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,

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0.5% Virol-Oxy
Skin Irritation Study in Rabbits
STUDY REPORT

Testing Laboratory
2634

Sponsor：Watch Water (S) Pte Ltd.

Testing Institution：Master Laboratory Co., Ltd.

June 2020

Report No.: MSA-202005-183-T01

Experimental starting date: 06.02.2020

Test article registration date: 06.05.2020

Animal in-housing: 06.02.2020

Extraction of test article: 06.06.2020

Test article administration date: 06.09.2020

Observation of ocular irritation reaction：06.09.2020-06.12.2020

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

Experiment executor: Bo Han Huang

Study Director

Jhen Ru Shih 06.16.2020

Jhen Ru Shih **Date**

Facility Management

Alan Hsieh 06.16.2020

Alan Hsieh **Date**

STUDY DIRECTOR 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), Good Laboratory Practice for Non-clinical Laboratory Studies (Food and Drug Administration, R.O.C., 2019) and the General Requirements for the Competence of Testing and Calibration Laboratories (ISO/IEC 17025:2017). Besides, there was no deviation from the approved study protocol and no adverse problems that would affect the integrity of the results or the interpretation of our conclusion. The test article is a proprietary product of the sponsor, therefore the sponsor will be responsible for the requirements listed under "0.5% Virol-Oxy" of the GLP regulation (21CFR P.58, FDA).

Study Director

Jhen Ru Shih
Jhen Ru Shih

06.16.2020
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).....	05.28.2020
Test (test substance data sheet, animal quarantine, standard operating procedures).....	06.09.2020
After the test (complete the original data, report reviews).....	06.16.2020

Quality Assurance unit in charge

Ying Chun Chen
Ying Chun Chen

06. 16. 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: Watch Water (S) Pte Ltd.

   b. Address: 25 International Business Park #01-26/29 German Centre Singapore 609916

CONTENTS

SUMMARY

The present study was to investigate the skin irritation responding to the test article "0.5% Virol-Oxy" 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 solution 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 h 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 "0.5% Virol-Oxy" 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 on 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	0.9% saline	0.5% Virol-Oxy extract by 0.9% saline
Number of animals	3	
Treated article	Cottonseed oil	0.5% Virol-Oxy extract by cottonseed oil

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

2. Feeding and care (MSAT-SOP-AM-001)

2.1. Housing: Rabbit room

2.2. Environment (SOP: Temperature: 19 ± 3℃; Humidity: 50 ± 20%)

a. Temperature: 20.8-21.8℃

b. Humidity: 54-58%

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

4.1. Test article: 0.5% Virol-Oxy

4.2. 0.9% saline: CHI SHENG CHEMICAL CORPORATION, Lot. N6012;
Solutio Natrii Chloridi Isotonica 0.9% 500 ml

4.3. Cottonseed oil: SIGMA-ALDRICH, Co., Lot. MKCB9547;
CAS No.: 8001-29-4

5. Administration of test article and control solution

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

a. Polar preparation: Measured the weight of test article, and immersed it in 0.9% saline for 72±2 hr at 50±2°C with constant agitation (100 rpm). The weight ratio of test article/0.9% saline was approximately 0.2 g/ml. After extraction, the extract solution was used immediately; the appearance of test article extract was clear and colorless without particulates present.

b. Non-polar preparation: Measured the weight of test article, and immersed it in cottonseed oil for 72±2 hr at 50±2°C with constant agitation (100 rpm). The weight ratio of test article/cottonseed oil was approximately 0.2 g/ml. After extraction, the extract solution 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 solution 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 and control solution were 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 solution administration: On the treatment day, 0.5 ml of 0.9% 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 and control solution.

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 1^{st}, 24^{th}, 48^{th} and 72^{nd} h after the removal of the test article and control

7.2. Score: After a single dose treatment, the skin responses at 24^{th}, 48^{th} and 72^{nd} h were checked and evaluated, according to "Grading system for skin reaction".(Appendix 2) PII was then 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 72^{nd} h, the observation and recordings should be continued until the 14^{th} 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 "0.5% Virol-Oxy" 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 "0.5% Virol-Oxy" extract did not cause skin irritation.

REFERENCES

1. Good Laboratory Practice for Nonclinical Laboratory Studies (2019) Food and Drug Administration, the Executive Yuan.
2. Guideline for the Nonclinical Pharmacology/Toxicology Studies Medicinal Products Applications (2013) Food and Drug Administration, the Executive Yuan.
3. 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.
4. Biological evaluation of medical devices-part 10: Test for irritation and skin sensitization. ISO 10993 (2010).
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.

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	0.9% saline	0.5% Virol-Oxy extract by 0.9% saline
Erythema and eschar	0/3	0/3
Edema	0/3	0/3

Remark：The control solution 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	0.5% Virol-Oxy extract by cottonseed oil
Erythema and eschar	0/3	0/3
Edema	0/3	0/3

Remark：The control solution 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

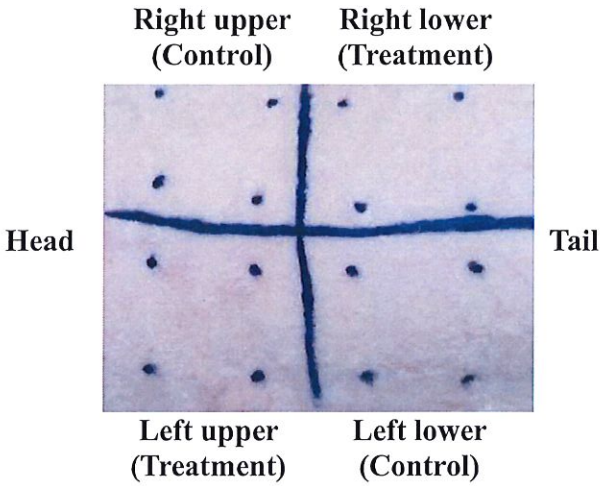

Figure 1.Observation at the 1st hr of Administration

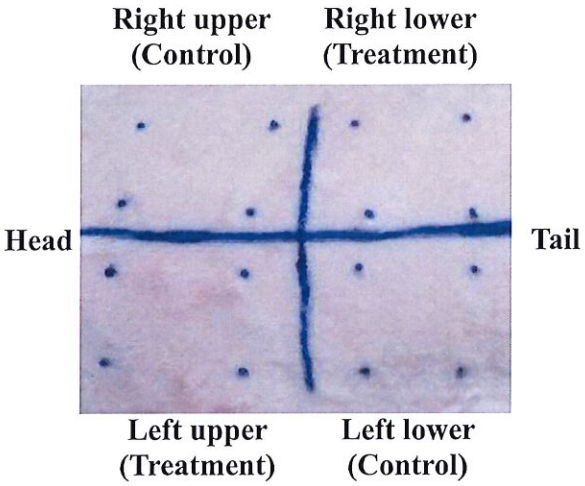

Figure 2.Observation at the 24th hr of Administration

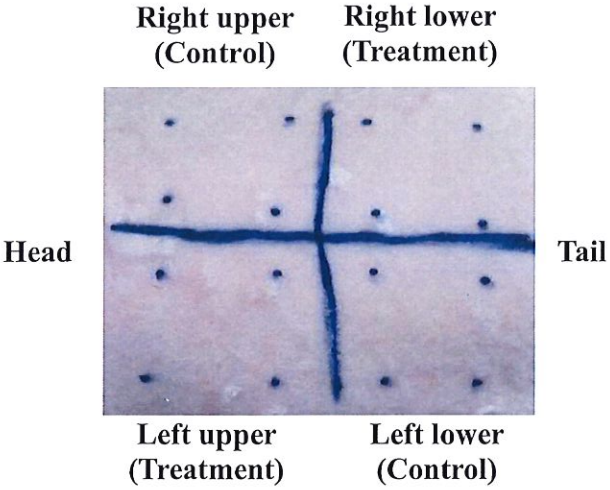

Figure 3.Observation at the 48th hr of Administration

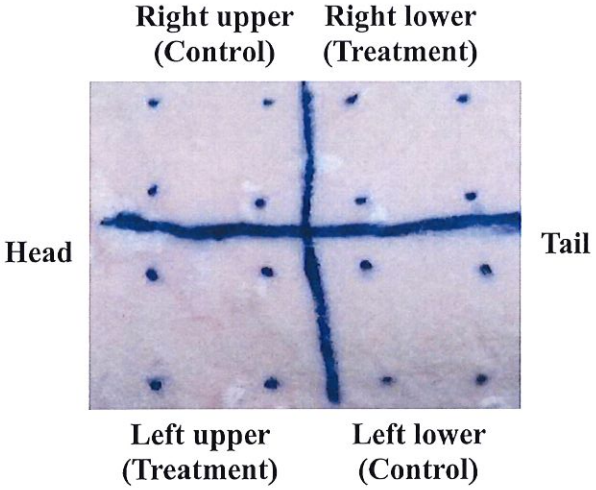

Figure 4.Observation at the 72nd hr of Administration

Non-Polar Group

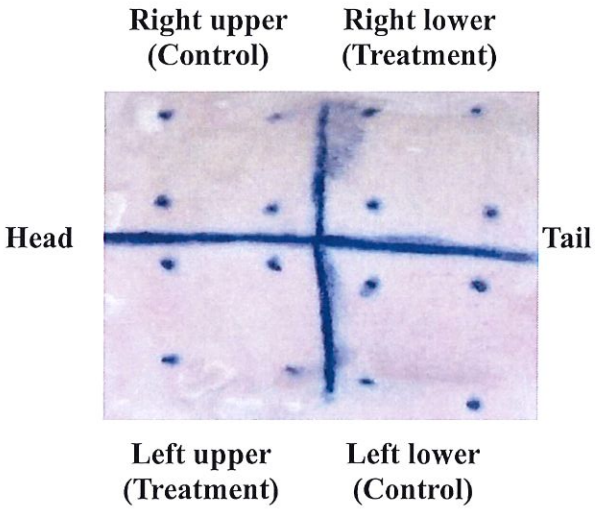

Figure 5.Observation at the 1st hr of Administration

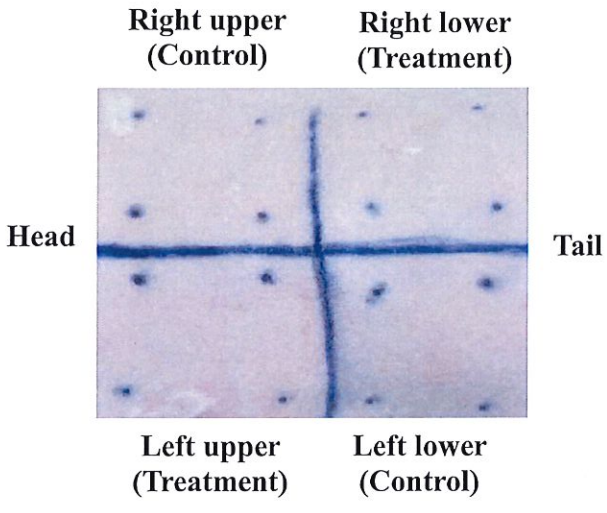

Figure 6.Observation at the 24th hr of Administration

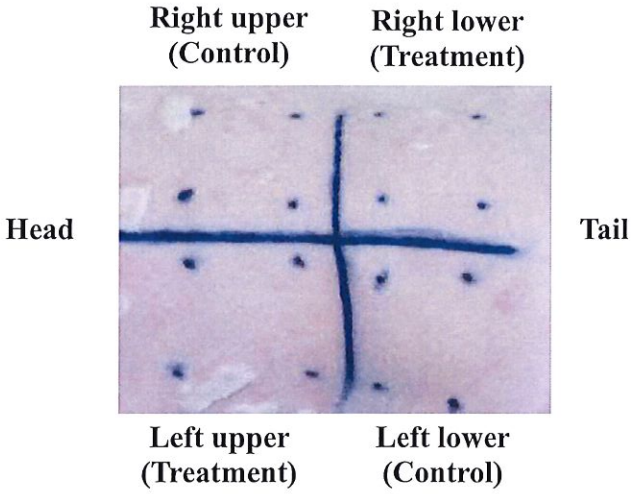

Figure 7.Observation at the 48th hr of Administration

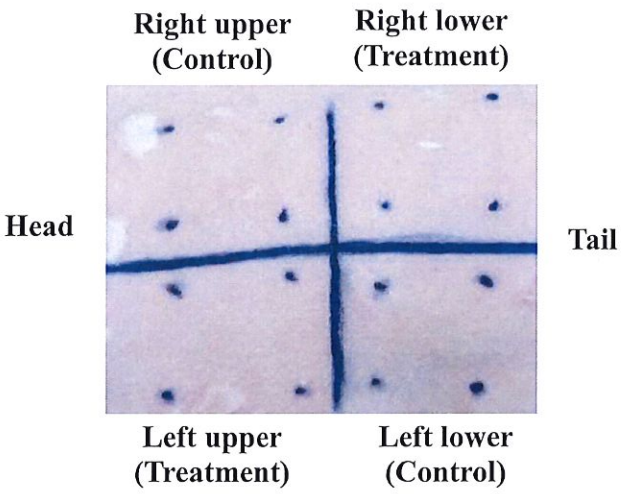

Figure 8.Observation at the 72nd hr of Administration

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 "0.5% Virol-Oxy" extract by "0.9% saline"	Male	183-1001	Erythema and eschar formation	0	0	0	0
				Edema formation	0	0	0	0
		Male	183-1002	Erythema and eschar formation	0	0	0	0
				Edema formation	0	0	0	0
		Male	183-1003	Erythema and eschar formation	0	0	0	0
				Edema formation	0	0	0	0
Right upper and Left lower backside	Control group "0.9% saline"	Male	183-1001	Erythema and eschar formation	0	0	0	0
				Edema formation	0	0	0	0
		Male	183-1002	Erythema and eschar formation	0	0	0	0
				Edema formation	0	0	0	0
		Male	183-1003	Erythema and eschar formation	0	0	0	0
				Edema formation	0	0	0	0

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 "0.5% Virol-Oxy" extract by "cottonseed oil"	Male	183-1004	Erythema and eschar formation	0	0	0	0
				Edema formation	0	0	0	0
		Male	183-1005	Erythema and eschar formation	0	0	0	0
				Edema formation	0	0	0	0
		Male	183-1006	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	183-1004	Erythema and eschar formation	0	0	0	0
				Edema formation	0	0	0	0
		Male	183-1005	Erythema and eschar formation	0	0	0	0
				Edema formation	0	0	0	0
		Male	183-1006	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

Dermal Reaction	Grade
Erythema and eschar formation	
· No erythema	0
· Very slight erythema	1
· Well defined erythema	2
· Moderate to severe erythema	3
· Severe erythema (beet redness) to slight eschar formation	4
Edema formation	
· No edema	0
· Very slight edema	1
· Slight edema (edges of area well defined by definite raising)	2
· Moderate edema (raised approximately 1 mm)	3
· Severe edema (raised more than 1 mm and extending beyond the area of exposure)	4

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} 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

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

Appendix 4. Test Article Information Sheet

表單編號：MS-A-QP03-TR004-20200605-01

Master Laboratory Co. Ltd.

Information for ☑Test Article /☐Control Article

Sponsor Company	Watch Water (S) Pte Ltd
Sponsor Address	25 International Business Park #01-26/29 German Centre Singapore 609916
Contract study item	☑ Base on the contract ☐ Others:
Name of test article	0.5% Virol-Oxy
Major components	1.Potassium Pentasulfate, 2.Hydrogen Peroxide, 3.Titanium Dioxide (Crystalline Powder), 4.Sulfamin Acid, 5. Sodium Chloride
Sample status	☐ Sterilized (☐Gamma ☐EO ☐Steam) ☑ Not Sterilized
Storage condition	☑ Room temperature (20°C~30°C) ☐ 4°C ☐ Dry ☐ Away from light ☐ Others:
Expiry day	3 Years Shelf-Life Time (Powder Form)
Specific requirement	
Batch/ Lot number	☑ Base on the specific number on the package:_____ ☐ Base on the date on the package ☐ Base on the arrived date ☐ Others:
Extract by	☑ Weight (0.2g/ml) Total weight of each test article:_ ☐ Surface (Sample thickness: ☐>1.0mm ☐0.5-1.0mm ☐<0.5mm) Total area surface of each test article:
Absorption	☑ Non absorption ☐ Water absorption rate: / Oil absorption rate:
Sponsor Signature	

MS-A(C)-QP03-TR004 v2.4

Appendix 5. Test Article