Report No.: MZ6-191000005001EN

SUPER LAB
because results matter

FINAL REPORT

Study title

Guinea Pig Skin Sensitization Test (Maximization Method) (Cottonseed oil)

Test article name: Thermoplastic sheet

Sponsor: HYMED Technology Co.
No. 3-3, Hongmao, Xinfeng Township, Hsinchu County 30472, Taiwan

Test facility: Super Laboratory Co., Ltd. Contract Research Organization
No. 21, Wugong 5th Rd., Xinzhuang Dist., New Taipei City 24890, Taiwan (R.O.C.)

Remark:

- This report contains a total of 26 pages. It will be invalid if separated and/or partially copied.
- This test does not involve sampling of test article, and the final report is only applicable to the test article provided by sponsor.

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Study statement and signature:

This study was conducted in accordance with study protocol, and no test deviation or incident that would affect the integrity of this study. This study was conducted with test article information which provided by the sponsor. This study was conducted in compliance with Good Laboratory Practice for Nonclinical Laboratory Studies, FDA (21 CFR Part 58, 2018); OECD Principles of Good Laboratory Practice (ENV/MC/CHEM (98) 17, 1998) and Good Laboratory Practice for Nonclinical Laboratory Studies, Taiwan Food and Drug Administration (2019). (The sections of regulations excluded from this study: OECD GLP Section Ⅱ 6.1.3, 6.2.2, 6.2.4 and 6.2.5, FDA GLP Subpart F Sec.58.105 (a), (b), (c) and Sec. 58.113 (a), TFDA GLP Part Ⅱ 6.1.3, 6.2.2, 6.2.4 and 6.2.5).

Record and specimen reserve:

All raw data, record, study protocol and final report generated as a result of the study will be retained in archives of Super Laboratory Co., Ltd. The sample of test article was retained in "Test article room". The control period will be based on rule of the Super Laboratory Co., Ltd.

Signature of final report:

Study director: Ya-lun Chen 2019/12/19

Yu-lun Chen, Ph.D., Associate Research Fellow

Test facility management: Yueh-ting Tsai 2019/12/20

Yueh-ting Tsai, Ph.D., Vice President

SUPER LAB
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Quality assurance statement:

The study in this laboratory was conducted with study protocol (PR-MZ6-191000005EN) and SuperLab standard operating procedures, except the characteristics of the test article was provided by the sponsor. The Quality Assurance Unit (QAU) of Super Laboratory is responsible for auditing the study, raw data and final report.

This study has been inspected by the QAU in accordance with standard operating procedure of Super Laboratory Co., Ltd. Results indicated no test deviation or incident that would affect the integrity of this study. The final report correctly described the methods and procedures used in the study, and accurately reflected the raw data generated during this study.

The QAU conducted inspections on the following dates. The findings were reported to the study director and test facility management. The following list of inspected items, types and related dates:

Inspected item	Type of inspection	Inspected date	Reported date	
			Study director	Test facility management
Study protocol	Study	2019/10/08	2019/10/08	2019/10/09
Raw data	Study	2019/11/22	2019/11/22	2019/11/27
Final report	Study	2019/11/22	2019/11/22	2019/11/27

Quality Assurance Unit: Ting-yu Li 2019/12/19

Ting-yu Li, Team Leader

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Guinea Pig Skin Sensitization Test (Maximization Method) for "Thermoplastic sheet" (Cottonseed oil)

Abstract

This study was conducted according to ISO 10993-10: 2010 guideline to evaluate the allergenic potential and sensitizing capacity of the "Thermoplastic sheet" (Specimen ID: MZ6-191000005) provided by HYMED Technology Co. Fifteen male Hartley strain guinea pigs were used in this study. According to the extraction methods for preparing test article proposed in ISO 10993-12: 2012 guideline, the test article was extracted with cottonseed oil at 37 ± 1°C for 72 hours, and the obtained extracts were used in this study. Before the test, the fur on the intrascapular region of test animal was shaved, and checked the skin with good condition. The test animals were exposed to the test article extracts or control article by intradermal injection and epidermal application, as induction phase. After 14 days, test article extracts loaded on the gauze patch was applied on the upper flank of all test animals, as challenge phase. The skin sensitization reaction of the test animals upper flank were evaluated at 24 and 48 hours after challenge phase. Results indicated that there was no hypersensitivity response on the upper flank skin of the test group and control group animals. The sensitization rate of test group and control group were zero (0% sensitized) and classified as weak allergenic potential. Therefore, the test article "Thermoplastic sheet" did not cause any skin sensitization response on guinea pigs under the conditions designed for this study.

1. **Objective:**

This study was conducted according to ISO 10993-10: 2010 guideline to evaluate the allergenic potential of the test article extracts to guinea pig.

2. **General information:**

2.1 Project No.: MZ6-191000005.

2.2 Sponsor information:

2.2.1 Sponsor title: HYMED Technology Co.

2.2.2 Sponsor address: No. 3-3, Hongmao, Xinfeng Township, Hsinchu County 30472, Taiwan.

2.2.3 Sponsor's representative: Hui-Yi Chiang.

2.3 Facility information:

2.3.1 Facility title: Super Laboratory Co., Ltd. Contract Research Organization.

2.3.2 Facility address: No.21, Wugong 5th Rd., Xinzhuang Dist., New Taipei City 24890, Taiwan (R.O.C.).

2.3.3 Study director: Yu-lun Chen, Associate Research Fellow.

2.3.4 Study person: Yu-lun Chen, Associate research fellow, Ming-yen Wu, Analyst, Jia-Yi Lin, Analyst, Meng-yen Su, Assistant analyst.

2.3.5 Study director and person address: The same as facility address.

2.4 Testing sites:

Guinea pig holding room C2A of Laboratory Animal Center of Super Laboratory Co., Ltd. 6F, No.21, Wugong 5th Rd., Xinzhuang Dist., New Taipei City 24890, Taiwan (R.O.C.).

3. **Study schedule:**

3.1 Study initiation date: 2019/10/08.

3.2 Experimental starting date: 2019/10/17.

3.3 Experimental completion date: 2019/11/11.

3.4 Study completion date: See study director's signature date in the final report.

4. **Test article (all information supplied by sponsor) and control article:**

4.1 Test article (Specimen ID: MZ6-191000005):

4.1.1 Article name: Thermoplastic sheet.

4.1.2 Receiving date: 2019/10/03.

4.1.3 Test article lot No., category, form, storage condition and expiration date were shown in Appendix 1 and 2.

4.2 Control article:

4.2.1 Article name: Cottonseed oil.

4.2.2 Manufacturer: Sigma-Aldrich Co. LLC.

4.2.3 Major ingredients: Cottonseed oil. Form: Liquid.

4.2.4 Storage condition: Room temperature.

5. Test system and condition:

5.1 Test system:

5.1.1 Fifteen male Hartley strain guinea pigs and 300 g to 500 g in weight were selected.

5.1.2 Source: National Laboratory Animal Center (No.128 Academia Road, Section 2, Nankang, Taipei City 115, Taiwan).

5.1.3 Quarantine and acclimation: This study was conducted according to SuperLab standard operating procedure SOPA-303. All animals from the supplier underwent quarantine and acclimate period for 7 days to ensure that no abnormal sign then could be used to test.

5.1.4 Animal identification: This study was conducted according to SuperLab standard operating procedure SOPA-203 and SOPP-315. All animals were housed in stainless steel cage. Designation cards were attached to the cage, marking the animal ID, number, sex, source, IACUC No. and study director.

5.1.5 IACUC No.: 108-3aa.

5.2 Condition:

5.2.1 This study was conducted according to SuperLab standard operating procedures SOPA-206.

5.2.2 Temperature: 21 ± 2°C. Relative humidity: 55 ± 15%. Light cycle: 12 hours light and 12 hours dark.

5.2.3 Housing: 5 ~ 10 animals per cage. Frequency of ventilation: 10 ~ 15 times/hour.

5.2.4 Feed: Guinea Pig Diet 5025 (Purina Mills, LLC, Missouri, U.S.A.).

5.2.5 Drinking water: Vitamin C was mixed into RO water, provided with water bottle.

6. Procedure:

6.1 Test article extraction:

6.1.1 The study was conducted according to SuperLab standard operating procedure SOPP-301.

6.1.2 Test article extracts were prepared according to ISO 10993-12: 2012 guideline, and the obtained extracts were used for this study. Cottonseed oil was served as the extraction vehicle. The form of test article was sheet and extracts was prepared with a ratio of 0.1 g/mL. The test articles were extracted for 72 hours at 37 ± 1°C with constant agitation of 100 rpm. Cottonseed oil without test article was placed in the same condition and served as control article.

6.2 Main study:

6.2.1 This study was conducted according to SuperLab standard operating procedure SOPP-309.

6.2.2 Before the test, 15 guinea pigs were grouped randomly, and divided into test group and control group. There were five animals in control group and ten animals in test group.

6.2.3 Before the test, 5 cm x 7 cm area over the intrascapular region of each test animal was shaved. The shaved area should not have any injury.

6.2.4 The treatment was divided into intradermal induction phase, topical induction phase and challenge phase.

6.2.5 Intradermal induction phase: On day 1, made a pair of 0.1 mL of the following articles was intradermal injections on the intrascapular region of test animals.

a. Emulsion of Freund's complete adjuvant (FCA, Difco/BD) with extraction solvent in 1 : 1 volume ratio.
b. Test article extracts (test group) or control article (control group).
c. Emulsion of either test article extracts or the control article in a 1 : 1 volume ratio stable emulsion of FCA and extraction solvent.

6.2.6 On day 6, the intrascapular region of the test animals were shaved and pretreated with 10% sodium dodecyl sulfate (SDS, J.T. Baker, Inc.) in petrolatum.

6.2.7 Topical induction phase: On day 7, a gauze patch (2 cm x 4 cm) was fully loaded with 0.5 mL test article extracts or control article, and applied onto the intrascapular regions of test group and control group animals and held in contact with a non-adhesive bandage. The dressing was left in place for 48 hours.

6.2.8 Challenge phase: On day 22, an approximately 5 cm x 3 cm area on the upper flanks of test animals were shaved. On day 23, a gauze patch loaded with 0.5 mL test article extracts was applied to the flank of test group and control group animals. The dressing was left in place for 24 hours.

6.2.9 The skin reaction at 24 and 48 hours after the patch removal was observed and recorded according to the grading table shown in "Magnusson and Kligman scale" (Appendix 3).

6.2.10 Animal weights were determined before the intradermal induction (Day 1) and at the end of the observation period (Day 26).

6.3 Evaluation criterion:

6.3.1 If grade of control group animals < 1, and the grade of test group animals ≧ 1 indicated hypersensitivity reaction.

6.3.2 If grade of control group animals ≧ 1, then the reaction of test group animals which exceeded most severe control reaction indicated hypersensitivity reaction.

6.3.3 The sensitization rate at 24 and 48 hours was calculated separately. The higher sensitization rate was used to classification the sensitizing capacity of the test article extracts according to the "Scoring system of Kligman" (Appendix 4).

7. Results:

7.1 The body weights of test and control group animals were shown in Table 1. Results indicated that the body weights of test animals were increased normally.

7.2 The skin sensitization reaction of test group and control group animals were shown in Table 2. Results indicated that there was no emergence of erythema and swelling on the upper flank skin of the test group and control group animals at 24 and 48 hours after the challenge phase (Figure 1).

7.3 Following challenge phase, the hypersensitivity percentage of the control group and test group animals were zero, and the test article extracts classified as weak allergenic potential according to the "Scoring system of Kligman" (Appendix 4).

8. Discussion:

8.1 Before the study, the average body weight was 446.93 ± 22.36 g in control group, 443.85 ± 28.08 g in test group. At the end of the study, the average body weight was 537.42 ± 36.14 g in control group, 535.82 ± 32.12 in test group. In body weight change, the animals of control and test groups were increased normally, ranged between 75.31 ~ 106.29 g in control group and 78.34 ~ 112.02 g in test group. These results indicated that the test article extracts administration did not affect the body weight of the test animal.

8.2 The upper flank of control group and test group animals were no erythema and swelling, thus the scores were 0. According to "6.3 evaluation criterion", the score of control group animals and test group animals were < 1, the test article extracts was considered as no allergenic potential on the skin of guinea pigs.

8.3 According to ISO 10993-10: 2010 guideline, validity of the study (positive control test) was performed once every six months. Positive control article was Hexyl cinnamic aldehyde (HCA). Results showed that the sensitization rate of positive group was 100%, and 0% in control group. The validity of the study and the technical of study person were well ensured.

9. Conclusion:

This study was conducted according to ISO 10993-10: 2010 guideline and SuperLab standard operating procedure SOPP-309. Results indicated that there was no hypersensitivity response on the upper flank skin of the test group and control group animals. The sensitization rate of test group and control group were zero (0% sensitized) and classified as weak allergenic potential. Therefore, the test article "Thermoplastic sheet" did not cause any sensitization response on the skin of guinea pigs under the conditions designed for this study.

10. References:

10.1 Taiwan Food and Drug Administration (TFDA). Good Laboratory Practice for Nonclinical Laboratory Studies. 2019.

10.2 Chinese National Standards (CNS). Biological evaluation of medical devices-Part 10: tests for irritation and sensitization, CNS 14393-10, 2005.

10.3 Food and Drug Administration (FDA). Good Laboratory Practice for Nonclinical Laboratory Studies. 21 CFR, Part 58, 2018.

10.4 International Organization for Standardization (ISO). Biological evaluation of medical devices-part 10: Tests for irritation and skin sensitization, ISO 10993-10, 2010.

10.5 International Organization for Standardization (ISO). Biological evaluation of medical devices-part 12: Sample preparation and reference materials, ISO 10993-12, 2012.

10.6 Organisation for Economic Co-operation and Development (OECD). OECD series on principles of Good Laboratory Practice and Compliance Monitoring, Number 1. OECD Principles of Good Laboratory Practice. ENV/MC/CHEM (98) 17. 1998.

Table 1: The body weight of the guinea pigs during the study period

Group	Sex	Animal ID	Animal Weight (g)		Weight Change [a] (g)
			Day 1	Day 26	
Control	Male	543	479.13	584.23	+ 105.10
		544	422.70	498.01	+ 75.31
		545	459.41	565.70	+ 106.29
		546	434.34	514.93	+ 80.59
		547	439.06	524.22	+ 85.16
Test	Male	548	423.31	506.92	+ 83.61
		549	444.56	533.61	+ 89.05
		550	415.44	506.62	+ 91.18
		551	437.10	532.26	+ 95.16
		552	430.26	521.43	+ 91.17
		553	452.69	531.03	+ 78.34
		554	425.42	505.43	+ 80.01
		555	487.90	588.08	+ 100.18
		556	424.22	536.24	+ 112.02
		557	497.62	596.62	+ 99.00

[a] Weight change: animal weight of Day 26 - animal weight of Day 1.

Table 2: Individual skin reaction of guinea pigs.

Group	Sex	Animal ID	Grade of skin reaction [a]		Sensitization Rate	Sensitizing Capacity [b]
			24 hours	48 hours		
Control	Male	543	0	0	0%	Weak
		544	0	0		
		545	0	0		
		546	0	0		
		547	0	0		
Test	Male	548	0	0	0%	Weak
		549	0	0		
		550	0	0		
		551	0	0		
		552	0	0		
		553	0	0		
		554	0	0		
		555	0	0		
		556	0	0		
		557	0	0		

[a] The skin reaction was grading according to "Magnusson and Kligman scale" (Appendix 3).

[b] The sensitizing capacity of the test article was classified according to the "Scoring system of Kligman" (Appendix 4).

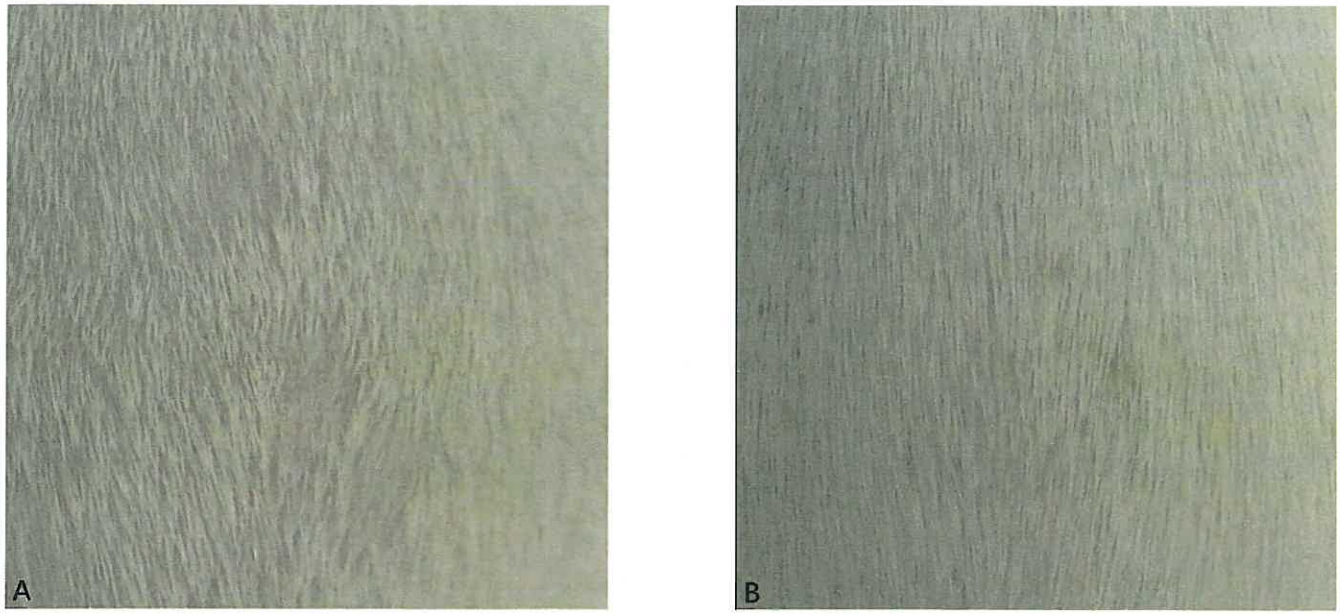
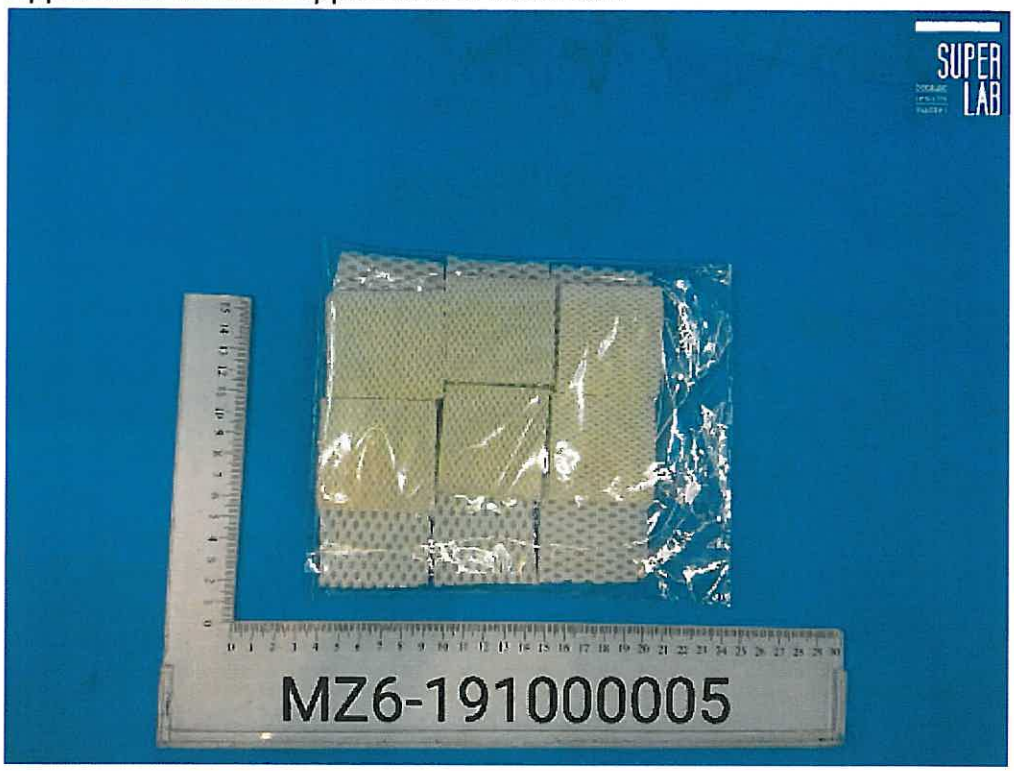


Figure 1: The result of guinea pig skin sensitization test. The skin reaction of all animals were no any sensitization response after challenge phase at 48 hours. A: Control group animal (Animal ID: 545). B: Test group animal (Animal ID: 552).

Appendix 1: The outer appearance of test article



Appendix 2: Test article information sheet (All data supplied by HYMED Technology Co.)

Test Article Information Sheet

Sponsor Information

Sponsor: HYMED Technology Co.	Sponsor's representative: Hui-Yi Chiang	
Sponsor's address: No. 3-3, Hongmao, Xinfeng Township, Hsinchu County 30472, Taiwan		
Sponsor's contact person: Kevin Yang	Tel: +886 910 784037	Fax:+886 3 5686961
Study item: Cytotoxicity Test、Rabbit Skin Irritation Test (Physiological Saline)、Rabbit Skin Irritation Test (Cottonseed Oil)、Guinea Pig Skin Sensitization Test (Maximization Method) (Physiological Saline)、Guinea Pig Skin Sensitization Test (Maximization Method) (Cottonseed Oil)		

Test Article Information

Article name: Thermoplastic sheet			
Lot No.:NA	Expiration date: NA (yyyy/mm/dd)	Color: Beige & White	
Article category:	☐ Food ☐ Health food ☐ Drug ■ Medical devices ☐ Chemical ☐ Pesticides ☐ Other		
Article form:	☐ Liquid ☐ Powder ■ Sheet ☐ Granular ☐ Cream ☐ Capsular ☐ Membrane ☐ Other		
Packing specification/amount: $25cm^2$/sheet × 15 sheets (ex: 100 g/battle × 10 battles)			
Major ingredients: Polyester & Polycaprolactone			
Sterilization:	■ No ☐ Yes, method: ☐ The article is aseptic and keep in aseptic condition		
Storage condition:	■ Room temperature ☐ Refrigeration (5 ± 3℃) ☐ Freezing (-20 ± 4 ℃) ☐ Protect from light ☐ Protect from moisture ☐ Other		
Disposal method for residual article:	■ Six months after the study was closed, discarded according to waste disposal standard operating procedures. ☐ Six months after the study was closed, discarded according to specified method by sponsor. Specified method: _____ ☐ Returned to sponsor		
Attachment	Stability test report	☐ Yes	■ No
	Certificate of analysis (COA)	☐ Yes	■ No
	Safety data sheet (SDS)	■ Yes	☐ No
	User's guide	☐ Yes	■ No
	Other:NA		
Precautions/Note:NA			

1. This is a required form which will be attached in final report. The unfilled field, please fill in NA.
2. "Sponsor's representative" must be the person who signs in study protocol.
3. This test article information sheet is an official document, please confirm the content was correct and sign.
4. The control date of retained article is five years from receiving date or based on the expiration date of test article.

Sponsor's representative sign and date (yyyy/mm/dd): Hui-Yi Chiang 2019/9/1

Appendix 3: Magnusson and Kligman scale

Skin reaction	Grade scale
No visible change	0
Discrete or patchy erythema	1*
Moderate and confluent erythema	2*
Intense erythema and swelling	3*

* Positive reaction.

Appendix 4: Scoring system of Kligman

Sensitization Rate (%)	Grade scale	Class
0 ~ 8	1	Weak
9 ~ 28	2	Mild
29 ~ 64	3	Moderate
65 ~ 80	4	Strong
81 ~ 100	5	Extreme

Appendix 5: Study protocol

Protocol No.: PR-MZ6-191000005EN

STUDY PROTOCOL

Study title

Guinea Pig Skin Sensitization Test (Maximization Method) (Cottonseed oil)

Test Article Name: Thermoplastic sheet

Sponsor: HYMED Technology Co.
No. 3-3, Hongmao, Xinfeng Township, Hsinchu County 30472, Taiwan

Test Facility: Super Laboratory Co., Ltd. Contract Research Organization
No.21, Wugong 5th Rd., Xinzhuang Dist., New Taipei City 24890, Taiwan (R.O.C.)

Protocol No.: PR-MZ6-191000005EN

Good laboratory practice statement:

This study will be conducted with test article information which provided by the sponsor. This study will be conducted in compliance with (1) Good Laboratory Practice for Nonclinical Laboratory Studies, Food and Drug Administration (FDA); (2) OECD Principles of Good Laboratory Practice, Organisation for Economic Co-operation and Development (OECD); (3) Good Laboratory Practice for Nonclinical Laboratory Studies, Taiwan Food and Drug Administration. (The sections of regulations excluded from this study: OECD GLP Section II 6.1.3, 6.2.2, 6.2.4 and 6.2.5, FDA GLP Subpart F Sec. 58.105 (a), (b), (c) and Sec. 58.113 (a), TFDA GLP Part II 6.1.3, 6.2.2, 6.2.4 and 6.2.5).

Record and specimen reserve:

All raw data, record, study protocol and final report generated as a result of the study will be retained in archives of Super Laboratory Co., Ltd. Expected archive file is shown in Appendix 1. The sample of test article will be retained in "Test article room" . The control period will be based on rule of the Super Laboratory Co., Ltd.

Study approve:

Sponsor title/Representative: Hui-Yi Chiang 2019/10/08

HYMED Technology Co., Hui-Yi Chiang

Study director: Yu-lun Chen 2019/10/08

Yu-lun Chen, Ph.D., Associate Research Fellow

Protocol No.: PR-MZ6-191000005EN

Guinea Pig Skin Sensitization Test (Maximization Method) for "Thermoplastic sheet" (Cottonseed oil)

1. **Objective:**

   This study will be conducted according to ISO 10993-10: 2010 guideline to evaluate the allergenic potential of the test article extracts to guinea pig.

2. **General information:**

   2.1 Sponsor information:

   Sponsor title: HYMED Technology Co.

   Sponsor address: No. 3-3, Hongmao, Xinfeng Township, Hsinchu County 30472, Taiwan.

   Sponsor's representative: Hui-Yi Chiang.

   2.2 Facility information:

   Facility title: Super Laboratory Co., Ltd. Contract Research Organization.

   Facility address: No.21, Wugong 5th Rd., Xinzhuang Dist., New Taipei City 24890, Taiwan (R.O.C.).

   Study director: Yu-lun Chen, Associate research fellow.

   Study director address: The same as facility address.

   2.3 Testing sites:

   Guinea pig holding room C2A of Laboratory Animal Center of Super Laboratory Co., Ltd. 6F, No.21, Wugong 5th Rd., Xinzhuang Dist., New Taipei City 24890, Taiwan (R.O.C.).

3. **Study schedule:**

   3.1 Experimental starting date: 2019/10/17.

   3.2 Experimental completion date: 2019/11/11.

4. **Test article (all information supplied by sponsor) and control article:**

   4.1 Test article (Specimen ID: MZ6-191000005):

   4.1.1 Article name: Thermoplastic sheet.

   4.1.2 Receiving date: 2019/10/03.

   4.1.3 Source: HYMED Technology Co.

   4.1.4 Lot No.: NA.

   4.1.5 Storage condition: Room temperature. Expiration date: NA.

4.2 Control article:

4.2.1 Article name: Cottonseed oil.

4.2.2 Manufacturer: Sigma-Aldrich Co. LLC.

4.2.3 Major ingredients: Cottonseed oil. Form: Liquid.

4.2.4 Storage condition: Room temperature.

5. Test system and condition:

5.1 Test system:

5.1.1 Fifteen Hartley strain guinea pigs in one sex and 300 g to 500 g in weight will be selected.

5.1.2 Source: National Laboratory Animal Center (No.128 Academia Road, Section 2, Nankang, Taipei City 115, Taiwan).

5.1.3 Quarantine and acclimation: The study will be conducted according to SuperLab standard operating procedure SOPA-303. All animals from the supplier will undergo quarantine and acclimate period for 7 days to ensure that no abnormal sign then can be used to test.

5.1.4 Animal identification: This study will be conducted according to SuperLab standard operating procedures SOPA-203 and SOPP-315. All animals will be housed in stainless steel cage. Designation cards are attached to the cage, and marking the animal ID, number, sex, source, IACUC No. and study director.

5.1.5 IACUC No.: 108-3aa.

5.2 Condition:

5.2.1 This study will be conducted according to SuperLab standard operating procedures SOPA-206.

5.2.2 Temperature: 21 ± 2°C. Relative humidity: 55 ± 15%. Light cycle: 12 hours light and 12 hours dark.

5.2.3 Housing: 5~10 animals per cage. Frequency of ventilation: 10 ~ 15 times/hour.

5.2.4 Feed: Guinea Pig Diet 5025 (Purina Mills, LLC, Missouri, U.S.A.).

5.2.5 Drinking water: Vitamin C will be mixed into RO water, provide with water bottle.

6. Procedure:

6.1 Test article extraction:

6.1.1 This study will be conducted according to SuperLab standard operating procedures SOPP-301.

6.1.2 Test article extracts are prepared according to ISO 10993-12: 2012 guideline, and the obtained extracts will be used for this study. Cottonseed oil is served as the extraction vehicle. The form of test article is sheet and extracts will be prepared with a ratio of 0.1 g/mL. The test article will be extracted for 72 ± 1 hours at 37 ± 1°C with constant

agitation of 100 rpm. Cottonseed oil without test article will be placed in the same condition and serve as control article.

6.2 Main study:

6.2.1 This study will be conducted according to SuperLab standard operating procedure SOPP-309.

6.2.2 Before the test, 15 guinea pigs are grouped randomly, and will divide into test group and control group. There are five animals in control group and ten animals in test group.

6.2.3 Before the test, 5 cm x 7 cm area over the intrascapular region of each test animal will be shaved. The shaved area shall not have any injury.

6.2.4 The treatment was divided into intradermal induction phase, topical induction phase and challenge phase. The detail information for applied article was tabulated in Table 1.

6.2.5 Intradermal induction phase: On day 1, make a pair of 0.1 mL of the following articles will be intradermal injections on the intrascapular of test animals.

a. Emulsion of Freund's complete adjuvant (FCA, Difco/BD) with extraction solvent in 1 : 1 volume ratio.

b. Test article extracts (test group) or control article (control group).

c. Emulsion of either test article extracts or the control article in a 1 : 1 volume ratio stable emulsion of FCA and extraction solvent.

6.2.6 On day 6, the intrascapular regions of the test animals will be shaved and pretreated with 10% sodium dodecyl sulfate (SDS, J.T. Baker, Inc.) in petrolatum.

6.2.7 Topical induction phase: On day 7, a gauze patch (2 cm x 4 cm) is fully loaded with 0.5 mL test article extracts or control article, and applied onto the intrascapular regions of test group and control group animals and held in contact with a non-adhesive bandage. The dressing will be left in place for 48 ± 1 hours.

6.2.8 Challenge phase: On day 22, an approximately 5 cm x 3 cm area on the upper flanks of test animals will be shaved. On day 23, a gauze patch loaded with 0.5 mL test article extracts will be applied to the flank of test group and control group animals. The dressing will be left in place for 24 ± 1 hours.

6.2.9 The skin reaction at 24 ± 1 and 48 ± 1 hours after the patch removal will be observed and recorded according to the grading table showed in "Magnusson and Kligman scale" (Table 2).

6.2.10 Animal weights are determined before the intradermal induction (Day 1) and at the end of the observation period (Day 26).

6.3 Evaluation criterion:

6.3.1 If grade of control group animals < 1, the grade of test group animals ≧ 1 indicates hypersensitivity reaction.

6.3.2 If grade of control group animals ≧ 1, then the reaction of test group animals which exceed most severe control reaction indicates hypersensitivity reaction.

6.3.3 The sensitization rate at 24 ± 1 and 48 ± 1 hours will be calculated separately. The higher sensitization rate will be used to classification the sensitizing capacity of the test article according to the "Scoring system of Kligman" (Table 3).

7. Study report:

The final report should including but not be limited to the following item:

7.1 The characteristics of the test article (or based on information provided by sponsor).

7.2 Test system, control article and procedure.

7.3 The data generation and analysis methods of the result.

7.4 Result and conclusion of the study.

7.5 Copy of the study protocol.

8. References:

8.1 Taiwan Food and Drug Administration (TFDA). Good Laboratory Practice for Nonclinical Laboratory Studies. 2019.

8.2 Chinese National Standards (CNS). Biological evaluation of medical devices-Part 10: tests for irritation and sensitization, CNS 14393-10, 2005.

8.3 Food and Drug Administration (FDA). Good Laboratory Practice for Nonclinical Laboratory Studies. 21 CFR, Part 58, 2018.

8.4 International Organization for Standardization (ISO). Biological evaluation of medical devices-part 10: Tests for irritation and skin sensitization, ISO 10993-10, 2010.

8.5 International Organization for Standardization (ISO). Biological evaluation of medical devices-part 12: Sample preparation and reference materials, ISO 10993-12, 2012.

8.6 Organisation for Economic Co-operation and Development (OECD). OECD series on principles of Good Laboratory Practice and Compliance Monitoring, Number 1. OECD Principles of Good Laboratory Practice. ENV/MC/CHEM (98) 17. 1998.

Table 1: Treatment strategy

Test period	Test group	Control group
Intradermal induction	a. Extraction solvent and FCA (1 : 1) b. Test article extracts c. Test article extracts and extraction solvent /FCA emulsion (1 : 1)	a. Extraction solvent and FCA (1 : 1) b. Control article c. Control article and extraction solvent /FCA emulsion (1 : 1)
Topical induction	Test article extracts	Control article
Challenge	Test article extracts	Test article extracts

Table 2: Magnusson and Kligman scale

Skin reaction	Grade scale
No visible change	0
Discrete or patchy erythema	1*
Moderate and confluent erythema	2*
Intense erythema and swelling	3*

* Positive reaction.

Table 3: Scoring system of Kligman

Sensitization rate (%)	Grade scale	Class
0 ~ 8	1	Weak
9 ~ 28	2	Mild
29 ~ 64	3	Moderate
65 ~ 80	4	Strong
81 ~ 100	5	Extreme

Appendix 1 : Expected archive file

Order	Name
1	Test service application form and attachment
2	Test article information Sheet
3	GLP laboratory Study director/ Quality assurance unit assignment
4	Safety data sheet
5	Study protocol
6	Test article inspection form
7	Test article extraction recording sheet
8	Guinea pig skin sensitization test recording sheet
9	Animal grouping recording sheet
10	Raw data
11	Record form
12	Final report

Appendix 6: IACUC approve

台美檢驗科技有限公司
實驗動物照護及使用委員會審查同意書
Affidavit of Approval of Animal Use Protocol
SUPER LABORATORY

動物實驗核准編號（IACUC No.）：108-3aa

計畫申請人： Principle Investigator（PI）	陳有倫 Yu-lun Chen	職 稱： Professional Title	副研究員 Associate Research Fellow
單 位： Corporation	台美檢驗科技有限公司 委託研究實驗室 Super Laboratory Co., Ltd. Contract Research Organization		
飼養/應用地點： Address of Animal Facility	台美實驗動物中心 Laboratory Animal Center of Super Laboratory Co., Ltd.		
計畫名稱： Protocol Title	天竺鼠皮膚過敏試驗(極大化法) –Thermoplastic sheet Guinea Pig Skin Sensitization Test (Maximization Method) –Thermoplastic sheet		

本計畫之「動物實驗申請表」經實驗動物照護及使用委員會■ 實質 □ 形式 審查通過
The animal experiment of this protocol approved by the Institutional Animal Care and Use Committee through ■substantive □formal review.

本計畫預定飼養應用之動物如下：
Expected feed animals of the protocol are shown in the following

動物種類 Animal Species	動物數量 Animal Amount	計畫執行期間 yyyy/mm/dd
天竺鼠(Narl: HARTLEY strain guinea pig)	15 隻天竺鼠 (15 Guinea pigs)	2019/10/08～2019/11/11

The animal use protocol listed below has been reviewed and approved by the Institutional Animal Care and Use Committee（IACUC）.

實驗動物照護及使用委員會召集人 日期

IACUC Chairman [signature] Date 2019/09/16,