



瑞德生物科技有限公司
MASTER LABORATORY CO.,LTD.

Skin Sensitization Study in Guinea Pigs (Maximization Test)

Master Laboratory Co., Ltd. Animal laboratory

Address: 3F, No. 221, Sec. 1, Zhongxing Rd., Zhudong Township,

Hsinchu County 31053, Taiwan (R.O.C.) TEL : (886-2)25176117



**Ear-loop
Skin Sensitization Study in Guinea Pigs
(Maximization Test)
STUDY REPORT**

Sponsor : CHIN HSIUNG FIBER CO., LTD

Testing Institution : Master Laboratory Co., Ltd.

November 2020



FINAL REPORT

Report No.: MSA-202010-409-T03

Report No.: MSA-202010-409-T03

Test article registration date: 10.22.2020

Experimental starting date: 10.27.2020

Animal in-housing: 10.27.2020

Extraction of test article date: 10.30.2020, 11.06.2020, 11.20.2020

Test article administration during induction phase: 11.02.2020, 11.09.2020

Test article administration during challenge phase: 11.23.2020

Observation of skin response: 11.25.2020-11.26.2020

Animal sacrifice date: 11.26.2020

Experimental complete date: 11.26.2020

Study Announcement

1. The study report is valid for the test article used only, and shall not be partly recopied or extracted for another object.
2. The study report is invalid without the endorsement of Master Laboratory Co., Ltd.



SIGNATURE OF STUDY PERSONNEL

Study Director

Bo Han Huang
Bo Han Huang

11. 27. 2020
Date

Facility Management

Alan Hsieh
Alan Hsieh

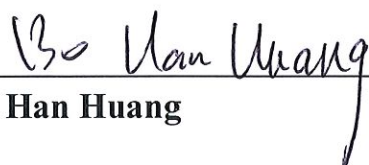
11. 27. 2020
Date



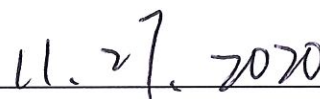
GLP COMPLIANCE STATEMENT

The study met with the technical requirements of the protocol, and all applicable guidance and regulations, which included the Good Laboratory Practice for Non-clinical Laboratory Studies (FDA, 21 CFR, Part 58, 2019) and Good Laboratory Practice for Non-clinical Laboratory Studies (Food and Drug Administration, R.O.C., 2019). There were no deviations from the approved study plan and no adverse problem that would affect the integrity of this study or the interpretation of the study result. Because the test article is a proprietary product of the sponsor, all the contents related with test article in 21 CFR Part 58 (US FDA) are not applicable to this study (21 CFR §58.105, §58.113, FDA).

Study Director



Bo Han Huang



Date



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QUALITY ASSURANCE STATEMENT

To comply with the “Good Laboratory Practice for Nonclinical Laboratory Study”, Quality Assurance Department has audited the facility, equipment, personnel, test methods, raw data, and records regularly.

The study report has been reviewed and approved. The experiments were conducted according to the protocol. All original records, raw data, and documents are truthfully transferred and addressed in the results of this report.

Inspection record:

<u>Inspection Contents</u>	<u>Date of inspection</u>
Before the test (test execution protocol, requisitions, contracts).....	10.27.2020
Test (test substance data sheet, animal quarantine, standard operating procedures).....	11.02.2020
After the test (complete the original data, report reviews).....	11.27.2020

Quality Assurance unit in charge

Ying Chun Chen
Ying Chun Chen

11. 27. 2020
Date



Contract Research Organization and Sponsor Information

1. CRO:

- a. Title: Master Laboratory Co., Ltd.-Animal laboratory
- b. Address: 3F, No. 221, Sec. 1, Zhongxing Rd., Zhudong Township, Hsinchu County 31053, Taiwan, R.O.C.

2. Sponsor:

- a. Title: CHIN HSIUNG FIBER CO., LTD
- b. Address: NO.37-12, Ta Hsin RD., Pu Yen Hsiang, Chang Hua Hsien, Taiwan



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SUMMARY

The present study was to investigate the skin sensitization response of “Ear-loop” extract in guinea pigs, the testing was performed in compliance with ISO 10993-10:2010. After the treatment of the test article (polar and non-polar extraction), the extracts were applied two times for induction phase and one time for challenge phase. 24 hr and 48 hr after challenge phase, no visible changes had been found on the treated areas of either the control or the treatment group (Table 1, Appendix 1 and Figure 1), according to the criteria of “Magnusson and Kligman scale”, ISO 10993-10:2010. The results indicated that the test article extract (polar or non-polar) did not cause delayed hypersensitivity on the skin of guinea pigs.



INTRODUCTION

This study was performed in compliance with ISO 10993-10:2010 (Biological evaluation of medical devices-Part 10: Tests for irritation and skin sensitization) to evaluate the possibility of delayed hypersensitivity after topical applications with test article “Ear-loop” extract (polar or non-polar) on the skin of guinea pigs.

MATERIALS AND METHODS

1. Animals

1.1. Species/Strain: Guinea Pig (Hartley strain)

1.2. Resource: Hui Jun

1.3. Body weights (Gender): 300-500 g (Male)

1.4. Quarantine/acclimation (MSAT-SOP-AM-001):

Totally 30 animals were introduced in-house, animals were subjected to be quarantined and acclimated before treatment. Veterinarian ensured the animal health status before the treatment.

1.5. Reasons chosen for animal experimentation: Guinea pigs were proven to be suitable for skin sensitization studies.

2. Feeding and care (MSAT-SOP-AM-002)

2.1. Housing: Guinea Pig room

2.2. Environment

a. Temperature: $23 \pm 3^{\circ}\text{C}$

b. Humidity: $50 \pm 20\%$

c. Light Cycle: 12 hours light and 12 hours dark

2.3. Cage and animal No.

a. Quarantine/acclimation: 2-3 male guinea pigs /cage

b. Study period: 2-3 male guinea pigs /cage



2.4. Feed

- a. Name: Lab Diet[®]5025
- b. Brand: Lab Diet[®], U.S.A.
- c. Way to supply: *ad libitum*
- d. Source: PMI Nutrition International, U.S.A.

2.5. Drinking water

- a. Sort: RO Water
- b. Way to supply: *ad libitum*

3. Individual and group identification (MSAT-SOP-AM-025)

- 3.1. Individual identification: Tested animals were identified by ear-punching.
- 3.2. Group identification: Cages were properly labeled for identification including the Study Title/No., Administration, Observation Period, Room No., Cage No., Quantity/cage, Species, Strain, Gender, In House Date, In House Age, Animal ID No., Keeper and Deputy.

4. Test article and control vehicles

- 4.1. Test article: Ear-loop
- 4.2. Saline: CHI SHENG CHEMICAL CORPORATION, Lot. O3149;
Solutio Natrii Chloridi Isotonica 0.9% 500 ml
- 4.3. Cottonseed oil: SIGMA-ALDRICH, Co., Lot. MKCB9547;
CAS No.: 8001-29-4
- 4.4. Positive Control: 1-chloro-2, 4-dinitrobenzene (DNCB), Lot. STBG2582V
SIGMA-ALDRICH, CAS No: 101-86-0



5. Administration of test article and control vehicles

5.1. Preparation (MAST-SOP-GE-014):

According to ISO 10993-12:2012 guideline, the test articles were extracted two times for the first and second induction phase and one time for challenge phase.

- a. Polar preparation: Measured the surface of test article, and immersed it in saline for 72 ± 2 hr at $50 \pm 2^\circ\text{C}$ with constant agitation (100 rpm). The surface ratio of test article/saline was approximately $3 \text{ cm}^2/\text{ml}$. After extraction, the extract was used immediately; the appearance of test article extract was clear and colorless without particulates present.
- b. Non-polar preparation: Measured the surface of test article, and immersed it in cottonseed oil for 72 ± 2 hr at $50 \pm 2^\circ\text{C}$ with constant agitation (100 rpm). The surface ratio of test article/cottonseed oil was approximately $3 \text{ cm}^2/\text{ml}$. After extraction, the extract was used immediately; the appearance of test article extract was clear and colorless without particulates present.

5.2. Method, route and frequency of administration:

- a. Induction phase: Two topical (upper backside) applications, intracutaneous and article spread to the dermal area. The area was from neck to scapular area.
- b. Challenge phase: One topical (lower backside) application, and article spread to the dermal area. The area was from scapular to hip area.



5.3. Groups

Polar Group “Saline”	Dose			Number of animals
	Induction phase I	Induction phase II	Challenge phase	Male
Control-1 group	0.1 ml	0.3 ml	0.3 ml	5
Treatment-1 group	0.1 ml	0.3 ml	0.3 ml	10

Non-polar Group “Cottonseed oil”	Dose			Number of animals
	Induction phase I	Induction phase II	Challenge phase	Male
Control-2 group	0.1 ml	0.3 ml	0.3 ml	5
Treatment-2 group	0.1 ml	0.3 ml	0.3 ml	10

6. Procedure (MSAT-SOP-ME-003)

6.1. Prior to the study, the furs of animal’s backside were clipped from neck to scapular area with an electric animal shaver. The animals with scratches or skin diseases were removed from this study. The clipped area of left or right side was $2 \times 2 \text{cm}^2$ each.

6.2. Induction phase I: On the treatment day, three kinds of solutions or emulsions were prepared from the control solution and test article extract. They were as follows:

6.2.1. Polar:

(A) Emulsion of Freund’s complete adjuvant (FCA) in saline and volume ratio of 1:1

(B) Solution of either test article extract or Saline



(C) Emulsion of either test article extract or saline in E-FCA in volume ratio of 1:1

0.1 ml of the respective solutions/emulsions of (A), (B) and (C) of the control solution and the test article extract were injected intradermally and symmetrically into right and left scapular regions.

6.2.2. Non-Polar:

(A) Emulsion of Freund's complete adjuvant (FCA) in cottonseed oil and volume ratio of 1:1

(B) Solution of either test article extract or cottonseed oil

(C) Emulsion of either test article extract or cottonseed oil in E-FCA in volume ratio of 1:1

0.1 ml of the respective solutions/emulsions of (A), (B) and (C) of the control solution and the test article extract were injected intradermally and symmetrically into right and left scapular regions.

Test periods	Treatment-1 group (10 animals)	Control-1 group (5 animals)
Induction (I)	A. Saline and FCA (1:1)	A. Saline and FCA (1:1)
	B. test article extract	B. Saline
	C. test article extract and E-FCA (1:1)	C. Saline and E-FCA (1:1)
Induction (II)	test article extract	Saline
Challenge	test article extract	Saline

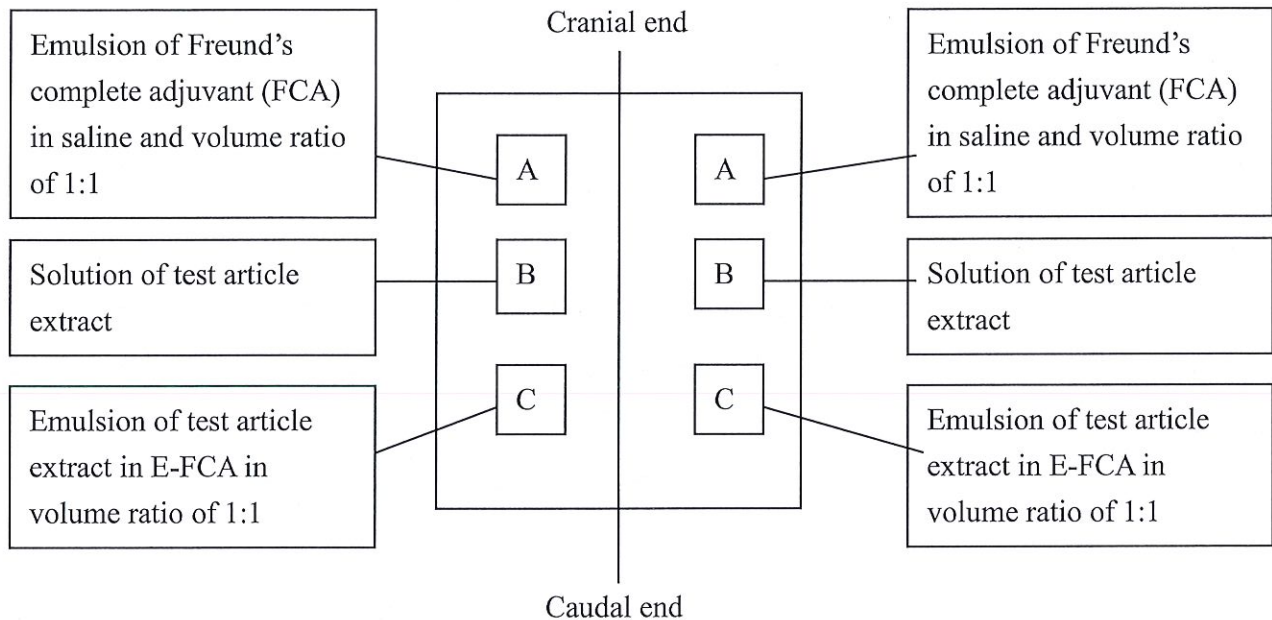
Test periods	Treatment-2 group (10 animals)	Control-2 group (5 animals)
Induction (I)	A. Cottonseed oil and FCA (1:1)	A. Cottonseed oil and FCA (1:1)
	B. test article extract	B. Cottonseed oil
	C. test article extract and E-FCA (1:1)	C. Cottonseed oil and E-FCA (1:1)
Induction (II)	test article extract	Cottonseed oil
Challenge	test article extract	Cottonseed oil



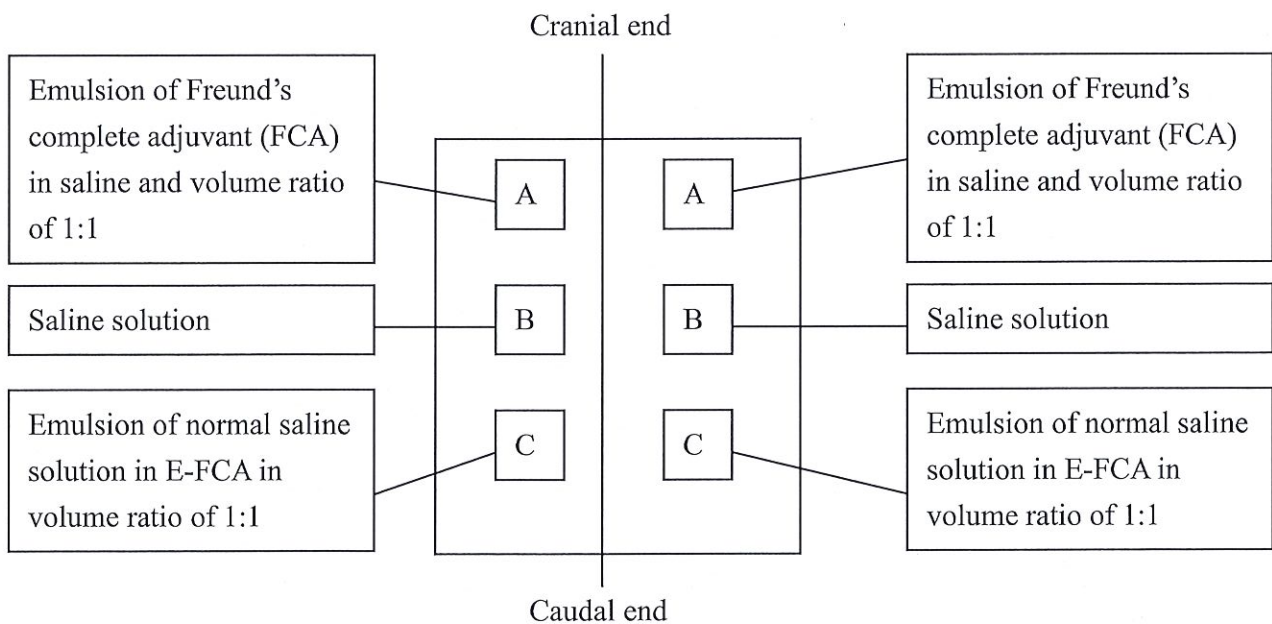
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The detail information for grouping and applied substance was tabulated above:



Polar : Treatment-1 group by 10 animals (solvent-Normal saline)

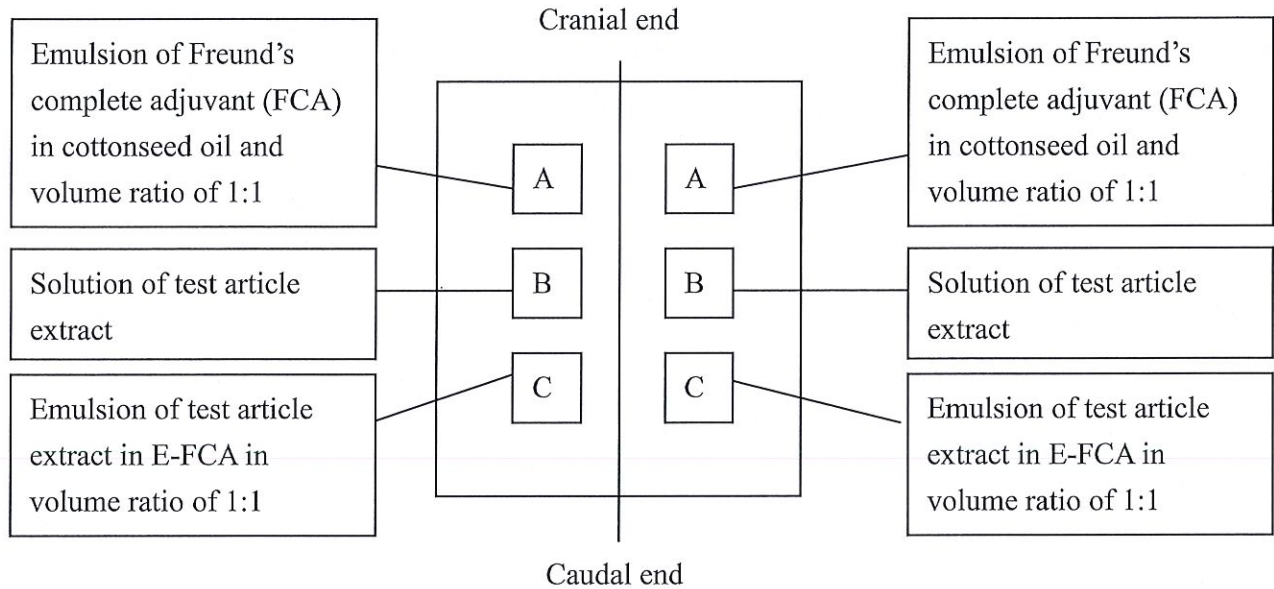


Polar : Control-1 group by 5 animals (saline)

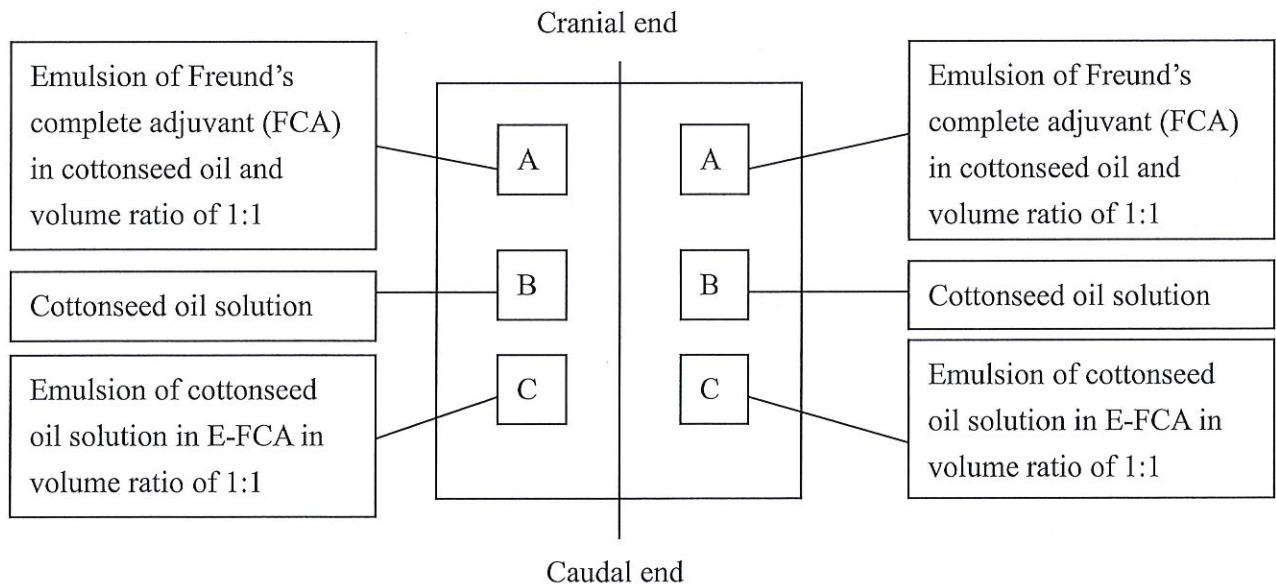


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Non-Polar : Treatment-2 group by 10 animals (solvent-cottonseed oil)



Non-Polar : Control-2 group by 5 animals (cottonseed oil)



6.3. Induction phase II: One week later, the injection sites were applied with 10 % of sodium dodecyl sulfate (SDS) for 24 hr if there were no irritation reaction. Then, injection sites were applied by the patches that soaked with 0.3 ml of the control solution and test article extracts for another 48 hr.

6.4. Challenge phase: On the challenge day (two weeks after induction phase II), the furs of lower backside of the animals were clipped from scapular to hip area. An appropriate site of this hairless area was selected and applied by the patches that soaked with 0.3 ml of the control solution and test article extracts. The patches were removed 24 hr later.

7. Animal observations and items for examination (MSAT-SOP-ME-003)

7.1. 24hr and 48hr after challenge phase, the skin response was graded according to the criteria of “Magnusson and Kligman scale, ISO 10993-10 : 2010” (Appendix 2).

8. Test positive control

8.1. The positive control testing for sensitization assay was performed using a test sample consisting of 1-Chloro-2,4-dinitrobenzene (DNCB), which was a typically used as a positive control in this test. The DNCB concentrations used for induction and challenge in the guinea pig maximization sensitization test were 0.5% and 0.1% w/v, respectively.

8.2. The positive control was tested completely within three months of test article results to confirm that tested with the same source and strain of animals, as well as the same methods.

8.3. Test period of positive control: 10.05.2020-10.29.2020; History record from Master Lab, Report No. MSA202010.



RESULTS

The study was to investigate the skin sensitization response of “Ear-loop” extracts in guinea pigs. Following the treatment of the test article (polar and non-polar extraction), the extracts were applied two times for induction phase and one time for challenge phase. 24 hr and 48 hr after challenge phase, no visible changes had been found on the treated areas of either the control or the treatment group (Table 1, Appendix 1 and Figure 1).

CONCLUSION

The present study was performed in compliance with ISO 10993-10 : 2010 to evaluate the possibility of delayed hypersensitivity after topical applications of test article extract (polar or non-polar) on the skin of guinea pigs. After 24 hr and 48 hr challenge phase, neither the control nor the treatment group showed visible changes of skin response on the treated areas. The results indicated that the test article “Ear-loop” extract (polar or non-polar) did not cause delayed hypersensitivity in the tested guinea pigs.



REFERENCES

1. Good Laboratory Practice for Nonclinical Laboratory Studies (2019) Food and Drug Administration, R.O.C.
2. Good Laboratory Practice for Nonclinical Laboratory Studies. Title 21 of the U.S. Code of Federal Regulations, Part 58 (2019) United States Food and Drug Administration.
3. Biological evaluation of medical devices-Part 10: Tests for irritation and skin sensitization, ISO 10993 (2010).
4. Biological evaluation of medical devices-part 10: Test for irritation and skin sensitization, EN ISO 10993 (2013).
5. Biological evaluation of medical devices-Part 12 : Sample preparation and reference materials ISO 10993 (2012).
6. ISO/IEC 17025:2017 General requirements for the competence of testing and calibration laboratories.
7. Handbook of Toxicology, Third Edition 2014, Edited by Michael J. Derelanko and Carol S. Auletta, Chapter 3. Dermal Toxicology

**Table 1. Skin Reaction in Guinea Pigs**

Polar Group (Saline)	Control	Treatment
Gender	Male	Male
Number of animals	5	10
Erythema and eschar	0/5	0/10
Edema	0/5	0/10

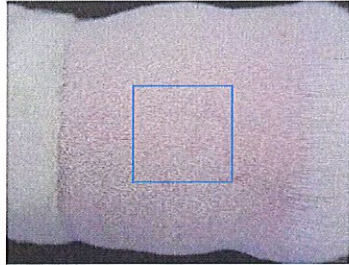
n/n : No. of guinea pigs with abnormal clinical signs / No. of guinea pigs per group

Non-Polar Group (Cottonseed oil)	Control	Treatment
Gender	Male	Male
Number of animals	5	10
Erythema and eschar	0/5	0/10
Edema	0/5	0/10

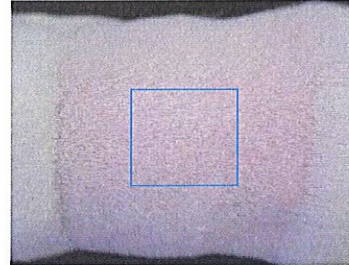
n/n : No. of guinea pigs with abnormal clinical signs / No. of guinea pigs per group



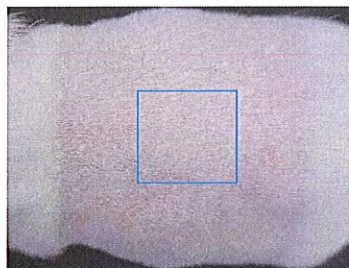
Figur1-1. Pictures for Observation of Skin Reaction (Saline)



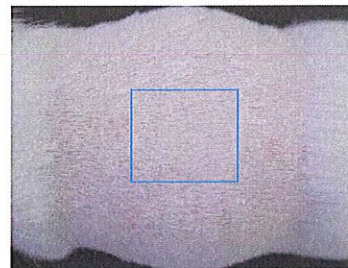
Observation at the 24th hr after administrated
Control-1 group



Observation at the 48th hr after administrated
Control-1 group

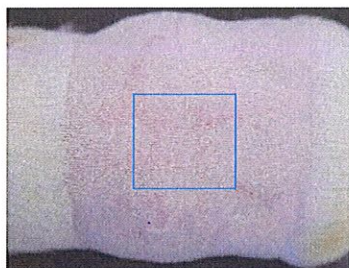


Observation at the 24th hr after administrated
Treatment-1 group

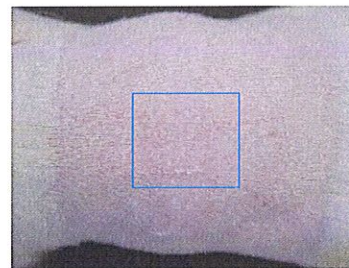


Observation at the 48th hr after administrated
Treatment-1 group

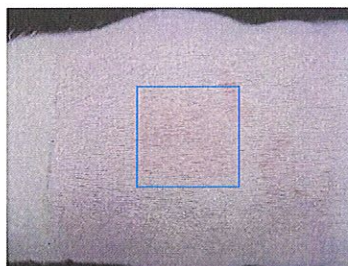
Figure1-2. Pictures for Observation of Skin Reaction (Cottonseed oil)



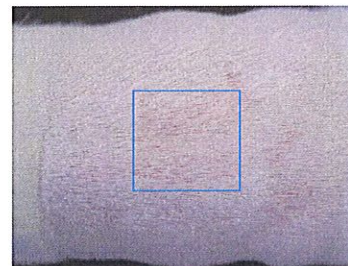
Observation at the 24th hr after administrated
Control-2 group



Observation at the 48th hr after administrated
Control-2 group



Observation at the 24th hr after administrated
Treatment-2 group

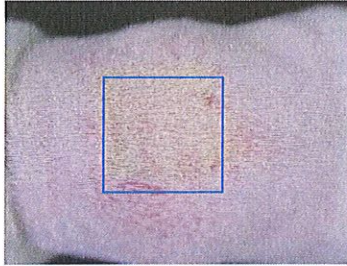


Observation at the 48th hr after administrated
Treatment-2 group



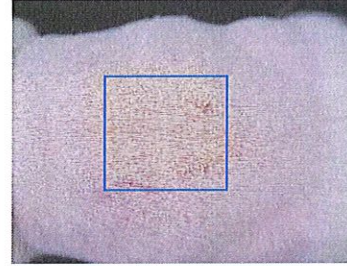
Figure1-3. Pictures for Observation of Skin Reaction

Positive control group



Observation at the 24th hr after administrated

Positive control group



Observation at the 48th hr after administrated

Positive control group

History record from Master Lab, Report No. MSA202010 (Test period: 10.05.2020-10.29.2020)



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Appendix 1-1. Individual Animal Grade in Skin Response of Guinea Pigs

Polar Groups	Gender	ID of animals	24 hr after challenge phase	48 hr after challenge phase
Control-1 "Saline"	Male	1001	0	0
		1002	0	0
		1003	0	0
		1004	0	0
		1005	0	0
Treatment-1 "Ear-loop" extract by "Saline"	Male	1006	0	0
		1007	0	0
		1008	0	0
		1009	0	0
		1010	0	0
		1011	0	0
		1012	0	0
		1013	0	0
		1014	0	0
		1015	0	0



Appendix 1-2. Individual Animal Grade in Skin Response of Guinea Pigs

Non-Polar Groups	Gender	ID of animals	24 hr after challenge phase	48 hr after challenge phase
Control-2 "Cottonseed oil"	Male	1016	0	0
		1017	0	0
		1018	0	0
		1019	0	0
		1020	0	0
Treatment-2 "Ear-loop" extract by "Cottonseed oil"	Male	1021	0	0
		1022	0	0
		1023	0	0
		1024	0	0
		1025	0	0
		1026	0	0
		1027	0	0
		1028	0	0
		1029	0	0
		1030	0	0



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Appendix 1-3. Individual Animal Grade in Skin Response of Guinea Pigs

Groups	Gender	ID of animals	24 hr after challenge phase	48 hr after challenge phase
Positive control "DNCB" Induction phase (0.5% DNCB) Challenge phase (0.1% DNCB)	Male	1006	1	1
		1007	1	1
		1008	2	2
		1009	1	1
		1010	2	2
		1011	1	1
		1012	1	2
		1013	1	2
		1014	1	1
1015	2	2		

History record from Master Lab, Report No. MSA202010 (Test period:10.05.2020-10.29.2020)

The positive control was tested completely within three months of test article results to confirm that was tested with the same source and strain of animals, as well as the same methods.

Appendix 2. Magnusson and Kligman Scale

ISO 10993-10:2010

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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Appendix 3. Test Article Information Sheet

Master Laboratory Co., Ltd.

Information for Test Article / Control Article

Sponsor Company	CHIN HSIUNG FIBER CO., LTD
Sponsor Address	NO.37-12, Ta Hsin RD., Pu Yen Hsiang, Chang Hua Hsien, Taiwan
Contract study item	<input checked="" type="checkbox"/> Base on the contract <input type="checkbox"/> Others:
Name of test article	Ear-loop
Major components	Spandex Yarn+ Nylon Yarn
Sample status	<input type="checkbox"/> Sterilized (<input type="checkbox"/> Gamma <input type="checkbox"/> EO <input type="checkbox"/> Steam) <input checked="" type="checkbox"/> Not Sterilized
Storage condition	<input checked="" type="checkbox"/> Room temperature (10°C~30°C) <input type="checkbox"/> 4°C <input type="checkbox"/> Dry <input checked="" type="checkbox"/> Away from light <input type="checkbox"/> Others:
Expiry day	
Specific requirement	
Batch/ Lot number	<input checked="" type="checkbox"/> Base on the specific number on the package: _____ <input type="checkbox"/> Base on the date on the package <input type="checkbox"/> Base on the arrived date <input type="checkbox"/> Others:
Extract by	<input type="checkbox"/> Weight (0.2g/ml) Total weight of each test article: <input checked="" type="checkbox"/> Surface (Sample thickness: <input checked="" type="checkbox"/> >1.0mm <input type="checkbox"/> 0.5-1.0mm <input type="checkbox"/> <0.5mm) Total area surface of each test article:
Absorption	<input checked="" type="checkbox"/> Non absorption <input type="checkbox"/> Water absorption rate: _____ / Oil absorption rate: _____
Sponsor Signature	Hank Shih



Appendix 4. Test Article

