Nelson Labs
A Sotera Health company

Sponsor:
Julia Madison
Armbrust Inc.
3813A Helios Way #290
Pflugerville, TX 78660

Kligman Maximization Test - ISO (GLP)

Test Article:	Model: USA Made Surgical Mask Part ID: AA-US-SURGICAL-01
Study Number:	1297407-S01.1 Amended
Study Received Date:	08 May 2020
Testing Facility:	Toxikon USA
Deviations:	None

Summary: Enclosed is the final report for the testing we coordinated for you. The information is retained by the testing laboratory.

Amendment Justification: The final report was amended to update the test article name, per sponsor request.

If you have any questions, please feel free to call or email any of our Subcontracting personnel at 801-290-7500 or subcontracting@nelsonlabs.com. Thank you for testing with Nelson Laboratories, LLC.

Mindy Schvaneveldt
Reviewed By Mindy L. Schvaneveldt, A.S.

30 NOV 2021
Amended Report Date

1297407-S01

TOXIKON
RIGHT. FROM THE START.SM

FINAL GLP REPORT: 20-01830-G1
AMENDED

Nelson Report Number: NL # 1297407

KLIGMAN MAXIMIZATION TEST – ISO

Test Article
Model: USA Made Surgical Mask
Part ID: AA-US-SURGICAL-01

21 CFR Part 58 Compliance
Good Laboratory Practice for Nonclinical Laboratory Studies

Final Report Date
7/16/2020

Amended Final Report Date
11/8/2021

Study Director
Sindhura Ramasahayam, Ph.D.

Sponsor
Nelson Laboratories, LLC
A Sotera Health Company
6280 South Redwood Road
Salt Lake City, UT 84123
USA

TABLE OF CONTENTS

TABLE OF CONTENTS (CONT.)

TABLE OF CONTENTS (CON'D)

List of Tables:

List of Appendices:

STUDY SUMMARY

The USP 0.9% Sodium Chloride for Injection (NaCl) and Cottonseed Oil (CSO) extracts of the test article, Model: USA Made Surgical Mask Part ID: AA-US-SURGICAL-01, elicited no reaction at the challenge (0% sensitization), following an induction phase. Therefore, as defined by the grading scale of the USP, the test article is classified as a non-sensitizer.

Based on the criteria of the protocol, the test article meets the requirements of the ISO 10993–10 guidelines.

QUALITY ASSURANCE STATEMENT

The Quality Assurance Unit conducted inspections on the following dates. The findings were reported to the Study Director and to Toxikon's Management.

The final report was reviewed to assure that the report accurately describes the methods and standard operating procedures. The reported results accurately reflect the raw data of the nonclinical study conducted per the protocol.

Phase	Inspection Date	Date Reported to Study Director	Date Reported to Management
DOSE ADMINISTRATION	6/17/2020	6/17/2020	6/17/2020
DATA	7/16/2020	7/16/2020	7/16/2020
FINAL REPORT	7/16/2020	7/16/2020	7/16/2020
AMENDED REPORT	11/8/2021	11/8/2021	11/8/2021

Stephanie McHugh

Stephanie McHugh, M.S.
Quality Assurance

11-8-21
Date

GLP COMPLIANCE STATEMENT

This study meets the technical requirements of the protocol.

This study was conducted in compliance with the current U.S. Food and Drug Administration 21 CFR, Part 58 Good Laboratory Practices for Nonclinical Laboratory Studies.

The sections of the regulations not performed by or under the direction of Toxikon Corporation, exempt from this Good Laboratory Practice Statement, included characterization and stability of the test article, 21 CFR, Part 58.105, and its mixture with carriers, 21 CFR, Part 58.113.

SIGNATURES

Signature Information	
Protocol Number	p19-1788-00d
Study Director	Sindhura Ramasahayam, Ph.D.
Study Supervisor	Allan Sleger, A.S., LAT
Company	Toxikon Corporation

VERIFICATION DATES

The study initiation day is the date the protocol is signed by the Study Director.

Verification Dates	
Test Article Receipt	5/22/2020
Project Log	5/22/2020
Study Initiation	5/26/2020
Study Completion	7/16/2020

Sindhura Ramasahayam, Ph.D.
Study Director

11/8/21
Date

Christopher Parker, M.S., MBA.

1.0 PURPOSE

The purpose of the study was to determine the potential allergenic or sensitizing capacity of the test article. The study was used as a procedure for screening of contact allergens in guinea pigs and extrapolating the results to humans, but it does not establish the actual risk of sensitization.

2.0 REFERENCES

The study was based upon the following references:

- ISO 10993–10, 2010, Biological Evaluation of Medical Devices – Part 10: Tests for Irritation and Skin Sensitization.
- United States Pharmacopeia 42, National Formulary 37, 2019. <1184> Sensitization Testing.
- Zhai, H., Wilhem, K–P, and H.I. Maibach, eds. Marzulli and Maibach's Dermatotoxicology. 7th edition Boca Raton: CRC Press, 2007. 443–444, 450–451.
- Magnusson, B. and A.M. Kligman. "The Identification of Contact Allergens by Animal Assay. The Guinea Pig Maximization Test." J. Invest. Dermatol. 52 (1969): 268–276.
- Magnusson, B. and A.M. Kligman, Allergic Contact Dermatitis in the Guinea Pig. Identification of Contact Allergens. Springfield, IL.: Thomas, 1970.
- ISO 10993–12, 2012, Biological Evaluation of Medical Devices – Part 12: Sample Preparation and Reference Materials.
- ISO/IEC 17025, 2017, General Requirements for the Competence of Testing and Calibration Laboratories.

3.0 COMPLIANCE

The study conformed to the current FDA 21 CFR, Part 58 – Good Laboratory Practice for Nonclinical Laboratory Studies.

4.0 IDENTIFICATION OF TEST AND CONTROL ARTICLES

The Sponsor supplied the following information on a Test Requisition Form or other correspondence, wherever applicable (excluding confidential or trade secret information). The Sponsor was responsible for all test article characterization data as specified in the GLP regulations.

4.1 Test Article:

Name: Model: USA Made Surgical Mask
Part ID: AA-US-SURGICAL-01

CAS/Code Number: Not Supplied by Sponsor (N/S)

Lot/Batch Number: 0506200101

Physical State: Insoluble

Color: N/S

Expiration Date: N/S

Density: Unknown

Stability: Unknown

Sterility: Not Sterile

Sterilization Conditions: N/S

Storage Condition: Room Temperature

Safety Precautions: Unknown

Intended Use: N/S

4.2 Negative Control Articles (Toxikon Supplied):

4.2.1 Negative Control Article 1:

Name: USP 0.9% Sodium Chloride for Injection (NaCl)

Toxikon QC Number: CSC-20-05-00113; CSC-20-06-00083; CSC-20-01-00111

4.2.2 Negative Control Article 2:

Name: Cottonseed Oil (CSO)

Toxikon QC Number: CSC-20-05-00112; CSC-20-06-00089

4.3 Positive Control Article (Toxikon Supplied):

Name: Dinitrochlorobenzene (DNCB)

Toxikon QC Number: CSC-17-12-00125

4.4 Reagents (Toxikon Supplied):

4.4.1 Reagent Name 1:

Name: Ethanol (EtOH)

Toxikon QC Number: CSC-19-10-00007

4.4.2 Reagent Name 2:

Name: USP Sterile Water for Injection (SWFI)

Toxikon QC Number: CSC-19-03-00140; CSC-20-04-00180

4.4.3 Reagent Name 3:

Name: Freund's Complete Adjuvant (FCA)

Toxikon QC Number: CSC-20-02-00172

4.4.4 Reagent Name 4:

Name: Sodium Dodecyl Sulfate (SDS)

Toxikon QC Number: CSC-18-01-00004

4.4.5 Reagent Name 5:

Name: Petrolatum

Toxikon QC Number: CSC-20-01-00078

5.0 IDENTIFICATION OF TEST SYSTEM

5.1 Animals Used in the Study:

Number and Species: 35 Hartley guinea pigs (*Cavia porcellus*)

Sex: 16 males and 19 females (females were non–pregnant and nulliparous)

Weight/Age Range: 326.5 – 453.9 grams / at least 26 days old (adult)
weighed to the nearest 0.1 g

Health Status: healthy, not previously used in other experimental procedures

Animal Purchase: Elm Hill Breeding Labs, Inc., Chelmsford, MA

Animal Identification: ear punch

Acclimation: minimum 5 days, under same conditions as for the actual test

Animal Selection: selected from larger pool and examined to ensure lack of adverse clinical signs

5.2 Animal Care and Maintenance:

Animal Room Target Temperature: 68 ± 5 °F

Animal Room Target Relative Humidity: 30–70%

Air Exchanges per Hour: a minimum of 10 changes per hour

Lights: 12–hour light/dark cycle, full spectrum fluorescent lights

Housing: group housed (per sex)

Cages: suspended stainless steel

Bedding: Alfa Cobs, ScottPharma Solutions, Marlborough, MA (non-contact)

Animal Rations: Teklad Hi–Fiber Guinea Pig Diet 2041, Envigo, Madison, WI, *ad libitum*

Water: tap water, *ad libitum*

There were no known contaminants present in the feed, water, or bedding expected to interfere with the test data.

The laboratory and animal rooms were maintained as limited–access facilities.

6.0 JUSTIFICATION OF TEST SYSTEM AND ROUTE OF ADMINISTRATION

6.1 Justification of Test System:

Historically, guinea pigs have been used in, and are generally regarded as the species of choice for, laboratory identification of skin allergens because the guidelines have no alternative (non–animal) methods.

6.2 Route of Administration:

Dermal application corresponds to a likely route of human exposure. The test article was extracted and administered *in vivo* through a medium compatible with the test system, as indicated on the Test Requisition Form.

7.0 EXPERIMENTAL DESIGN AND DOSAGE

7.1 Preparation of Test and Control Articles:

7.1.1 Preparation, Extraction Medium, and Extraction Conditions:

The test article (307.58 cm^2 as per Sponsor) was combined with 102.5 mL of vehicle following an ISO 10993–12 ratio of 3 cm^2 per 1 mL. The test article was separately extracted in NaCl and CSO at 50 ± 2 °C for 72 ± 2 hours under dynamic conditions. A total of 6 units of the test article was used for testing.

7.1.2 Addition of Extraction Medium:

Properly prepared test articles were filled with the appropriate medium.

7.1.3 Control Conditions:

An untreated control (blank) was prepared for parallel treatment and comparison. The untreated control was the extraction medium that was subjected to a similar incubation as used for the test article.

7.1.4 Extract Agitation:

Each extract was agitated vigorously prior to administration.

7.1.5 Extract Examination:

The test article appeared slight reddish/brown discoloration post extraction in NaCl for topical and challenge phases. However, the test article was unchanged by the CSO extraction procedure. The test article appeared unchanged by the extraction procedure. The extracts were clear and free of particulates and the color of the vehicle unchanged.

7.1.6 Extract Manipulation:

The extracts were not filtered, centrifuged, or pH adjusted.

7.1.7 Extract Storage:

After the completion of the extraction, the extracts were kept at room temperature and were used the same day as the extraction was completed. Fresh extracts were created for each dosing phase of the study. No storage of the extracts occurred.

7.1.8 Positive Control:

The positive control, DNCB, was dissolved in 95% EtOH to a final concentration of 0.1%.

7.1.9 Other Test Article Preparation:

All other test article preparation was as specified by the Sponsor.

7.2 Pre–Dose Procedure:

The test animals were weighed and the application sites were prepared by shaving the animals with clippers to render the test sites free of any hair. On Day 0 and Day 6, a 5 cm × 7 cm area (approximate) over the shoulder region was prepared. On Day 23, a 4 cm × 4 cm area (approximate) of the flank was prepared.

7.3 Dose Administration:

7.3.1 Distribution of Animals:

(1)	Experimental	(10 animals per extract)
(2)	Negative Controls	(5 animals per extract)
(3)	Positive Controls	(5 animals per study)

7.3.2 Primary Irritation Phase:

As the test article was extracted, a Primary Irritation Phase was not performed. The extracts were used at 100% concentration for the remainder (i.e., sensitization phase) of the study.

7.3.3 Induction/Intradermal Application:

Three pairs of intradermal injections were made so that on each side of the midline there was one row of three injections each. Injections 1 and 2 were given in close proximity to each other cranially. Injection 3 was located caudally. The injection sites (6) were just within the boundaries of a 2 cm × 4 cm patch, which were applied one week following the injections. The dosing solutions were as follows:

7.3.3.1 Experimental Group (Day 0):

(1) 0.1 mL FCA 1:1 with vehicle
(2) 0.1 mL test article extract
(3) 0.1 mL test article extract 1:1 with FCA

7.3.3.2 Negative Control Group (Day 0):

(1) 0.1 mL FCA 1:1 vehicle
(2) 0.1 mL blank vehicle
(3) 0.1 mL vehicle 1:1 with FCA

7.3.3.3 Positive Control Group (Day 0):

(1) 0.1 mL FCA 1:1 NaCl
(2) 0.1 mL 0.1% DNCB in 95% EtOH
(3) 0.1 mL 0.1% DNCB in 95% EtOH 1:1 with FCA

The extracts were used neat when preparing the dosing solutions/dilutions for injection.

7.3.4 Topical Application:

On Day 6, animals that showed no signs of irritation or corrosion after the induction application were pretreated with 10% Sodium Dodecyl Sulfate (SDS) in Petrolatum 24 hours before the topical induction application. If irritation or corrosion was present, no pretreatment occurred.

7.3.4.1 Experimental Group (Day 7):

Approximately 0.3 mL of test article extract was used to "saturate" a 2 cm x 4 cm piece of absorbable material. The patch was secured with an occlusive wrapping or guinea pig jacket and left in place for 48 hours.

7.3.4.2 Negative Control Group (Day 7):

The animals were exposed to the vehicle without the test article using the same procedure utilized for the experimental group.

7.3.4.3 Positive Control Group (Day 7):

The animals were exposed to 0.1% DNCB solution in 95% EtOH, using the same procedures applied to the experimental group.

The extracts were used neat when preparing the dosing solutions/dilutions.

7.3.5 Challenge Application:

7.3.5.1 Experimental Group (Day 23):

Extract "saturated" pieces of appropriate absorbable material, measuring 2 cm × 2 cm or, a Hill Top Chamber®, was secured to a previously unexposed area of the animal for 24 hours with the same type of occlusive bandage or guinea pig jacket that was used for the Topical Induction Application. Approximately 0.3 mL of test article extract, negative control vehicle, or 0.1% DNCB in 95% EtOH was used to "saturate" the 2 cm x 2 cm piece of absorbable material or the Hill Top Chamber®.

7.3.5.2 Negative Control Group (Day 23):

For the negative control animals, the patch was saturated with the vehicles without the test article.

7.3.5.3 Positive Control Group (Day 23):

For the positive control animals, the patch was saturated with 0.1% DNCB in 95% EtOH.

The extracts were used neat when preparing the dosing solutions/dilutions.

7.4 Post Dose Procedures:

7.4.1 Skin Readings (Day 25, 26, and 27):

After removing the patches on Day 24, the challenge sites were immediately cleaned. Skin readings were taken at 24, 48, and 72 hours after the challenge exposure period (Days 25, 26, and 27). The evaluation of skin reactions used the four–point scale described in Table 1.

Any animal showing a skin reaction score of 1 or greater (at any time point) was considered positive.

7.4.2 Clinical Observations:

Daily observations were made for clinical signs.

7.4.3 Scoring:

Using the Scoring System of Magnusson and Kligman (Table 1), the allergenic potential of a test article was classified based on the percent of responsive animals as described in Table 2:

TABLE 1:
Magnusson and Kligman Scale

Reaction	Grading Scale
No Visible Change	0
Discrete or Patch Erythema	1*
Moderate and Confluent Erythema	2*
Intense Erythema and Swelling	3*

* Denotes a positive response.

TABLE 2:
Sensitization Classification

Positives in Test Group (%)	Assigned Grade	Assigned Class
0	–	Nonsensitizer
< 10	1	Weak
10–30	2	Mild
31–60	3	Moderate
61–80	4	Strong
81–100	5	Extreme

The test results were interpreted based upon the percentage sensitization observed.
Note: Table 2 obtained from USP <1184>.

7.4.4 Mortality/Morbidity:

All animals survived the duration of the study.

7.4.5 Necropsy:

At the end of the observation period, animals were sacrificed by carbon dioxide (CO_2) inhalation.

8.0 EVALUATION CRITERIA

8.1 Evaluation of Data:

A sensitizer is a test article with which a positive response is observed in at least 10% of the test animals, as described in Table 2.

8.2 Control of Bias Statement:

The study as designed employed methodology to minimize uncertainty of measurement and to control bias for data collection and analysis, which included but was not limited to: concurrent control data, system suitability assessment, randomization, and method controls such as blanks and replicates.

9.0 RESULTS

9.1 Animal Weights (Table 3):

All animals were within the specified range of body weights (300–500 g) at the initiation of the study (Day 0).

9.2 Clinical Observations (Table 3):

No systemic signs of toxicity were observed in treated or control animals.

9.3 Sensitization (Table 4):

None of the treated (NaCl or CSO extracts) or negative control animals exhibited any reaction at the challenge (0% sensitized). The positive control article elicited discrete (Grade 1) reactions in all animals (100% sensitized).

10.0 CONCLUSION

The USP 0.9% Sodium Chloride for Injection (NaCl) and Cottonseed Oil (CSO) extracts of the test article, Model: USA Made Surgical Mask Part ID: AA-US-SURGICAL-01, elicited no reaction at the challenge (0% sensitization), following an induction phase. Therefore, as defined by the grading scale of the USP, the test article is classified as a non-sensitizer.

Based on the criteria of the protocol and these results, the test article meets the requirements of the ISO 10993–10 guidelines.

11.0 RECORDS

- Original raw data will be archived by Toxikon Corporation.
- The original final report and any report amendments will be archived by Toxikon Corporation.
- A copy of the final report and a copy of any protocol amendments or deviations will be forwarded to the Sponsor.
- The test article will be disposed by Toxikon.
- Test article retention upon study completion is the responsibility of the Sponsor.

12.0 CONFIDENTIALITY AGREEMENT

Per corporate policy, confidentiality shall be maintained in general, and in specific accordance with any relevant agreement specifically executed between Toxikon and the Sponsor.

13.0 ANIMAL WELFARE STATEMENT

The Sponsor assured that, to the best of their knowledge, this study did not unnecessarily duplicate previous testing and that there were no non–animal alternatives acceptable for the evaluation of this test article as defined by the protocol.

FCA-induced lesions at or near the FCA injection sites are an expected feature of the study protocol. FCA lesion sites judged to be large or excessive are monitored closely and reported

to Veterinary Staff. The large FCA lesion sites are also recorded and reported to an Institutional Official (IO) and Institutional Animal Care and Use Committee (IACUC).

Any evidence of pain and distress was reported to the Veterinarian and/or Study Director during the course of this study.

Toxikon strictly adheres to the following standards in maintaining the animal care and use program:

United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service, 9 CFR Ch. 1, Subchapter A–Animal Welfare.

"Guide for the Care and Use of Laboratory Animals," National Research Council, 2011.

Office for Laboratory Animal Welfare (OLAW), "Public Health Service Policy on Humane Care and Use of Laboratory Animals," Health Research Extension Act of 1985 (Public Law 99–158 November 20, 1985), revised 2015.

ISO 10993–2, 2006, Biological Evaluation of Medical Devices – Part 2: Animal Welfare Requirements.

AAALAC International accreditation.

14.0 UNFORESEEN CIRCUMSTANCES

Any unforeseen circumstances were documented in the raw data. However, no unforeseen circumstances that affected the integrity of the study were noted.

15.0 PROTOCOL AMENDMENTS/DEVIATIONS

There were no protocol amendments or deviations. No changes to the protocol were required.

TABLE 3:
Animal Weights and Clinical Observations

Group	Animal #	Sex	Body Weight (g) Day 0 6/17/2020	Signs of Toxicity*
Test Article (NaCl Extract)	1	Male	437.9	None
	2	Male	433.8	None
	3	Male	400.6	None
	4	Male	397.9	None
	5	Male	440.5	None
	6	Female	339.2	None
	7	Female	397.3	None
	8	Female	401.1	None
	9	Female	413.6	None
	10	Female	414.6	None
Test Article (CSO Extract)	11	Male	453.9	None
	12	Male	402.6	None
	13	Male	425.1	None
	14	Male	410.4	None
	15	Male	401.2	None
	16	Female	388.2	None
	17	Female	386.7	None
	18	Female	371.3	None
	19	Female	388.9	None
	20	Female	421.8	None
Negative Control (NaCl)	21	Male	430.4	None
	22	Male	439.1	None
	23	Female	399.3	None
	24	Female	385.7	None
	25	Female	398.2	None
Negative Control (CSO)	26	Male	390.7	None
	27	Male	397.3	None
	28	Female	413.8	None
	29	Female	372.8	None
	30	Female	326.5	None
Positive Control (DNCB)	31	Male	400.3	None
	32	Male	429.9	None
	33	Female	412.6	None
	34	Female	410.6	None
	35	Female	354.0	None

* Summary of Clinical Observations - Day 0 through Day 27, excluding skin reactions.

TABLE 4:
Skin Examination Data

Group	Animal #	Sex	Scores Day 25 7/12/2020	Scores Day 26 7/13/2020	Scores Day 27 7/14/2020	Percent Animals Sensitized	Allergenic Potential
Test Article (NaCl Extract)	1	Male	0	0	0	0%	Non Sensitizer
	2	Male	0	0	0		
	3	Male	0	0	0		
	4	Male	0	0	0		
	5	Male	0	0	0		
	6	Female	0	0	0		
	7	Female	0	0	0		
	8	Female	0	0	0		
	9	Female	0	0	0		
	10	Female	0	0	0		
Test Article (CSO Extract)	11	Male	0	0	0	0%	Non Sensitizer
	12	Male	0	0	0		
	13	Male	0	0	0		
	14	Male	0	0	0		
	15	Male	0	0	0		
	16	Female	0	0	0		
	17	Female	0	0	0		
	18	Female	0	0	0		
	19	Female	0	0	0		
	20	Female	0	0	0		
Negative Control (NaCl)	21	Male	0	0	0	0%	Non Sensitizer
	22	Male	0	0	0		
	23	Female	0	0	0		
	24	Female	0	0	0		
	25	Female	0	0	0		
Negative Control (CSO)	26	Male	0	0	0	0%	Non Sensitizer
	27	Male	0	0	0		
	28	Female	0	0	0		
	29	Female	0	0	0		
	30	Female	0	0	0		
Positive Control (DNCB)	31	Male	1	1	1	100%	Extreme Sensitizer
	32	Male	1	1	1		
	33	Female	1	1	1		
	34	Female	1	1	1		
	35	Female	1	1	1		

REPORT AMENDMENT PAGE

SPONSOR:

Nelson Laboratories, LLC
A Sotera Health Company
6280 South Redwood Road
Salt Lake City, UT 84123
USA

TESTING LABORATORY:

Toxikon Corporation
15 Wiggins Avenue
Bedford, MA 01730

Test Article Name: Model: USA Made Surgical Mask
Part ID: AA-US-SURGICAL-01

CAS/Code #: Not Supplied by Sponsor (N/S)

Lot/Batch Number: 0506200101

AMENDMENT:

Per Sponsor request, Test Article Name and Lot/Batch Number have been changed from:

Name: Lot # 6

Lot/Batch Number: 6

To:

Name: Model: USA Made Surgical Mask
Part ID: AA-US-SURGICAL-01

Lot/Batch Number: 0506200101

This amendment does not affect the integrity of the study.

AUTHORIZED PERSONNEL:

for

Sindhura Ramasahayam, Ph.D.
Study Director

11/8/21
Date

Christopher Baker, M.S., MB.A.

APPENDIX I:
Software Systems

Software	Use	21 CFR Part 11 Status	Publisher/ Vendor	Location
Adobe Acrobat 8, 9, and 10 Professional	Document preparation	Not Applicable	Adobe Systems, Inc.	San José, CA
Matrix Gemini 5.3.19	Laboratory Information Management System	Compliant	Autoscribe Limited	Reading, UK
MS Office 2010 Small Business Suite and MS Office 2013 Professional Suite and higher	Business software (suite includes Word, Excel, PowerPoint, Outlook, Publisher, Office tools)	Not Applicable	Microsoft Corporation	Redmond, WA
Rees Scientific Centron Presidio 3.0	Automated Environmental Monitoring	Compliant	Rees Scientific	Trenton, NJ
TMS Web 7	Document management for SOPs and training records management software system	Compliant	Quality Systems Integrators	Eagle, PA
Toxikon Protocol Manager 1.0	Protocol requisition application	Not Applicable	Toxikon Corporation	Bedford, MA