STUDY TITLE

Liquid Calcium-Dry Ground:
Acute Oral Toxicity – Up-And-Down Procedure in Rats

DATA REQUIREMENT

U.S. EPA Health Effects Test Guidelines, OPPTS 870.1100 (2002)

AUTHOR

Carolyn Lowe, LATG

STUDY COMPLETED ON

July 26, 2019

PERFORMING LABORATORY

Product Safety Labs

LABORATORY STUDY NUMBER

49591

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-Dry 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: 7/26/2019

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 11, 2019	Apr 2, 2018; Feb 11, 2019
Critical phase inspection: *Day 14 in-life observations and body weight for Animal #3102 & 3103*	B. Simms	Feb 1, 2019	Feb 1, 2019
Raw data audit	M. Zakrzewski	Feb 11, 2019	Feb 11, 2019
Draft report review	M. Zakrzewski	Feb 11, 2019	Feb 11, 2019

Final report reviewed by:

Barbara Simms
Quality Assurance Auditor
Product Safety Labs

7/20/19
Date

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

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LIQUID CALCIUM-DRY GROUND: ACUTE ORAL TOXICITY – UP-AND-DOWN PROCEDURE IN RATS

PROTOCOL NO.:	P320.UDP
STUDY NUMBER:	49591
SPONSOR:	OR-CAL, Inc. 29454 Meadowview Road Junction City, OR 97448
TEST SUBSTANCE IDENTIFICATION:	Liquid Calcium-Dry Ground Formula: 11-19-18-F
DATE RECEIVED:	December 14, 2018
PSL REFERENCE NO.:	181214-2R
STUDY INITIATION DATE:	December 19, 2018
DATES OF TEST:	January 8 - February 1, 2019
NOTEBOOK NO.:	49591: pages 1-21

1. PURPOSE

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

2. SUMMARY

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

An initial limit dose of 5000 mg/kg was administered to one healthy female rat by oral gavage. Due to the absence of mortality in this animal, two additional females received the same dose level, simultaneously. Since these animals survived, no additional animals were tested. Females were selected for the test because they are frequently more sensitive to the toxicity of test compounds than males. All animals were observed for mortality, signs of gross toxicity, and behavioral changes at least once daily for 14 days after dosing. Body weights were recorded prior to administration (initial) and again on Days 7 and 14 (terminal) following dosing. Necropsies were performed on all animals at terminal sacrifice.

All animals survived test substance administration, gained body weight, and appeared active and healthy during the study. There were no signs of gross toxicity, adverse clinical effects, or abnormal behavior. 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-Dry Ground, Formula: 11-19-18-F, was received on December 14, 2018, and was further identified with PSL Reference Number 181214-2R. 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: 3

3.B.2 Sex: Female, nulliparous and non-pregnant.

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

3.B.4 Age/Body Weight: Young adult (10-12 weeks)/208-229 grams at experimental start.

3.B.5 Source: Received from SAGE® Labs on December 19, 2018.

4. METHODS

A. Husbandry

4.A.1 Housing: The animals were 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). Animals were group housed, except on the day of administration, at which time they were single housed and until the animals were deemed acceptable, based on observations, to return to group housing. 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: 19-22°C and 31-63%, 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: 20 or 30 days

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

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, dose level, 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 rat. This number, together with a sequential animal number assigned to study number 49591, constituted unique identification. Only the sequential animal number is presented in this report.

5. PROCEDURE

A. Selection of Animals

Prior to each dosing, experimentally naive rats were fasted overnight by removing the feed from their cages. During the fasting period, the rats were examined for health and weighed (initial). Three healthy, naive female rats (not previously tested) were selected for test.

B. Preparation of Test Substance

The test substance was administered 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. Dosing

The test substance was administered to the stomach using a stainless steel ball-tipped gavage needle attached to an appropriate syringe. Following administration, each animal was returned to its designated cage. Feed was replaced approximately 3-4 hours after dosing.

Individual animals were dosed as follows:

Limit Test

Dosing Sequence	Animal No.	Dose Level (mg/kg)	Short-Term Outcome	Long-Term Outcome
1	3101	5000	S	S
2	3102		S	S
3	3103		S	S

S – Survival

E. In-life Observations

The animals were observed for mortality, signs of gross toxicity, and behavioral changes approximately 30 minutes post-dosing, during the first several hours post-dosing and at least once daily thereafter for 14 days after dosing. 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.

F. Body Weights

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

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 Harry Maselli, ALAT, with contributions from Jonathan Bozzick, AS, Amber Norton, BS, Xiomara Portuguez, BS, and Katherine Sibley, 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 870.1100 (2002)

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. DEVIATIONS FROM THE PROTOCOL

None.

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, gained body weight, and appeared active and healthy during the study. There were no signs of gross toxicity, adverse clinical effects, or abnormal behavior. 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 acute oral LD_{50} of Liquid Calcium-Dry Ground is greater than 5000 mg/kg of body weight in 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-Dry 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

Carolyn Lowe, LATG
Study Director
Product Safety Labs

7/26/2019

Date

TABLE 1: INDIVIDUAL BODY WEIGHTS AND DOSES

Animal No.	Sex	Dose Level (mg/kg)	Body Weight (g)			Dose[1]
			Initial	Day 7	Day 14	mL
3101	F	5000	208	234	247	0.67
3102	F		228	256	266	0.73
3103	F		229	249	253	0.74

[1] The test substance was administered as received. Density – 1.559 g/mL.

TABLE 2: INDIVIDUAL IN-LIFE OBSERVATIONS

Animal Number	Animal Sex	Dose Level (mg/kg)	Observation	Day of Observation (x=observation is present)														
				0(0.5 hr)	0(3 hrs)	1	2	3	4	5	6	7	8	9	10	11	12	13	14
3101	F	5000	Active and healthy	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
3102	F	5000	Active and healthy	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
3103	F	5000	Active and healthy	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x

TABLE 3: INDIVIDUAL NECROPSY OBSERVATIONS

Animal Number	Animal Sex	Dose Level (mg/kg)	Organ / Tissue	Observation
3101	F	5000	All tissues and organs	No gross abnormalities
3102	F	5000	All tissues and organs	No gross abnormalities
3103	F	5000	All tissues and organs	No gross abnormalities