Institute for Industrial Research & Toxicology
औद्योगिक अनुसंधान एवं विष विज्ञान संस्थान
Registration No. 1303/C/09/CPCSEA (Ministry of Environment & Forests, Government of India)
GLP Certified, NABL (ISO/IEC 17025) Accredited and FDA Approved (Drug & Cosmetics)
AN ISO 9001 : 2015, ISO 14001 : 2015, ISO 45001 : 2018 Certified Organization

SKIN SENSITIZATION OF "GEL -ARTICLE NO - 10252203174" IN GUINEA PIG

(ISO 10993-10)

SPONSORED BY

NUTRIN GMBH & GALAXA PHARMA APS

CRO

MITTAL GLOBAL CLINICAL TRIAL SERVICES (MGCTS)

DATA REQUIREMENTS

ISO GUIDELINE 10993 PART 10 BIOLOGICAL EVALUATION OF MEDICAL DEVICES- TEST FOR IRRITATION AND SKIN SENSITIZATION

TESTING LABORATORY

INSTITUTE FOR INDUSTRIAL RESEARCH & TOXICOLOGY
F-209, U.P.S.I.D.C., M.G. ROAD,
GHAZIABAD-201302, INDIA

PROJECT NO	**:**	**202010-052**
REPORT NO	**:**	**IIRT/MD/202010/528/SST**
ULR NO	**:**	**TC661219000000528F**
DATE	**:**	**24-08-2020**

TEST COMPOUND : GEL " ARTICLE NO - 10252203174"
SPONSORED BY : NUTRIN GMBH & GALAXA PHARMA APS
CRO : MITTAL GLOBAL CLINICAL TRIAL SERVICES (MGCTS)
STUDY : SKIN SENSITIZATION IN GUINEA PIG
PROJECT NO. : 202010-052
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1. GLP COMPLIANCE STATEMENT

I, undersigned hereby declare that **Project No. 202010-052/Report No. IIRT/MD/202010/528/SST** Entitled **Skin Sensitization of "GEL -ARTICLE NO - 10252203174" in Guinea Pig** was performed in accordance with the standard operating procedures of ***Toxicology Department, Institute for Industrial Research & Toxicology***, as well as the approved study plan.

I hereby attest the authenticity of the study and guarantee that this report represents a true and accurate record of results obtained and shall not be reproduced except in full, without the written approval of the Sponsor.

This is certified that this study was conducted in compliance with ISO 10993-10 Biological Evaluation of Medical Devices following Good Laboratory Practice.

All original raw data including documentation, the draft report, a copy of the final report and the representative test item are archived in the archives at **Toxicology Department, Institute For Industrial Research & Toxicology.** There were no known circumstances that may have affected the quality or integrity of the study.

Dr. Amit Kumar Pal		**24-08-2020**
Study Director	**Signature**	**Date**

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2. STATEMENT BY TEST FACILITY MANAGEMENT

Management of the test facility has made available all the resources to the Study Director necessary for conduct of the present study in compliance with the principles of GLP.

I, the undersigned, take overall responsibility for the reliability of the work described in the report with compliance of Good laboratory Practice.

Laboratory In-charge

24-08-2020

Date

3. Quality Assurance Report

This **Project No.202010-522/Report No. IIRT/MD/202010/528/SST** entitled **Skin Sensitization of "GEL - ARTICLE NO - 10252203174" in Guinea Pig** (ISO Guideline: 10993-10) was subjected to inspections by the Quality Assurance Unit.

This report has been audited by the Quality Assurance Unit, and is considered to be an accurate account of the data generated and of the procedures followed. In each case, the outcome of QA evaluation is reported to the Study Director and Management on the day of evaluation. Audits of study documentation, and process inspections appropriate to the type and schedule of this study were as follows:

Standard Test Method Compliance Audit	:	21-07-2020
Test Material Preparation	:	22-07-2020
Date of Testing	:	24-07-202
Draft Report Audit	:	14-08-2020
Final Report Date	:	24-08-2020

Ms. Shalini Mishra		**24-08-2020**
Quality Assurance Head	**Signature**	**Date**

4. Summary

A Guinea Pig Maximization Test (GPMT) of **"GEL - ARTICLE NO - 10252203174"** was conducted to evaluate the potential for dermal contact sensitization. This study was conducted based on the requirements of the International Organization for Standardization 10993: Biological Evaluation of Medical Devices, Part 10: Tests for Irritation and Skin Sensitisation.

The test article was extracted in 0.9% sodium chloride USP (NSE-normal saline extract) for polar and cottonseed oil (COE) for non polar. NSE and COE was intradermally injected and occlusively patched to ten test Guinea pigs in an attempt to induce sensitization separately. The vehicle control (NSE and COE) was similarly injected and occlusively patched to five Guinea pigs. Following a recovery period, the test and control animals received a challenge patch of the test article extract and the reagent control. All sites were scored at 24 and 48 hours after patch removal.

Under the conditions of this study, the test article extract showed **no evidence** of causing dermal contact sensitization in the Guinea pig.

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5. Introduction

a. **Purpose**

A Guinea Pig Maximization Test of the material identified below was conducted to evaluate the potential to cause Skin sensitization.

b. Testing Guideline

The study was conducted based on the International Organization for Standardization 10993, Biological Evaluation of Medical Devices, Part 10- Test for Irritation and Skin Sensitisation.

c. GLP Compliance

The study was conducted in accordance with the provisions of the Good Laboratory Practice (GLP) Regulations, 21 CFR 58. A Statement of Quality Assurance Activities was issued with this report.

d. Duplication of Experimental Work

By signature on the protocol, the sponsor confirmed that the conduct of this study did not unnecessarily duplicate previous experiments.

6. Materials and Method

Sample Selection: We randomly selected the test material from the one of the gel tubes from the test samples sent by the manufacturer for the Biocompatibility test.

The test Article provided by the sponsor was identified and handled as follows.

Test Article Name	:	**"GEL - ARTICLE NO - 10252203174""**
Batch No.	:	rd20014
Storage Condition	:	Room Temperature
Control Article	:	0.9% Sodium Chloride Solution (USP) and Cottonseed oil
Control Article Stability testing	:	

Control Article

1. **Normal saline**

   (Sodium Chloride) Strength: Not applicable, No active components in formulation, Purity: Meets the requirement of USP Sodium chloride for injection and is certified as USP Grade. 0.9% NaCl ± 5.0% of Label claim, balance is water, Composition: Sodium Chloride /Water.

2. **Cottonseed oil**

   Cottonseed oil was procured from Sigma-Aldrich, Inc for the study

Preparation:

Ten gm sample from the tube supplied by sponsor of was taken out for extraction in normal saline and also in cottonseed oil separately. The tube was sealed as necessary to avoid loss of vehicle during extraction. The vehicle (without test article) was similarly prepared to serve as the reagent control.

Extraction condition:

Test article/Vehicle	Extraction condition
Normal Saline Control	(50 ± 2) °C for (72 ± 2) h
Normal Saline Extract (NSE)	(50 ± 2) °C for (72 ± 2) h
Cottonseed Oil Control	(50 ± 2) °C for (72 ± 2) h
Cottonseed Oil Extract (COE)	(50 ± 2) °C for (72 ± 2) h

Additional Materials:

Freund's Complete Adjuvant (FCA) was mixed 50:50 (v/v) with the chosen vehicle and used at induction I. A 10% (w/w) sodium lauryl sulphate (SLS) suspension in petrolatum was used for induction II. These materials were provided by the test facility.

7. Test System

Species : Guinea Pig (*Caviaporcellus*)

Source : Institute for Industrial Research & Toxicology

Strain : Dunkin Hartley

Sex : Female (nulliparous)

Body weight Range : 305 to 400gm

Acclimatization : Minimum 5 days

No. of Animal : Thirty

Identification Method : Ear Punch

a. Justification of Test System

The Dunkin Hartley albino Guinea pig has been used historically for sensitization studies (Magnusson and Kligman, 1972). The Guinea pig is believed to be the most sensitive animal model for this type of study.

8. Animal Management

Husbandry:

The Conditions conformed to IIRT Standard Operating System that is based on the "*Committee for the Purpose of Control and Supervision of Experiments on Animal"* guideline for the laboratory animal facility.

Food:

A commercially available guinea pig feed was provided daily.

Water:

Potable water was provided *ad libitum* through species appropriate water container or delivered through an automatic watering system.

Environmental conditions:

Air conditioned rooms with 10-15 air changes per hour, temperature between 22 ± 3^0C, relative humidity 40-60% and illumination cycle set to 12 hours artificial fluorescent light and 12 hours dark.

Selection: Only healthy previously unused, animal free from irritation or other dermatological lesions that could not interfere with test were selected.

Personal:

Associates involved were appropriately qualified and well trained.

Veterinary Care:

Standard veterinary medical care was provided in this study.

IAEC:

This procedure has been approved by IIRT Institutional Animal Ethical Committee and is reviewed at least annually by the same committee.

9. Method

On the first day of treatment, thirty Guinea pigs (twenty tests, ten controls) were weighed and identified. The fur over the dorsoscapular region was removed with an electric clipper. The animals were randomly grouped as mentioned in Table 1.

Table 1

Study designed

Group	No of Animals	Extracted test Article	Treated/Control
Group I	5	Normal Saline Control	Control
Group II	10	Normal Saline Extract (NSE)	Treated
Group III	5	Cottonseed oil Control	Control
Group IV	10	Cottonseed Oil Extract (COE)	Treated

Induction I: Intradermal Injections

The test animals were injected with the test article extract and the control animals were injected with the reagent control. To minimize tissue sloughing the "a" and "c" injections were slightly deeper than "b". Site "c" was injected slightly more caudal than site "b".Three rows of intradermal injections (two per row) were given to each animal within an approximate 2 cm x 4 cm boundary of the fur clipped area as illustrated below:

4cm

a.	a.
b.	b.
c.	c.

2 cm

Day 0 – Treated/Control groups

Control:

a. 0.1 mL of 50:50 (v/v) mixture of FCA and the vehicle.

b. 0.1 mL of vehicle (Normal Saline/Cottonseed Oil)

c. 0.1 mL of 1:1 mixture of the 50:50 (v/v) vehicle FCA mixture and the vehicle

Test Animals:

a. 0.1 mL of 50:50 (v/v) mixture ofFCA and the vehicle

b. 0.1 mL of Normal Saline Extract (NSE)/Cottonseed Oil Extract (COE)

c. 0.1 mL ofa 1:1 mixture of the 50:50 (v/v) vehicle FCA mixture and the test extract

Induction II: Topical Application

Day 5-7 -Treated and Control groups

The day prior to conducting the Induction II patch, the fur over the dorsoscapular region (same area as used during induction I)was removed with an electric clipper and the area was treated with a 10% sodium lauryl sulphate (SLS) suspension in petrolatumsufficient to coat the skin. The SLS suspension, applied to provoke a mild acute inflammation, was massaged into the skin overthe injection site. The area was left uncovered.

Day 6-8 - Treated and Control groups

At 7 days (±1 day) after completion of the Induction I injection, any remaining SLS residue was gently removed with a gauzepad. A 2 cm x 4 cm section of filter paper, saturated with approximately 0.3 mL of freshly prepared test article extract, was thentopically applied to the previously injected sites of the test animals. The control animals were similarly patched with the reagentcontrol. Each patch was secured with a nonreactive tape and the trunk of each animal was wrapped with an elastic bandage. At48 hours, the binders and patches were removed.

Challenge: Topical Application

Day 20-22 -Treated and Control groups

At 14 days (±1 day) after unwrap the Induction II wraps; the fur was removed from the sides and flank areas with an electric clipper. The non-woven cotton disk contained in a Hill Top Chamber was saturated with 0.3 mL of the test article extract or reagent control. The test extract was applied to the right flank of each animal and the control vehicle was applied to the left flank of each animal. Each patch was secured to the skin with semi occlusive hypo-allergenic adhesive tape. The trunk of each animal was wrapped with an elastic bandage to maintain well-occluded sites. At 24 hours, the wraps and patches were removed and anyresidue remaining at the sites was removed.

10. Laboratory Observations:

a) Animals were observed daily for general health.

b) Body weights were recorded at pre-treatment.

c) Observations for dermal reactions were conducted at 24 and 48 hours after challenge patch removal. Prior to each scoring interval, the sites were wiped with 35% isopropyl alcohol. If necessary, the fur was clipped from each site to facilitate scoring. Scores were recorded in accordance with the criteria shown below.

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

11. Evaluation and Statistical Analysis

The responses from the challenge phase were compared within the test animal group and between test and control conditions. Control conditions were the vehicle control solution on the test animals and the test extract, control solution and biomaterial on the control animals.

In the final analysis of data, consideration was given to the overall pattern, intensity, duration and character of reactions of the test as compared to the control conditions. Statistical manipulation of data was not applicable to this study. Grades of one or greater in the test group generally indicated sensitization, provided that grades of less than 1 were observed on the control animals. If grades of 1 or greater were noted on control animals, then the reactions of test animals that exceeded the most severe control reaction were considered to be due to sensitization.

Body Weights and Clinical Observations

Individual body weights are presented in Appendix No I. All animals appeared clinically normal throughout the study.

Dermal Observations

Individual results of dermal scoring for the challenge phase appear Appendix No II. No any evidence of sensitization was found.

12. Conclusion

From the conditions and result of above study, the normal saline extract and cottonseed oil extract test article i.e **Gel- Article No 10252203174** showed **no evidence** of causing Skin contact sensitization in the guinea pigs. Results and conclusions apply only to the test article tested.

Any extrapolation of these data to other samples is the sponsor's responsibility.

13. Proposed Date

The study dates were finalized by the study director following receipt of the sponsor approved protocol and appropriate material for the study. Initiation of the study was the date on which the study director signed the GLP protocol. Projected dates for starting the study (first treatment) and for the completion of the study (final report release) were provided to the sponsor (or representative of the sponsor)

14. Archive

The raw data, sample of the test substance, study report and other material were retained for nine year at Institute for Industrial Research and Toxicology, Ghaziabad on completion of the study.

15. References

- International Organization for Standardization (ISO) 10993, Biological Evaluation of Medical Devices Part-2, Animal Welfare Requirements (2006)
- International organization for standardization (ISO) 10993, Biological evaluation of Medical Devices Part-12, Sample Preparation and Reference Materials (2004)
- International organization for standardization (ISO) 10993, Biological evaluation of Medical Devices Part-10, Test for Irritation and Skin Sensitization (2010)
- Magnusson, B. and A. Kligman, *Allergic Contact Dermatitis in the Guinea Pig* (Spring field: C.H. Thomas, 1972).

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APPENDIX –I:

Individual Body Weights and Clinical Observations

Group	Treated/Control	Animal ID	Pretreatment Body Weight	Clinical Observation
I	Normal Saline Control	202010-052-01	342	Clinically Normal
		202010-052-02	332	Clinically Normal
		202010-052-03	312	Clinically Normal
		202010-052-04	358	Clinically Normal
		202010-052-05	347	Clinically Normal
II	Normal Saline Extract	202010-052-06	335	Clinically Normal
		202010-052-07	371	Clinically Normal
		202010-052-08	335	Clinically Normal
		202010-052-09	329	Clinically Normal
		202010-052-10	330	Clinically Normal
		202010-052-11	321	Clinically Normal
		202010-052-12	354	Clinically Normal
		202010-052-13	329	Clinically Normal
		202010-052-14	341	Clinically Normal
		202010-052-15	342	Clinically Normal

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APPENDIX –I:

Individual Body Weights and Clinical Observations (Continued)

Group	Treated/Control	Animal ID	Pretreatment Body Weight	Clinical Observation
III	Cottonseed Oil Control	202010-052-16	350	Clinically Normal
		202010-052-17	328	Clinically Normal
		202010-052-18	358	Clinically Normal
		202010-052-19	330	Clinically Normal
		202010-052-20	335	Clinically Normal
IV	Cottonseed Oil Extract	202010-052-21	328	Clinically Normal
		202010-052-22	325	Clinically Normal
		202010-052-23	355	Clinically Normal
		202010-052-24	356	Clinically Normal
		202010-052-25	335	Clinically Normal
		202010-052-26	351	Clinically Normal
		202010-052-27	366	Clinically Normal
		202010-052-28	310	Clinically Normal
		202010-052-29	333	Clinically Normal
		202010-052-30	357	Clinically Normal

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APPENDIX –II:

Dermal Reactions – Challenge

Group	Treated/Control	Animal ID	Hours Following Patch Removal			
			24 Hour Score		48 hour Score	
			Control	Test	Control	Test
I	Normal Saline Control	202010-052-01	0	0	0	0
		202010-052-02	0	0	0	0
		202010-052-03	0	0	0	0
		202010-052-04	0	0	0	0
		202010-052-05	0	0	0	0
II	Normal Saline Extract	202010-052-06	0	0	0	0
		202010-052-07	0	0	0	0
		202010-052-08	0	0	0	0
		202010-052-09	0	0	0	0
		202010-052-10	0	0	0	0
		202010-052-11	0	0	0	0
		202010-052-12	0	0	0	0
		202010-052-13	0	0	0	0
		202010-052-14	0	0	0	0
		202010-052-15	0	0	0	0

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APPENDIX –II:

Dermal Reactions – Challenge (Continued)

Group	Treated/Control	Animal ID	Hours Following Patch Removal			
			24 Hour Score		48 hour Score	
			Control	Test	Control	Test
III	Cottonseed Oil Control	202010-052-16	0	0	0	0
		202010-052-17	0	0	0	0
		202010-052-18	0	0	0	0
		202010-052-19	0	0	0	0
		202010-052-20	0	0	0	0
IV	Cottonseed Oil Extract	202010-052-21	0	0	0	0
		202010-052-22	0	0	0	0
		202010-052-23	0	0	0	0
		202010-052-24	0	0	0	0
		202010-052-25	0	0	0	0
		202010-052-26	0	0	0	0
		202010-052-27	0	0	0	0
		202010-052-28	0	0	0	0
		202010-052-29	0	0	0	0
		202010-052-30	0	0	0	0