STUDY TITLE

Atmosphere:
Primary Skin Irritation in Rabbits

DATA REQUIREMENT

U.S. EPA Health Effects Test Guidelines, OPPTS 870.2500 (1998)

AUTHOR

Carolyn Lowe, LATG

STUDY COMPLETED ON

July 9, 2019

PERFORMING LABORATORY

Product Safety Labs

LABORATORY STUDY NUMBER

50237

SPONSOR

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Submitter: Mel Jones Date: June 28, 2019

Name of Signer: Mel Jones

Name of Company: Atmosphere Global LLC

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

Atmosphere

This study meets the requirements of U.S. EPA GLP: Pesticide Programs (FIFRA): 40 CFR Part 160, 1989, U.S. EPA GLP: Toxic Substances Control Act (TSCA): 40 CFR Part 792, 1989, and U.S. FDA GLP: 21 CFR Part 58, 1987. 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: 7/9/2019

Name of Signer: Carolyn Lowe, LATG

Name of Company: Product Safety Labs

Sponsor: Mel Jones Date: June 28, 2019

Name of Signer: Mel Jones

Name of Company: Atmosphere Global LLC

Submitter: Shane Ormsby Date: 27 June 2019

Name of Signer:

Name of Company: Atmosphere Global 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	R. Krick; B. Simms	Jun 26, 2018[1]; Jun 20, 2019	Jun 26, 2018; Jun 20, 2019
Critical phase inspection: *24-hour scoring*	M. Zakrzewski	Apr 25, 2019	Apr 25, 2019
Raw data audit	B. Simms	Jun 20, 2019	Jun 20, 2019
Draft report review	B. Simms	Jun 20, 2019	Jun 20, 2019

Final report reviewed by:

Ronda S. Krick for BS
Barbara Simms
Quality Assurance Auditor
Product Safety Labs

Date July 9, 2019

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

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ATMOSPHERE: PRIMARY SKIN IRRITATION IN RABBITS

PROTOCOL NO.:	P326
STUDY NUMBER:	50237
SPONSOR:	Atmosphere Global LLC 55 W Goethe St., Unit 1241 Chicago, IL 60610
TEST SUBSTANCE IDENTIFICATION:	Atmosphere Lot #: TC0408191
DATE RECEIVED:	April 11, 2019
PSL REFERENCE NO.:	190411-1D
STUDY INITIATION DATE:	April 16, 2019
DATES OF TEST:	April 24 - May 22, 2019
NOTEBOOK NO.:	50237: pages 1-30

1. PURPOSE

To provide information on the skin irritation likely to arise from a single topical exposure to Atmosphere.

2. SUMMARY

A primary skin irritation test was conducted with rabbits to determine the potential for Atmosphere to produce irritation after a single topical application. Under the conditions of this study, the test substance is classified as moderately irritating to the skin.

Five-tenths of a milliliter of the test substance was applied to the skin of three healthy rabbits for 4 hours. Following exposure, dermal irritation was evaluated by the Draize method of scoring (Draize, Woodard, & Calvery, 1944; see Table 5).

For the first 72 hours of patch removal, all three treated sites exhibited very slight to well-defined erythema and very slight to slight edema. The overall incidence and severity of irritation decreased gradually with time. Hyperkeratosis was noted at all three sites between Days 7 and 21. Eschar was noted at two sites between Days 10 and/or Day 21. Dark discoloration was noted at all sites between 30-60 minutes and/or Day 7. All animals were free of erythema and edema by Day 28 (study termination).

The incidence, severity and reversibility of irritation are detailed below:

	Incidence of Irritation	
Time After Patch Removal	**Erythema**	**Edema**
30-60 minutes	3/3	3/3
24 hours	3/3	3/3
48 hours	3/3	3/3
72 hours	3/3	3/3
Day 7	3/3	3/3
Day 10	0/3	3/3
Day 14	0/3	3/3
Day 17	0/3	3/3
Day 21	0/3	2/3
Day 28	0/3	0/3

Time After Patch Removal	Severity of Irritation – Mean Score
30-60 minutes	3.3
24 hours	3.4
48 hours	3.0
72 hours	3.7
Day 7	2.0
Day 10	1.0
Day 14	1.0
Day 17	1.0
Day 21	0.7
Day 28	0.0

The Primary Dermal Irritation Index (PDII) calculated for this test substance was 3.4.

3. MATERIALS

A. Test Substance

The test substance, identified as Atmosphere, Lot #: TC0408191, was received on April 11, 2019, and was further identified with PSL Reference Number 190411-1D. The test substance was stored in ambient conditions. 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: Maquat LC 12S-50% Quatenary Ammonium - 7.5%, CAS #10324-3
Other ingredients - 92.5%

Physical Description: Clear/yellowliquid

pH: 8.10

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: Rabbit/New Zealand albino.

3.B.4 Age/Body Weight: Young adult (12 weeks)/2466-2750 grams at experimental start.

3.B.5 Source: Received from Robinson Services, Inc. on April 17, 2019.

4. METHODS

A. Husbandry

4.A.1 Housing: The animals were singly housed in suspended stainless steel 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. Litter paper was placed beneath the cage and was changed at least three times per week.

4.A.2 Animal Room Temperature and Relative Humidity Ranges: 18-26°C and 52-66%, respectively. Temperature was above the targeted upper limit for one day during the study. This excursion was considered minor and had no impact on this study.

4.A.3 Animal Room Air Changes/Hour: 12 and 13. 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: Certified RSI 5025 High Fiber Rabbit Diet (Rowe Nutrition, LLC). A designated amount of diet (approximately 150 grams/day) and Alfalfa Timothy Hay Cubes (Standlee Premium Western Forage) were available to each rabbit.

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 rabbit 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 50237, constituted unique identification. Only the sequential animal number is presented in this report.

5. PROCEDURE

A. Preparation and Selection of Animals

On the day before application, a group of animals was prepared by clipping the dorsal area of the trunk. On the day of dosing, but prior to application, the animals were examined for health and the skin checked for any abnormalities. Three healthy, naive animals (not previously tested) without pre-existing skin irritation were selected for test.

B. Preparation of Test Substance

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

The pH was determined for the test substance prior to the application and was within a pH range of 2 and 11.5, therefore testing proceeded. The procedure used and the results are retained in the raw data.

C. Application of Test Substance

Five-tenths of a milliliter of the test substance was applied to one 6-cm^2 intact dose site on each animal and covered with a 1-inch x 1-inch, 4-ply gauze pad. The pad and entire trunk of each animal were then wrapped with semi-occlusive 3-inch Micropore tape to avoid dislocation of the pad. Elizabethan collars were placed on each rabbit and they were returned to their designated cages.

After 4 hours of exposure to the test substance, the pads and collars were removed and the dose sites were gently cleansed with a 3% soap solution followed by tap water and a clean paper towel to remove any residual test substance.

D. Evaluation of Dose Sites

Individual dose sites were scored according to the Draize scoring system (Draize et al., 1944; see Table 5) at approximately 30-60 minutes, 24, 48, and 72 hours and 7, 10, 14, 17, 21 and 28 days after patch removal.

The classification of irritancy was obtained by adding the average erythema and edema scores for the 30-60 minute, 24, 48, and 72-hour scoring intervals and dividing by the number of evaluation intervals (4).

The resulting Primary Dermal Irritation Index (PDII) was classified as follows:

PDII	Classification
0	Non-irritating
> 0 - 2.0	Slightly irritating
2.1 - 5.0	Moderately irritating
> 5.0	Severely irritating

E. In-life Observations

The animals were observed for signs of gross toxicity and behavioral changes at least once daily during the test period.

F. Body Weights

Individual weights of animals were recorded shortly before application of the test substance (initial) and at the completion of testing (terminal).

G. Study Termination

Once testing was complete, the animals were released for euthanasia and humanely euthanized.

6. STATISTICAL ANALYSIS

Statistical analysis was limited to the calculation of the mean irritation scores.

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 was Carolyn Lowe, LATG. The primary scientist for this study was Xiomara Portuguez, BS, with contributions from Cindy Bodnar, Harry Maselli, ALAT, Amber Norton, BS, Mark Schooley, Katherine Sibley, BS, and Matthew Sorber, 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
- U.S. EPA GLP: Toxic Substances Control Act (TSCA): 40 CFR Part 792, 1989
- U.S. FDA GLP: 21 CFR Part 58, 1987

and based on the following testing guideline:

- U.S. EPA Health Effects Test Guidelines, OPPTS 870.2500 (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 will be forwarded to the Sponsor. A copy of the signed report, together with the protocol and all raw data generated at Product Safety Labs, will be maintained in the Product Safety Labs' Archives. PSL will maintain these records for a period of at least five

years. After this time, the Sponsor will be offered the opportunity to take possession of the records or may request continued archiving by PSL.

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

12. RESULTS

Individual body weights are presented in Table 1. Individual in-life observations are presented in Table 2. Individual skin irritation scores are presented in Table 3. A summary of primary skin irritation scores used for calculation of Primary Dermal Irritation Index is presented in Table 4. The Draize Primary Skin Irritation Scoring System is presented in Table 5.

All animals appeared active and healthy and gained body weight during the study. Apart from the dermal irritation noted below, there were no other signs of gross toxicity, adverse clinical effects, or abnormal behavior.

For the first 72 hours of patch removal, all three treated sites exhibited very slight to well-defined erythema and very slight to slight edema. The overall incidence and severity of irritation decreased gradually with time. Hyperkeratosis was noted at all three sites between Days 7 and 21. Eschar was noted at two sites between Days 10 and/or Day 21. Dark discoloration was noted at all sites between 30-60 minutes and/or Day 7. All animals were free of erythema and edema by Day 28 (study termination).

The Primary Dermal Irritation Index for Atmosphere is 3.4.

13. CONCLUSION

Under the conditions of this study, Atmosphere is classified as moderately irritating to the skin.

14. REFERENCES

Draize, J.H., Woodard, G., & Calvery, H.O. (1944). Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. *J. Pharmacol. Exp. Ther.*, 82, 377-390.

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

Seabaugh, V., & Vocci, F. (1988). *Pesticide assessment guidelines: Subdivision F, hazard evaluation, human and domestic animals: Addendum 3 on data reporting: Series 81-5, dermal irritation.* Washington, DC: U.S. Environmental Protection Agency.

SIGNATURE

Atmosphere

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

7/9/2019

Date

TABLE 1: INDIVIDUAL BODY WEIGHTS

Animal No.	Sex	Body Weight (g)	
		Initial	Terminal
3501	F	2466	3076
3502	F	2750	3504
3503	F	2726	3580

TABLE 2: INDIVIDUAL IN-LIFE OBSERVATIONS

Animal Number	Animal Sex	Observation	Day of Observation (x=observation is present)													
			0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
3501	F	Active and healthy	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
3502	F	Active and healthy	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
3503	F	Active and healthy	x	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	Observation	Day of Observation (x=observation is present)												
			15	16	17	18	19	20	21	22	23	24	25	26	27	28
3501	F	Active and healthy	x	x	x	x	x	x	x	x	x	x	x	x	x	x
3502	F	Active and healthy	x	x	x	x	x	x	x	x	x	x	x	x	x	x
3503	F	Active and healthy	x	x	x	x	x	x	x	x	x	x	x	x	x	x

TABLE 3: INDIVIDUAL SKIN IRRITATION SCORES

ERYTHEMA/EDEMA

Animal No.	Sex	Time After Patch Removal									
		30-60 min	24 hrs[1]	48 hrs[1]	72 hrs[1]	Day 7[2]	Day 10[3]	Day 14[3]	Day 17[3]	Day 21[3]	Day 28
3501	F	1/2[1]	2/2	2/1	2/2	1/1[3]	0/1[4]	0/1[4]	0/1[4]	0/1[4]	0/0
3502	F	1/2	1/2	1/2	1/2	1/1[1]	0/1[4]	0/1[4]	0/1[2]	0/1[2]	0/0
3503	F	2/2[1]	2/1	2/1	2/2	1/1[3]	0/1[2]	0/1[2]	0/1[2]	0/0[2]	0/0
Total		4/6	5/5	5/4	5/6	3/3	0/3	0/3	0/3	0/2	0/0
Mean		1.3/2.0	1.7/1.7	1.7/1.3	1.7/2.0	1.0/1.0	0.0/1.0	0.0/1.0	0.0/1.0	0.0/0.7	0.0/0.0

[1] Small area of dark discoloration.

[2] Desquamation at the dose sites.

[3] Hyperkeratosis.

[4] Eschar.

TABLE 4: SUMMARY OF PRIMARY SKIN IRRITATION SCORES[1]

	Time After Patch Removal									
	30-60 min	**24 hrs**	**48 hrs**	**72 hrs**	**Day 7**	**Day 10**	**Day 14**	**Day 17**	**Day 21**	**Day 28**
Erythema	1.3	1.7	1.7	1.7	1.0	0.0	0.0	0.0	0.0	0.0
Edema	2.0	1.7	1.3	2.0	1.0	1.0	1.0	1.0	0.7	0.0
TOTAL (PDI)[2]	3.3	3.4	3.0	3.7	2.0	1.0	1.0	1.0	0.7	0.0

$$\text{Primary Dermal Irritation Index (PDII)}: \frac{\text{PDI for 30 - 60 minutes, 24, 48 and 72 hours}}{4} = 3.4$$

Classification: Moderately irritating

CLASSIFICATION SYSTEM[3]

PDII	Classification
0	Non-irritating
> 0 - 2.0	Slightly irritating
2.1 - 5.0	Moderately irritating
> 5.0	Severely irritating

[1] Average values for three rabbits.

[2] PDI = Average Erythema + Average Edema

[3] Seabaugh & Vocci, 1988.

TABLE 5: PRIMARY SKIN IRRITATION SCORING SYSTEM[1]

Evaluation of Skin Reactions	Value
Erythema and eschar formation:	
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4
Edema formation:	
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well defined by definite raising)	2
Moderate edema (raised approximately 1 millimeter)	3
Severe edema (raised more than 1 millimeter and extending beyond the area of exposure)	4

[1] Draize et al., 1944