Product Safety Labs

SYN549522

SYN549522 FS (A22417C) - Primary Skin Irritation in Rabbits

Final Report

DATA REQUIREMENT(S): OECD 404 (2015)
EPA 870.2500 (1998)
JMAFF 12-Nousan-8147 (2000)
EC No. 440/2008

AUTHOR(S): Jennifer Durando, BS

COMPLETION DATE: April 26, 2019

PERFORMING LABORATORY: Product Safety Labs
2394 Highway 130
Dayton, NJ 08810 USA

LABORATORY PROJECT ID: Report Number: 49510
Study Number: 49510
Task Number: TK0317077

SPONSOR(S): Syngenta Crop Protection, LLC
410 Swing Road
Post Office Box 18300
Greensboro, NC 27419-8300 USA

VOLUME 1 OF 1 OF STUDY

STATEMENT OF DATA CONFIDENTIALITY CLAIMS

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GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study meets the requirements of U.S. EPA GLP: Pesticide Programs (FIFRA): 40 CFR Part 160, 1989, which are compatible with OECD Principles of GLP (as revised in 1997): ENV/MC/CHEM(98)17, OECD, Paris, 1998, Japanese Ministry of Agriculture, Forestry and Fisheries: No. 23-Syouan-5173, 2 February, 2012, and EC Directive 2004/10/EC, Official Journal of the European Union, L50/44, Feb. 20, 2004. 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).

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.

Jennifer Durando, BS
Study Director, Product Safety Labs

04/26/2019
Date

Performing Laboratory: Product Safety Labs
2394 Highway 130
Dayton, NJ 08810 USA

FLAGGING STATEMENT

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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 30, 2018[1]; Feb 1, 2019	Apr 30, 2018; Feb 1, 2019
Critical phase inspection: *Day 1 in-life observations*	M. Zakrzewski	Jan 4, 2019	Jan 4, 2019
Raw data audit	B. Simms	Feb 1, 2019	Feb 1, 2019
Draft report review	B. Simms	Feb 1, 2019	Feb 1, 2019

Final report reviewed by:

Barbara Simms
Quality Assurance Auditor
Product Safety Labs

04/25/2019
Date

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

GENERAL INFORMATION

Contributors

The following contributed to this report in the capacities indicated:

Name	Title
Jennifer Durando, BS	Study Director
Monique Inforzato, BS	Syngenta Study Monitor
Xiomara Portuguez, BS	Primary Scientist
Cindy Bodnar	Scientist
Matthew Sorber, BS	Scientist

Study dates

Study initiation date: December 11, 2018
Experimental start date: January 3, 2019
Experimental termination date: January 10, 2019

Deviations from the Guidelines

None

Amendments to Final Protocol

None

Deviations from Final Protocol

None

Retention of samples

The test substance is retained for at least 3 months following submission of the final report, unless otherwise specified by the Sponsor. All remaining test substance will be returned to the Sponsor or properly disposed. Records of sample disposition are maintained by Product Safety Labs (PSL).

Other

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 and electronic copies (in Microsoft Word and pdf) of the final report, including the signed QA and GLP Compliance pages will be sent to the Sponsor. A copy of the signed report, together with the protocol (P326 SYN), associated amendments and/or deviations if applicable, and all raw data generated at PSL, is maintained in the PSL Archives in Notebook No. 49510: pages 1-28. 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.

Performing laboratory test substance reference number

181105-1H

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1.0 EXECUTIVE SUMMARY

1.1 Study Design

A primary skin irritation test was conducted with rabbits to determine the potential for SYN549522 FS (A22417C) to produce irritation after a single topical application.

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).

1.2 Results

Within 24 hours of patch removal, all three treated sites exhibited very slight to well-defined erythema and/or very slight to slight edema. The overall incidence and severity of irritation decreased gradually with time. All animals were free of erythema and edema by Day 7 (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	1/3
48 hours	2/3	1/3
72 hours	2/3	1/3
Day 7	0/3	0/3

Time After Patch Removal	**Severity of Irritation – Mean Score**
30-60 minutes	3.4
24 hours	2.7
48 hours	1.0
72 hours	1.0
Day 7	0.0

The Primary Dermal Irritation Index (PDII) calculated for this test substance was 2.0.

1.3 Conclusion

Under the conditions of this study, SYN549522 FS (A22417C) is classified as slightly irritating (Seabaugh, V., & Vocci, F., 1988) to the skin.

2.0 INTRODUCTION

2.1 Purpose

This study was conducted to provide information on the potential skin irritation from a single topical exposure to SYN549522 FS (A22417C).

2.2 Regulatory Guidelines

The procedures as described in this protocol are based on the most recent version of the following testing guidelines:

- OECD Guidelines for Testing of Chemicals, Test No. 404 (2015)
- U.S. EPA Health Effects Test GuideLines, OPPTS 870.2500 (1998)
- JMAFF 12-Nousan-8147 (2000)
- Official Journal of the European Communities. Methods for the Determination of Toxicity and Other Health Effects, Part B.4 (Dermal Irritation/Corrosion), Commission Regulation (EC) No. 440/2008.

2.3 Test Facility

This study was conducted at Product Safety Labs' (PSL) test facility at 2394 US Highway 130, Dayton, New Jersey 08810. In the opinion of the Sponsor and the Study Director, this study did not unnecessarily duplicate any previous work.

3.0 MATERIALS AND METHODS

3.1 Test Substance

The test substance was identified as: SYN549522 FS
A22417C
Batch ID SMU8IP001

It was received on November 5, 2018, and was further identified with PSL Reference Number 181105-1H. 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.

Characterization of the test substance was provided to PSL by the Sponsor (see Appendix 1):

Composition: SYN549522 (498 g/L), 41.4% w/w (a mixture of SYN547386 and SYN548941):
SYN547386 (448 g/L), 37.2% w/w
SYN548941 (50.0 g/L), 4.16% w/w

Physical Description: Red liquid

pH: Not available

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

Recertification Date: End of October 2021

3.2 Experimental Design

3.2.1 Animals

Species/Strain: Rabbit/New Zealand albino

Number of Animals: 3

Sex: Female, nulliparous and non-pregnant.

Age/Body Weight: Young adult (13 weeks)/2223-2432 grams at experimental start.

Source: Received from Robinson Services, Inc. on December 28, 2018.

Justification of Test System and Route of Exposure: An *in vitro* skin irritation study was performed prior to treatment of any animal. The results from the in vitro skin irritation study (Study number: 18/279-043B) in the Episkin™ Model, in accordance with the guidance from the OECD 439 for this method, indicated that the test item is not irritating to the skin. The rabbit was the system of choice because, historically, it has been the preferred and most commonly used species for primary skin irritation tests. The topical route of administration was used because human exposure may occur via this route.

3.2.2 Husbandry

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.

Animal Room Temperature: 19-22ºC

Animal Room Relative Humidity: 42-69%

Animal Room Air Changes: 13/hour. Airflow measurements are evaluated regularly and the records are kept on file at PSL.

Photoperiod: 12-hour light/dark cycle

Acclimation Period: 6 days

3.2.3 Food and feeding

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.

Water: Filtered tap water was supplied *ad libitum*.

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 PSL.

3.2.4 Identification

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

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 number 49510, constituted unique identification. Only the sequential animal number is presented in this report.

3.3 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 was checked for any abnormalities. Three healthy, naive animals (not previously tested) without pre-existing skin irritation were selected for test.

3.4 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.

3.5 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.

3.6 Evaluation of Dose Sites

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

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

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

3.7 In-life Observations

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

3.8 Body Weights

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

3.9 Study Termination

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

3.10 Statistical Analysis

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

4.0 RESULTS AND DISCUSSION

Individual body weights and individual in-life observations are presented in Tables 1 and 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. The Certificate of Analysis is presented in Appendix 1.

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.

Within 24 hours of patch removal, all three treated sites exhibited very slight to well-defined erythema and/or very slight to slight edema. The overall incidence and severity of irritation decreased gradually with time. All animals were free of erythema and edema by Day 7 (study termination).

The Primary Dermal Irritation Index for SYN549522 FS (A22417C) is 2.0.

5.0 CONCLUSIONS

Under the conditions of this study, SYN549522 FS (A22417C) is classified as slightly irritating (Seabaugh, V., & Vocci, F., 1988) to the skin.

6.0 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*: Eighth Edition. Washington, D.C.: 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.

US EPA (2018). *Label Review Manual, Chapter 7 Precautionary Statements,* revised March 2018. Washington, D.C.: U.S. Environmental Protection Agency, Office of Prevention, Pesticides & Toxic Substances. https://www.epa.gov/sites/production/files/2018-04/documents/chap-07-mar-2018.pdf

TABLES SECTION

TABLE 1 Individual Body Weights

Animal No.	Sex	Body Weight (g)	
		Initial	Terminal
3501	F	2302	2477
3502	F	2223	2465
3503	F	2432	2570

F - Female

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
3501	F	Active and healthy	x	x	x	x	x	x	x	x
3502	F	Active and healthy	x	x	x	x	x	x	x	x
3503	F	Active and healthy	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[1]	24 hrs[1]	48 hrs[1]	72 hrs[1]	Day 7
3501	F	1/2	2/2	1/1	1/1	0/0
3502	F	2/2	2/0	1/0	1/0	0/0
3503	F	2/1	2/0	0/0	0/0	0/0
Total		5/5	6/2	2/1	2/1	0/0
Mean		1.7/1.7	2.0/0.7	0.7/0.3	0.7/0.3	0/0

[1] Red staining at the dose site.

TABLE 4 Summary of Primary Skin Irritation Scores[1]

	Time After Patch Removal				
	30-60 min	**24 hrs**	**48 hrs**	**72 hrs**	**Day 7**
Erythema	1.7	2.0	0.7	0.7	0.0
Edema	1.7	0.7	0.3	0.3	0.0
TOTAL (PDI)[2]	3.4	2.7	1.0	1.0	0.0

$$\text{Primary Dermal Irritation Index (PDII):} \frac{\text{Sum of PDI for 30 - 60 minutes, 24, 48 and 72 hours}}{4} = 2.0$$

Classification: Slightly 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 Score + Average Edema Score

[3] Seabaugh, V., & Vocci, F. (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.

APPENDICES SECTION

APPENDIX 1 Certificate of Analysis

syngenta

Syngenta Crop Protection AG
GLP Testing Facility WMU
Analytical Development & Product Chemistry
Breitenloh 5
4333 Münchwilen, Switzerland

Certificate of Analysis

A22417C
SYN549522 FS (500)
SMU8IP001

Batch Identification	**SMU8IP001**
Other Batch ID	1058463
Product Code	**A22417C**
Other Product Code(s)	SYN549522 FS (500)
Chemical Analysis (Active Ingredient content)	
– **Identity of the Active Ingredient(s)***	**confirmed**
– **Content of SYN549522***	**41.4 % w/w corresponding to 498 g/l**
– **Content of SYN547386***	**37.2 % w/w corresponding to 448 g/l**
– **Content of SYN548941***	**4.16 % w/w corresponding to 50.0 g/l**
	The Active Ingredient(s) content is within the FAO limits.
Methodology used for Characterization / Recertification	HPLC, chiral HPLC, oscillating density meter
Physical Analysis	
– **Appearance**	**red liquid**
– **Density***	**1203 kg/m³**
Stability:	
– **Storage Temperature**	**< 30 °C**
– **Recertification Date**	**End of October 2021**

If stored under the conditions given above, this test substance can be considered stable until the recertification date is reached.
This Certificate of Analysis summarizes data which originates either from a single study or from several individual studies. Tests marked with an asterisk (*) have been conducted in compliance with GLP.
Raw data, documentation, study plans, any amendments to study plans and reports pertaining to this/these study/studies are stored under the study number(s) referenced below within the archives of the GLP Testing Facility WMU at Syngenta Crop Protection AG, Switzerland.

Study number of batch characterization: CHMU180671
Study number(s) of batch recertification: ---

Authorization: 17-Oct-2018

Daniel Jenniches
Analytical Development & Product Chemistry

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