Data Review	2018.6.15	2018.6.15	2018.6.15
Final Report Review	2018.7.15	2018.7.15	2018.7.15

Based on a review of this study, it has been concluded that this report accurately describes the methods and standard operating procedures, and that the reported results accurately reflect the raw data of the study. This study has been reviewed in accordance with the provisions of the FDA Good Laboratory Practice Regulations (21 CFR, Part 58).

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