STUDY TITLE

Solbere:
Acute Dermal Toxicity in Rats

DATA REQUIREMENT

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

AUTHOR

Melissa Slonina, BS

STUDY COMPLETED ON

February 11, 2019

PERFORMING LABORATORY

Product Safety Labs

LABORATORY STUDY NUMBER

49550

SPONSOR

CO2 Solved, LLC
30301 Riverview Drive
Junction City, OR 97448

Product Safety Labs
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Dayton, NJ 08810
USA

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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: CO2 Solved, LLC

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

Solbere

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: M. Slonina Date: 2/11/19

Name of Signer: Melissa Slonina, BS

Name of Company: Product Safety Labs

Sponsor:_____ Date:_____

Name of Signer:_____

Name of Company: CO2 Solved, LLC

Submitter:_____ Date:_____

Name of Signer:_____

Name of Company: CO2 Solved, LLC

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; B. Simms	Apr 2, 2018[1]; Jan 31, 2019	Apr 2, 2018; Jan 31, 2019
Critical phase inspection: *Day 14 in-life observations and body weights*	B. Simms	Jan 16, 2019	Jan 16, 2019
Raw data audit	B. Simms	Jan 31, 2019	Jan 31, 2019
Draft report review	B. Simms	Jan 31, 2019	Jan 31, 2019

Final report reviewed by:

Barbara Simms
Quality Assurance Auditor
Product Safety Labs

02/11/2019
Date

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

TABLE OF CONTENTS

SOLBERE: ACUTE DERMAL TOXICITY IN RATS

PROTOCOL NO.:	P322.RAT
STUDY NUMBER:	49550
SPONSOR:	CO2 Solved, LLC 30301 Riverview Drive Junction City, OR 97448
TEST SUBSTANCE IDENTIFICATION:	Solbere Formula: 11-30-18-F Dry Ground
DATE RECEIVED:	December 11, 2018
PSL REFERENCE NO.:	181211-4R
STUDY INITIATION DATE:	December 14, 2018
DATES OF TEST:	January 2 - January 16, 2019
NOTEBOOK NO.:	49550: pages 1-20

1. PURPOSE

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

2. SUMMARY

An acute dermal toxicity test was conducted with rats to determine the potential for Solbere 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 the dose sites on Day 1, 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 Solbere, Formula: 11-30-18-F Dry Ground, was received on December 11, 2018, and was further identified with PSL Reference Number 181211-4R. 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: 11 HX-Calcium Carbonate - 71.94%, CAS #471-34-1
TiO_2 - 3.5%, CAS #13463-67-7
Inert ingredients - 24.56%

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 246-268 grams and females 158-173 grams at experimental start.

3.B.5 Source: Received from SAGE® Labs on December 26, 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 application, at which time they were singly housed 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: 20-21°C and 36-53%, 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 49550, 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.

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 Melissa Slonina, BS. The primary scientist for this study was Matthew Sorber, BS, with contributions from Cindy Bodnar, Harry Maselli, ALAT, Amber Norton, BS, Matthew Notta, 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. 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 and gained body weight during the study. Other than the dermal irritation noted at all the dose sites on Day 1, 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 Solbere 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

Solbere

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.

M Slonina
Melissa Slonina, BS
Study Director
Product Safety Labs

2/11/19
Date

TABLE 1: INDIVIDUAL BODY WEIGHTS AND DOSES

Animal No.	Sex	Body Weight (g)			Dose[1]
		Initial	Day 7	Day 14	mL
3201	M	246	286	317	0.79
3202	M	263	296	345	0.84
3203	M	264	309	327	0.85
3204	M	268	303	345	0.86
3205	M	264	301	336	0.85
3206	F	158	174	202	0.51
3207	F	165	185	204	0.53
3208	F	169	199	215	0.54
3209	F	173	191	219	0.56
3210	F	167	185	213	0.54

[1] The test substance was applied as received. Density - 1.560 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)	0(3 hrs)	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	x	x	x	x	x
			Erythema at the dose site				x													
			Staining at the dose site	Yellow			x	x	x	x	x	x	x	x	x	x	x	x	x	x
3202	M	5000	Active and healthy		x	x		x	x	x	x	x	x	x	x	x	x	x	x	x
			Erythema at the dose site				x													
			Staining at the dose site	Yellow			x	x	x	x	x	x	x	x	x	x	x	x	x	x
3203	M	5000	Active and healthy		x	x		x	x	x	x	x	x	x	x	x	x	x	x	x
			Erythema at the dose site				x													
			Staining at the dose site	Yellow			x	x	x	x	x	x	x	x	x	x	x	x	x	x
3204	M	5000	Active and healthy		x	x		x	x	x	x	x	x	x	x	x	x	x	x	x
			Erythema at the dose site				x													
			Staining at the dose site	Yellow			x	x	x	x	x	x	x	x	x	x	x	x	x	x
3205	M	5000	Active and healthy		x	x		x	x	x	x	x	x	x	x	x	x	x	x	x
			Erythema at the dose site				x													
			Staining at the dose site	Yellow			x	x	x	x	x	x	x	x	x	x	x	x	x	x

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)	0(3 hrs)	1	2	3	4	5	6	7	8	9	10	11	12	13	14
3206	F	5000	Active and healthy		x	x		x	x	x	x	x	x	x	x	x	x	x	x	x
			Erythema at the dose site				x													
			Staining at the dose site	Yellow			x	x	x	x	x	x	x	x	x	x	x	x	x	x
3207	F	5000	Active and healthy		x	x		x	x	x	x	x	x	x	x	x	x	x	x	x
			Erythema at the dose site				x													
			Staining at the dose site	Yellow			x	x	x	x	x	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	x	x	x	x
			Erythema at the dose site				x													
			Staining at the dose site	Yellow			x	x	x	x	x	x	x	x	x	x	x	x	x	x
3209	F	5000	Active and healthy		x	x		x	x	x	x	x	x	x	x	x	x	x	x	x
			Erythema at the dose site				x													
			Staining at the dose site	Yellow			x	x	x	x	x	x	x	x	x	x	x	x	x	x
3210	F	5000	Active and healthy		x	x		x	x	x	x	x	x	x	x	x	x	x	x	x
			Erythema at the dose site				x													
			Staining at the dose site	Yellow			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
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