STUDY TITLE

Liquid Calcium-Wet Ground:
Acute Dermal Toxicity in Rats

DATA REQUIREMENT

U.S. EPA Health Effects Test Guidelines, OPPTS 870.1200 (1998)

AUTHOR

Carolyn Lowe, LATG

STUDY COMPLETED ON

February 8, 2019

PERFORMING LABORATORY

Product Safety Labs

LABORATORY STUDY NUMBER

49590

SPONSOR

OR-CAL, Inc.
29454 Meadowview Road
Junction City, OR 97448

Product Safety Labs
2394 US Highway 130
Dayton, NJ 08810
USA

732-438-5100
psl@productsafetylabs.com
www.productsafetylabs.com

NO CLAIM OF CONFIDENTIALITY

No claim of confidentiality, on any basis whatsoever, is made for any information contained in this document. I acknowledge that information not designated as within the scope of FIFRA sec. 10(d)(1)(A), (B), or (C) and which pertains to a registered or previously registered pesticide is not entitled to confidential treatment and may be released to the public, subject to the provisions regarding disclosure to multinational entities under FIFRA 10(g).

Submitter:_____ Date:_____

Name of Signer:_____

Name of Company: OR-CAL, Inc.

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

Liquid Calcium-Wet Ground

This study meets the requirements of U.S. EPA GLP: Pesticide Programs (FIFRA): 40 CFR Part 160, 1989. Specific information related to the characterization of the test substance as received and tested is the responsibility of the study Sponsor (see Test Substance section).

Study Director: Carolyn Lowe Date: 2/8/2019

Name of Signer: Carolyn Lowe, LATG

Name of Company: Product Safety Labs

Sponsor:_____ Date:_____

Name of Signer:_____

Name of Company: OR-CAL, Inc.

Submitter:_____ Date:_____

Name of Signer:_____

Name of Company: OR-CAL, Inc.

QUALITY ASSURANCE STATEMENT

The Product Safety Labs' Quality Assurance Unit has reviewed this final study report to assure the report accurately describes the methods and standard operating procedures, and that the reported results accurately reflect the raw data of the study.

QA activities for this study:

QA Activity	Performed By	Date Conducted	Date Findings Reported To Study Director And Management
Protocol review	M. Zakrzewski; M. Zakrzewski	Apr 2, 2018[1]; Feb 5, 2019	Apr 2, 2018; Feb 5, 2019
Critical phase inspection: *Day 14 in-life observations and body weights*	B. Simms	Jan 23, 2019	Jan 23, 2019
Raw data audit	M. Zakrzewski	Feb 5, 2019	Feb 5, 2019
Draft report review	M. Zakrzewski	Feb 5, 2019	Feb 5, 2019

Final report reviewed by:

Maryann Zakrzewski
Quality Assurance Auditor
Product Safety Labs

February 8, 2019
Date

[1] PSL's "generic" protocol used for this study was reviewed by the Quality Assurance group on this date.

TABLE OF CONTENTS

LIQUID CALCIUM-WET GROUND: ACUTE DERMAL TOXICITY IN RATS

PROTOCOL NO.:	P322.RAT
STUDY NUMBER:	49590
SPONSOR:	OR-CAL, Inc. 29454 Meadowview Road Junction City, OR 97448
TEST SUBSTANCE IDENTIFICATION:	Liquid Calcium-Wet Ground Lot #: 11-19-18-B
DATE RECEIVED:	December 14, 2018
PSL REFERENCE NO.:	181214-1R
STUDY INITIATION DATE:	December 19, 2018
DATES OF TEST:	January 9 - January 23, 2019
NOTEBOOK NO.:	49590: pages 1-22

1. PURPOSE

To provide information on health hazards likely to arise from a short-term exposure to Liquid Calcium-Wet Ground by the dermal route.

2. SUMMARY

An acute dermal toxicity test was conducted with rats to determine the potential for Liquid Calcium-Wet Ground to produce toxicity from a single topical application. Under the conditions of this study, the single dose acute dermal LD_{50} of the test substance is greater than 5000 mg/kg of body weight in male and female rats.

Five thousand milligrams of the test substance per kilogram of body weight was applied to the skin of ten healthy rats for 24 hours. The animals were observed for mortality, signs of gross toxicity, and behavioral changes at least once daily for 14 days. Body weights were recorded prior to application (initial) and again on Days 7 and 14 (terminal). Necropsies were performed on all animals at terminal sacrifice.

All animals survived test substance administration and gained body weight during the study. Other than the dermal irritation noted at all dose sites between Days 1 and 11, there were no other adverse clinical findings recorded for any animal over the course of the study. No gross abnormalities were noted for any of the animals when necropsied at the conclusion of the 14-day observation period.

3. MATERIALS

A. Test Substance

The test substance, identified as Liquid Calcium-Wet Ground, Lot #: 11-19-18-B, was received on December 14, 2018, and was further identified with PSL Reference Number 181214-1R. The test substance was stored at room temperature. Documentation of the methods of synthesis, fabrication, or derivation of the test substance is retained by the Sponsor.

The following information related to the characterization of the test substance was provided by the Sponsor:

Composition: Calcium Carbonate - 52%, CAS #471-34-1
Inert ingredients - 48%

Physical Description: White liquid

pH: 8.6 - 9.6

Stability: Test substance was expected to be stable for the duration of testing.

Expiration Date: Not applicable

B. Animals

3.B.1 Number of Animals: 10

3.B.2 Sex: 5 Males and 5 Females. Females assigned to test were nulliparous and non-pregnant.

3.B.3 Species/Strain: Rats/Sprague-Dawley derived, albino

3.B.4 Age/Body Weight: Young adult (8-9 weeks)/males 253-272 grams and females 174-189 grams at experimental start.

3.B.5 Source: Received from SAGE® Labs on January 2, 2019.

4. METHODS

A. Husbandry

4.A.1 Housing: The animals were singly housed in caging which conforms to the size recommendations in the most recent *Guide for the Care and Use of Laboratory Animals* (Natl. Res. Council, 2011). Enrichment (e.g., toy) was placed in each cage and litter was changed at least once per week.

4.A.2 Animal Room Temperature and Relative Humidity Ranges: 20-22°C and 31-51%, respectively.

4.A.3 Animal Room Air Changes/Hour: 12. Airflow measurements are evaluated regularly and the records are kept on file at Product Safety Labs.

4.A.4 Photoperiod: 12-hour light/dark cycle

4.A.5 Acclimation Period: 7 days

4.A.6 Food: Envigo Teklad Global 16% Protein Rodent Diet® #2016. The diet was available *ad libitum*.

4.A.7 Water: Filtered tap water was supplied *ad libitum*.

4.A.8 Contaminants: There were no known contaminants reasonably expected to be found in the food or water at levels which would have interfered with the results of this study. Analyses of the food and water are conducted regularly and the records are kept on file at Product Safety Labs.

B. Identification

4.B.1 Cage: Each cage was identified with a cage card indicating at least the study number and identification and sex of the animal.

4.B.2 Animal: A number was allocated to each rat on receipt and a stainless steel ear tag bearing this number was attached to the animal. This number, together with a sequential animal number assigned to study 49590, constituted unique identification. Only the sequential animal number is presented in this report.

5. PROCEDURE

A. Preparation and Selection of Animals

On the day prior to application, a group of animals was prepared by clipping the dorsal area and the trunk. After clipping and prior to application, the animals were examined for health, weighed (initial) and the skin checked for any abnormalities. Ten healthy, naive rats (five males and five females; not previously tested) were selected for test.

B. Preparation of Test Substance

The test substance was applied as received and mixed well prior to use.

C. Dose Calculations

Individual doses were calculated based on the initial body weights, taking into account the density (determined by PSL) of the test substance.

D. Application of Test Substance

Five thousand milligrams of the test substance per kilogram of body weight was applied evenly over a dose area of approximately 2 inches x 3 inches (approximately 10% of the body surface) and covered with a 2-inch x 3-inch, 4-ply gauze pad. The gauze pad and entire trunk of each animal were then wrapped with 3-inch Durapore tape to avoid dislocation of the pad and to minimize loss of the test substance. The rats were then returned to their designated cages. The day of application was considered Day 0 of the study.

After 24 hours of exposure to the test substance, the pads were removed and the test sites were gently cleansed with a 3% soap solution followed by tap water and a clean paper towel to remove any residual test substance.

E. In-life Observations

The animals were observed for mortality, signs of gross toxicity, and behavioral changes during the first several hours after application, after patch removal, and then at least once daily thereafter for 14 days. Observations included gross evaluation of skin and fur, eyes and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and behavior pattern. Particular attention was directed to observation of tremors, convulsions, salivation, diarrhea, and coma (see Section 10).

F. Body Weights

Individual body weights of the animals were recorded prior to test substance application (initial) and again on Days 7 and 14 (terminal).

G. Necropsy

All rats were euthanized via CO_2 inhalation at the end of the 14-day observation period. Gross necropsies were performed on all animals. Tissues and organs of the thoracic and abdominal cavities were examined.

6. STATISTICAL ANALYSIS

Statistical analysis was limited to the calculation of the mean density value for dosing.

7. STUDY CONDUCT

This study was conducted at Product Safety Labs' (PSL) test facility at 2394 US Highway 130, Dayton, New Jersey 08810. The Study Director for this study was Carolyn Lowe, LATG. The primary scientist for this study was Matthew Sorber, BS, with contributions from Cindy Bodnar, Jonathan Bozzick, AS, Amber Norton, BS, Katherine Sibley, BS, and Shannon Stevens, BS. This study was conducted to comply with the Good Laboratory Practice (GLP) regulations as defined in:

- U.S. EPA GLP: Pesticide Programs (FIFRA): 40 CFR Part 160, 1989

and based on the following testing guideline:

- U.S. EPA Health Effects Test Guidelines, OPPTS 870.1200 (1998)

8. QUALITY ASSURANCE

The final report was audited for agreement with the raw data records and for compliance with the protocol, Product Safety Labs Standard Operating Procedures and appropriate Good Laboratory Practice Standards. Dates of inspections and audits performed during the study and the dates of reporting of the inspection and audit findings to the Study Director and Facility Management are presented in the Quality Assurance Statement.

9. AMENDMENTS TO THE PROTOCOL

None.

10. DEVIATION FROM THE PROTOCOL

The protocol states that all animals will be observed during the first several hours post dosing on Day 0. Due to an oversight, the Scientist only performed the one hour clinical observations post dosing for all animals. All animals were observed to be active and healthy one hour after dosing and the day after the missed observations, therefore it is likely that the animals were active and healthy at the time of the missed clinical observations. Furthermore, in accordance with Product Safety Labs' SOP, the animals were observed for viability in the morning and afternoon. This deviation had no impact on the overall results of the study.

11. FINAL REPORT AND RECORDS TO BE MAINTAINED

Information on care of the test system, equipment maintenance and calibration, storage, usage, and disposition of the test substance, and all other records that would demonstrate adherence to

the protocol will be maintained. Facility records which are not specific to the subject study will be maintained by the testing facility and archived according to PSL SOP.

The original, signed final report together with the original protocol, raw data, and associated documents, will be sent to the Sponsor. Electronic copies of the documents will be retained by PSL.

Any electronic raw data generated will be maintained on-site in accordance with GLP archiving procedures.

12. RESULTS

Individual body weights and doses are presented in Table 1. Individual in-life and necropsy observations are presented in Tables 2 and 3, respectively.

All animals survived test substance administration and gained body weight during the study. Other than the dermal irritation noted at all dose sites between Days 1 and 11, there were no other adverse clinical findings recorded for any animal over the course of the study. No gross abnormalities were noted for any of the animals when necropsied at the conclusion of the 14-day observation period.

13. CONCLUSION

Under the conditions of this study, the single dose acute dermal LD_{50} of Liquid Calcium-Wet Ground is greater than 5000 mg/kg of body weight in male and female rats.

14. REFERENCES

National Research Council. (2011). *Guide for the Care and Use of Laboratory Animals (8^{th} ed.)*. Washington, DC: The National Academies Press.

SIGNATURE

Liquid Calcium-Wet Ground

I, the undersigned, declare that the methods, results and data contained in this report faithfully reflect the procedures used and raw data collected during the study.

Carolyn Lowe, LATG
Study Director
Product Safety Labs

2/8/2019
Date

TABLE 1: INDIVIDUAL BODY WEIGHTS AND DOSES

Animal No.	Sex	Body Weight (g)			Dose[1]
		Initial	Day 7	Day 14	mL
3201	M	257	290	333	0.84
3202	M	253	281	313	0.83
3203	M	270	315	349	0.88
3204	M	272	296	329	0.89
3205	M	260	285	313	0.85
3206	F	189	199	228	0.62
3207	F	174	189	203	0.57
3208	F	186	204	224	0.61
3209	F	189	206	224	0.62
3210	F	174	193	207	0.57

[1] The test substance was applied as received. Density - 1.531 g/mL.

TABLE 2: INDIVIDUAL IN-LIFE OBSERVATIONS

Animal Number	Animal Sex	Dose Level (mg/kg)	Observation	Color	Day of Observation (x=observation is present)														
					0(1 hr)[1]	1	2	3	4	5	6	7	8	9	10	11	12	13	14
3201	M	5000	Active and healthy		x						x	x	x	x	x	x	x	x	x
			Staining at the dose site	Yellow		x	x	x	x	x	x	x	x	x	x	x	x	x	x
			Erythema at the dose site			x	x	x	x	x									
3202	M	5000	Active and healthy		x					x	x	x	x	x	x	x	x	x	x
			Staining at the dose site	Yellow		x	x	x	x	x	x	x	x	x	x	x	x	x	x
			Erythema at the dose site			x	x	x	x										
3203	M	5000	Active and healthy		x					x	x	x	x	x	x	x	x	x	x
			Staining at the dose site	Yellow		x	x	x	x	x	x	x	x	x	x	x	x	x	x
			Erythema at the dose site			x	x	x	x										
3204	M	5000	Active and healthy		x					x	x	x	x	x	x	x	x	x	x
			Staining at the dose site	Yellow		x	x	x	x	x	x	x	x	x	x	x	x	x	x
			Erythema at the dose site			x	x	x	x										

[1] See Section 10

TABLE 2 (cont.): INDIVIDUAL IN-LIFE OBSERVATIONS

Animal Number	Animal Sex	Dose Level (mg/kg)	Observation	Color	Day of Observation (x=observation is present)														
					0(1 hr)[1]	1	2	3	4	5	6	7	8	9	10	11	12	13	14
3205	M	5000	Active and healthy		x					x	x	x	x	x	x	x	x	x	x
			Staining at the dose site	Yellow		x	x	x	x	x	x	x	x	x	x	x	x	x	x
			Erythema at the dose site			x	x	x	x										
			Desquamation at the dose site					x	x										
3206	F	5000	Active and healthy		x												x	x	x
			Staining at the dose site	Yellow		x	x	x	x	x	x	x	x	x	x	x	x	x	x
			Erythema at the dose site			x	x	x	x	x	x	x							
			Eschar at the dose site					x	x	x	x	x	x	x	x	x			
3207	F	5000	Active and healthy		x												x	x	x
			Staining at the dose site	Yellow		x	x	x	x	x	x	x	x	x	x	x	x	x	x
			Erythema at the dose site			x	x	x	x	x	x	x	x						
			Desquamation at the dose site					x	x	x	x	x	x	x	x	x			
3208	F	5000	Active and healthy		x					x	x	x	x	x	x	x	x	x	x
			Staining at the dose site	Yellow		x	x	x	x	x	x	x	x	x	x	x	x	x	x
			Erythema at the dose site			x	x												
			Desquamation at the dose site					x	x										

[1] See Section 10.

TABLE 2 (cont.): INDIVIDUAL IN-LIFE OBSERVATIONS

Animal Number	Animal Sex	Dose Level (mg/kg)	Observation	Color	Day of Observation (x=observation is present)														
					0(1 hr)[1]	1	2	3	4	5	6	7	8	9	10	11	12	13	14
3209	F	5000	Active and healthy		x									x	x	x	x	x	x
			Staining at the dose site	Yellow		x	x	x	x	x	x	x	x	x	x	x	x	x	x
			Erythema at the dose site			x	x	x	x	x	x	x							
			Eschar at the dose site					x	x	x									
			Desquamation at the dose site					x	x	x	x	x	x						
3210	F	5000	Active and healthy		x												x	x	x
			Staining at the dose site	Yellow		x	x	x	x	x	x	x	x	x	x	x	x	x	x
			Erythema at the dose site			x	x	x	x	x	x	x							
			Eschar at the dose site							x	x	x	x	x	x	x			
			Desquamation at the dose site					x	x										

[1] See Section 10.

TABLE 3: INDIVIDUAL NECROPSY OBSERVATIONS

Animal Number	Animal Sex	Dose Level (mg/kg)	Organ / Tissue	Observation
3201	M	5000	All tissues and organs	No gross abnormalities
3202	M	5000	All tissues and organs	No gross abnormalities
3203	M	5000	All tissues and organs	No gross abnormalities
3204	M	5000	All tissues and organs	No gross abnormalities
3205	M	5000	All tissues and organs	No gross abnormalities
3206	F	5000	All tissues and organs	No gross abnormalities
3207	F	5000	All tissues and organs	No gross abnormalities
3208	F	5000	All tissues and organs	No gross abnormalities
3209	F	5000	All tissues and organs	No gross abnormalities
3210	F	5000	All tissues and organs	No gross abnormalities