



瑞德生物科技有限公司
MASTER LABORATORY CO.,LTD.

Skin Irritation Study in Rabbits

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 Irritation Study in Rabbits
STUDY REPORT**

Sponsor : CHIN HSIUNG FIBER CO., LTD

Testing Institution : Master Laboratory Co., Ltd.

November 2020



FINAL REPORT

Report No.: MSA-202010-408-T01

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Test article registration date: 10.22.2020

Experimental starting date: 10.20.2020

Animal in-housing: 10.20.2020

Extraction of test article: 10.23.2020

Test article administration date: 10.26.2020

Observation of dermal reaction: 10.26.2020-10.29.2020

Animal sacrifice date: 10.29.2020

Experimental complete date: 10.29.2020

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

11.10.2020
Date

Facility Management

Alan Hsieh
Alan Hsieh

11.10.2020
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
Bo Han Huang

11.10.2020
Date



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:

Inspection Contents	Date of inspection
Before the test (test execution protocol, requisitions, contracts).....	10.20.2020
Test (test substance data sheet, animal quarantine, standard operating procedures).....	10.26.2020
After the test (complete the original data, report reviews).....	11.06.2020

Quality Assurance unit in charge

Ying Chun Chen
Ying Chun Chen

11.10.2020
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 irritation responding to the test article “Ear-loop” extracts in New Zealand White Rabbits. The testing was performed in compliance with ISO 10993-10:2010. The furs of animals were shaved, and divided four regions. The left upper and right lower backsides of the skin were applied with the test article extract, and the control vehicle was applied on the right upper and left lower backsides. After 4 hours, the application was rinsed thoroughly by distilled water, and dermal reactions were observed at the time points of 1st, 24th, 48th and 72nd hr thereafter. The results showed that there were no erythema and edema findings in either the control or treatment group, and there were no mortalities (Table 1, Figure 1-8 and Appendix 1). Furthermore, the PII values were 0. Therefore, a single topical application of 0.5 ml of “Ear-loop” extract did not cause skin irritation.



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 local irritant reaction after a single topical application of test article extract in New Zealand White Rabbits.

MATERIALS AND METHODS

1. Animals

1.1. Species/Strain: New Zealand White Rabbit

1.2. Resource: Hui Jun

1.3. Body weights (Gender): >2 kg (Male)

1.4. Quarantine/acclimation: (MSAT-SOP-AM-001) 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: New Zealand White Rabbit was proven to be suitable for skin irritation studies, and widely used in single dose skin irritation studies.

1.6. Groups: A total of 6 rabbits were used in this study.

Group	Control	Treatment
Number of animals		3
Treated article	Saline	Ear-loop extract by saline
Number of animals		3
Treated article	Cottonseed oil	Ear-loop extract by cottonseed oil

Remark: The control vehicle and the test article extract were applied on the same rabbit, but the applied sites were different.



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

2.1. Housing: Rabbit room

2.2. Environment

a. Temperature: $19 \pm 3^{\circ}\text{C}$

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

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

2.3. Cage and animal No.

a. Quarantine/acclimation: 1 rabbit/cage

b. Study period: 1 rabbit/cage

2.4. Feed

a. Name: Prolab Rabbit Diet

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

3.1. Individual identification: Tested animals were identified by ear-marking.

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, Lot. MKCB9547; CAS No.8001-29-4

5. Administration of test article and control vehicles

5.1. Preparation (MSAT-SOP-GE-014) According to ISO 10993-12:2012 guideline.

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 single dose of test article and control vehicle was applied on left upper and right lower, and right upper and left lower of each rabbit's skin, respectively.

5.3. Volume of administration: Test article extract or control vehicle was 0.5 ml.

6. Procedure (MSAT-SOP-ME-001)

6.1. Pre-treatment, the furs of animal were shaved with electric animal shaver. If skin surface had dermatitis or scar, this animal dose not to be used. The clipped areas of right upper and lower, and left upper and lower were 2.5×2.5 cm each.



- 6.2. Test article administration: On the treatment day, 0.5 ml of the test article (polar or non-polar) extract was applied on both left upper and right lower backsides of the skin.
- 6.3. Control vehicle administration: On the treatment day, 0.5 ml of control vehicle (saline or cottonseed oil) was applied on both right upper and left lower backsides of the skin.
- 6.4. Elastic and porous bandages were wrapped around the application sites.
- 6.5. After 4 hours, distilled water was used to rinse off the test article extract and control vehicle.
7. Animal observations and items for examination (MSAT-SOP-ME-001)
- 7.1. Animal observation: The dermal reactions at the treated areas were observed and recorded, including erythema, edema, irritation, corrosion, recovery and other toxicity, at the time points of 1st, 24th, 48th and 72nd hr after the removal of the test article extract and control vehicle.
- 7.2. Score: After a single dose treatment, the skin responses at 24th, 48th and 72nd hr were checked and evaluated, according to “Grading system for skin reaction” (Appendix 2). Then PII was calculated from the erythema and edema scores (Appendix 3) to evaluate the dermal response.
- 7.3. Irritation reaction: If the test article caused the irritant reaction at 72nd hr, the observation and recordings should be continued until the 14th day and to determine the reversibility of the dermal injury.
- 7.4. Animal management: For the animals that skin irritation lasted for more than 72 h, the euthanasia would be performed after the observation on day 14, and the study would be terminated.



RESULTS

The present study was to investigate the skin irritation responding to the test article “Ear-loop” extract in New Zealand White Rabbits. The results showed that there were no erythema and edema findings in either the control or treatment group, and there were no mortalities (Table 1, Figure 1-8 and Appendix 1). Furthermore, the PII values were 0. Therefore, a single topical application of 0.5 ml of the test article extract did not cause skin irritation.

CONCLUSION

The study results showed that there were no erythema and edema findings in either the control or treatment group, and there were no mortalities (Table 1, Figure 1-8 and Appendix 1). Furthermore, the Primary Irritation Index values were 0. Therefore, a single topical application of 0.5 ml test article “Ear-loop” extract did not cause skin irritation.



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: Test 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. General requirements for the competence of testing and calibration laboratories. ISO/IEC 17025 (2017).



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Table 1. Incidence of Clinical Observation in Rabbits

Polar Group	Control (right upper and left lower backside)	Treatment (left upper and right lower backside)
Number of animals		3
Treated article	Saline	Ear-loop extract by saline
Erythema and eschar	0/3	0/3
Edema	0/3	0/3

Remark : The control vehicle and test article extract were applied on the same rabbit.
n/n : No. of rabbits with abnormal clinical signs/No. of rabbits per group

Non-polar Group	Control (right upper and left lower backside)	Treatment (left upper and right lower backside)
Number of animals		3
Treated article	Cottonseed oil	Ear-loop extract by cottonseed oil
Erythema and eschar	0/3	0/3
Edema	0/3	0/3

Remark : The control vehicle and test article extract were applied on the same rabbit.
n/n : No. of rabbits with abnormal clinical signs/No. of rabbits per group



Polar Group

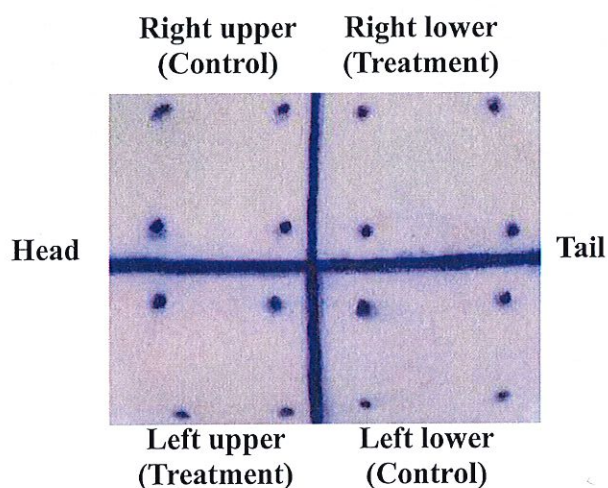


Figure 1. Observation at the 1st hr of Administration

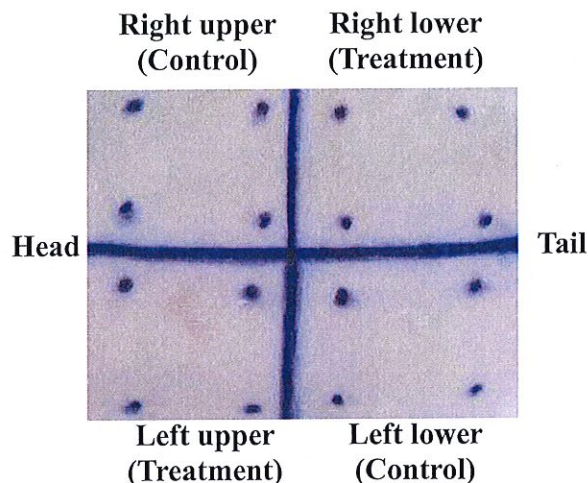


Figure 2. Observation at the 24th hr of Administration

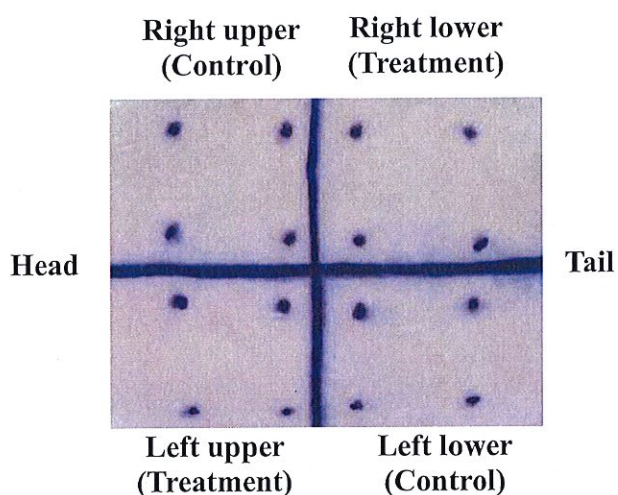


Figure 3. Observation at the 48th hr of Administration

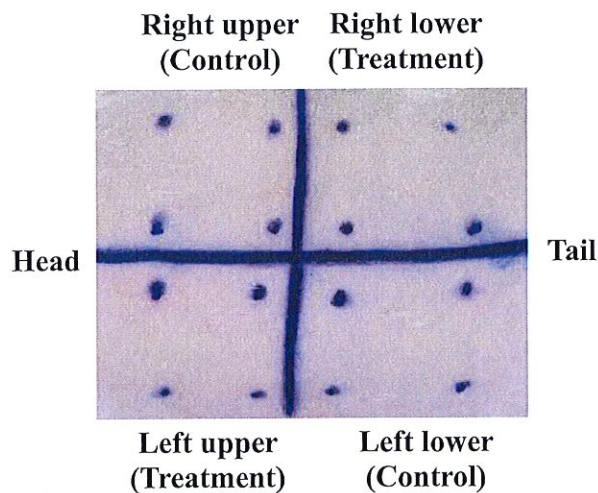


Figure 4. Observation at the 72nd hr of Administration



Appendix 1-1. Individual Animal Grade in Clinical Observation of Rabbits

Applied Regions (Dorsal Skin)	Treated article	Gender	Animal No.	Items for Grading	Clinical Observation (time point/hr)			
					1	24	48	72
Left upper and Right lower backside	Treatment group “Ear-loop” extract by “Saline”	Male	1001	Erythema and eschar formation	0	0	0	0
				Edema formation	0	0	0	0
		Male	1002	Erythema and eschar formation	0	0	0	0
				Edema formation	0	0	0	0
		Male	1003	Erythema and eschar formation	0	0	0	0
				Edema formation	0	0	0	0
Right upper and Left lower backside	Control group “Saline”	Male	1001	Erythema and eschar formation	0	0	0	0
				Edema formation	0	0	0	0
		Male	1002	Erythema and eschar formation	0	0	0	0
				Edema formation	0	0	0	0
		Male	1003	Erythema and eschar formation	0	0	0	0
				Edema formation	0	0	0	0



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Appendix 1-2. Individual Animal Grade in Clinical Observation of Rabbits

Applied Regions (Dorsal Skin)	Treated article	Gender	Animal No.	Items for Grading	Clinical Observation (time point/hr)			
					1	24	48	72
Left upper and Right lower backside	Treatment group “Ear-loop” extract by “Cottonseed oil”	Male	1004	Erythema and eschar formation	0	0	0	0
				Edema formation	0	0	0	0
		Male	1005	Erythema and eschar formation	0	0	0	0
				Edema formation	0	0	0	0
		Male	1006	Erythema and eschar formation	0	0	0	0
				Edema formation	0	0	0	0
Right upper and Left lower backside	Control group “Cottonseed oil”	Male	1004	Erythema and eschar formation	0	0	0	0
				Edema formation	0	0	0	0
		Male	1005	Erythema and eschar formation	0	0	0	0
				Edema formation	0	0	0	0
		Male	1006	Erythema and eschar formation	0	0	0	0
				Edema formation	0	0	0	0



Appendix 2. Grading System for Skin Reaction
ISO 10993-10:2010

Reaction	Numerical grading
Erythema and eschar formation	
· No erythema	0
· Very slight erythema (barely perceptible)	1
· Well-defined erythema	2
· Moderate erythema	3
· Severe erythema (beet-redness) to eschar formation preventing grading of erythema	4
Oedema formation	
· No oedema	0
· Very slight oedema (barely perceptible)	1
· Well-defined oedema (edges of area well-defined by definite raising)	2
· Moderate oedema (raised approximately 1 mm)	3
· Severe oedema (raised more than 1 mm and extending beyond exposure area)	4
Maximal possible score for irritation	8
Other adverse changes at the injection sites shall be recorded and reported.	

**Appendix 3. Evaluation Table of Single Dermal Irritation**

Primary Irritation Index (PII)	Irritation Interpretation
0~0.4	Non-irritant
0.5~1.9	Slightly irritant
2.0~4.9	Moderately irritant
5.0~8.0	Severely irritant

PIS (Primary Irritation Scores)

$PIS (24^{th}, 48^{th}, 72^{nd} \text{ h time point}) = (A+B) - (C+D)$

A, B = scores of test article, left upper and right lower backside

C, D = scores of right upper and left lower backside

$$PII = \frac{\text{Total scores of } 24^{th}, 48^{th}, 72^{nd} \text{ hr PIS}}{3}$$



Appendix 4. 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 5. Test Article

