Product Safety Labs

SYN549522

SYN549522 FS (A22417C) - Primary Eye Irritation in Rabbits

Final Report

DATA REQUIREMENT(S): OECD 405 (2017)
EPA 870.2400 (1998)
JMAFF 12-Nousan-8147 (2000)
EC No 2017/735, B.5 (2017)

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COMPLETION DATE: April 26, 2019

PERFORMING LABORATORY: Product Safety Labs
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LABORATORY PROJECT ID: Report Number: 49509
Study Number: 49509
Task Number: TK0317074

SPONSOR(S): Syngenta Crop Protection, LLC
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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 14, 2019	Apr 30, 2018; Feb 14, 2019
Critical phase inspection: *72-hour scoring*	M. Zakrzewski	Jan 10, 2019	Jan 10, 2019
Raw data audit	B. Simms	Feb 14, 2019	Feb 14, 2019
Draft report review	B. Simms	Feb 14, 2019	Feb 14, 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

Study dates

Study initiation date: December 11, 2018
Experimental start date: January 7, 2019
Experimental termination date: January 24, 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 (P324 SYN), associated amendments and/or deviations if applicable and all raw data generated at PSL, is maintained in the PSL Archives in Notebook No. 49509: pages 1-38. 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 eye irritation test was conducted with rabbits to determine the potential for SYN549522 FS (A22417C) to produce irritation from a single instillation via the ocular route.

The study was conducted in a stepwise fashion. Initially, one-tenth of a milliliter of the test substance was instilled into the conjunctival sac of the right eye of one healthy rabbit by pulling the lower lid away from the eyeball. The upper and lower lids were then gently held together for about one second before releasing to minimize loss of the test substance. The left eye remained untreated and served as a control. Ocular irritation was evaluated by the Draize scoring system (Draize, et al., 1944; see Table 5). Since there was no significant irritation observed in this animal, the test was completed on two additional animals, as described above, treated with the test substance sequentially.

1.2 Results

One hour after test substance instillation, 'positive' conjunctivitis was noted for one treated eye. There was no corneal opacity or iritis observed in any treated eye during this study. The overall incidence and severity of irritation decreased with time. Positive irritation cleared from the treated eye by 24 hours. All animals were free of ocular irritation by 48 hours.

The incidence of positive effects, severity and reversibility are detailed below:

Animal No.		Scores after treatment *				Mean scores (24-72 h)	Reversible – grade 0 (day)
		1 h	24 h	48 h	72 h		
3401	Corneal opacity	0	0	0	0	0	-
	Iritis	0	0	0	0	0	-
	Redness conjunctivae	1	1	0	0	0.3	2
	Chemosis conjunctivae	2	1	0	0	0.3	2
	Discharge	2	0	0	0	0	1
3402	Corneal opacity	0	0	0	0	0	-
	Iritis	0	0	0	0	0	-
	Redness conjunctivae	1	1	0	0	0.3	2
	Chemosis conjunctivae	1	1	0	0	0.3	2
	Discharge	1	0	0	0	0	1
3403	Corneal opacity	0	0	0	0	0	-
	Iritis	0	0	0	0	0	-
	Redness conjunctivae	1	1	0	0	0.3	2
	Chemosis conjunctivae	1	0	0	0	0	1
	Discharge	1	0	0	0	0	1

* scores in the range of 0 to 4 for cornea opacity and chemosis, 0 to 3 for redness of conjunctivae and 0 to 2 for iritis

The control eye of each animal was symptom-free during the study.

No clinical signs of systemic toxicity were observed in any animal in this study.

No mortality occurred during the study.

The body weights of all rabbits were considered to be within the normal range of variability.

1.3 Conclusion

The test item, SYN549522 FS (A22417C) was graded as minimally irritating to the rabbit eye according to the modified Kay & Calandra classification system.

2.0 INTRODUCTION

2.1 Purpose

This study was conducted to provide information on potential irritation from exposure of the eyes 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 the Testing of Chemicals, Section 4, Test No. 405: Acute Eye Irritation/Corrosion (2017)
- U.S. EPA Health Effects Test Guidelines, OPPTS 870.2400, Acute Eye Irritation (1998)
- JMAFF Appendix to Director General Notification, No. 12-Nousan-8147 (2000)
- Commission Regulation (EU) No 2017/735, B.5 (L 112/1, 14 February 2017) amending Regulation (EC) No 440/2008

2.3 Test Facility

This study was conducted at Product Safety Labs' 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. All animals assigned to test were nulliparous and non-pregnant.

Age/Body Weight: Young adult (11-14 weeks)/1947-2550 grams at experimental start.

Source: Received from Robinson Services, Inc. on December 28, 2018 and January 9, 2019.

Justification of Test System and Route of Exposure: An *in vitro* eye irritation study was performed prior to treatment of any animal. The results from the in vitro eye irritation study (Study number: 18/279-038CS) in the Isolated Chicken Eye model with test item, in accordance with the guidance from the OECD 438 for this method, indicated that the test item is not classified as a severe irritant and not classified as non-irritant. It is concluded that further information is required for classification.

The rabbit was the system of choice because, historically, it has been the preferred and most commonly used species for primary eye irritation tests.

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: 19-23ºC

Animal Room Relative Humidity: 39-69%

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: 10-17 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 49509, constituted unique identification. Only the sequential animal number is presented in this report.

3.3 Preparation and Selection of Animals

Prior to test initiation, both eyes of a group of animals were examined using a white light source and a fluorescein dye procedure. One drop of ophthalmic fluorescein sodium dye was instilled into both eyes of each rabbit. The eyes were rinsed with physiological saline (0.9%

NaCl) after instillation of the fluorescein and then evaluated for corneal damage using an ultraviolet light source. Prior to test substance instillation, the eyes were re-examined and scored for abnormalities according to the Draize "Scale for Scoring Ocular Lesions" (see Table 5). Three healthy, naive rabbits (not previously tested) without pre-existing ocular irritation were selected for test.

Initially, only one rabbit was placed on test. In the absence of significant irritation in this animal, the remaining two animals were tested sequentially to confirm the result.

A systemic analgesic (Buprenorphine SR®) was administered to relieve potential discomfort associated with eye irritation which provides therapeutic relief for periods of up to 76 hours. Prior to test substance instillation, 0.1 mg/kg of body weight of the analgesic was administered to the animals and at appropriate intervals to maintain therapeutic blood levels.

3.4 Preparation of Test Substance

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

The pH was determined for the test substance prior to the instillation 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 Instillation

An in vitro eye irritation study was conducted prior to the initition of this test. The results of the study were non-irritating. Therefore, the preliminary eye irritaion study was initiated and conducted in a stepwise fashion.

Prior to instillation, 1-2 drops of ocular anesthetic (Tetracaine Hydrochloride Ophthalmic Solution USP, 0.5%) were placed into both the treated and control eye of each animal. One-tenth of a milliliter of the test substance was then instilled into the conjunctival sac of the right eye of each rabbit by pulling the lower lid away from the eyeball. The upper and lower lids were then gently held together for about one second before releasing to minimize loss of the test substance. The other eye of each rabbit remained untreated with the test substance and served as a control. The rabbits were then returned to their designated cages.

3.6 Ocular Scoring

Ocular irritation was evaluated using a white light in accordance with the Draize “Scale for Scoring Ocular Lesions” (see Table 5) at 1, 24, 48, and 72 hours post-instillation. The fluorescein dye evaluation procedure described in Section 3.3 was used at 24 hours to verify the absence of corneal damage. In addition to observations of the cornea, iris and conjunctivae, any other observed lesions were noted. The average score for all rabbits at each scoring period was calculated to aid in data interpretation.

3.7 Classification of Eye Scores

The time point with the highest mean score (Maximum Mean Total Score - MMTS) for all rabbits, was used to further classify the test substance by the system of Kay and Calandra (Kay, J.H., & Calandra, J.C., 1962).

3.8 In-life Observations

The animals were observed for signs of gross toxicity and behavioral changes at least once daily during the test period (see Table 2).

3.9 Body Weights

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

3.10 Study Termination

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

3.11 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. A summary of severity of irritation is presented in Table 3. Individual ocular irritation scores are presented in Table 4. The Draize Scale for Scoring Ocular Lesions is presented in Table 5. The Kay and Calandra scheme for classifying eye irritants is presented in Table 6. 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 eye irritation noted below, there were no other signs of gross toxicity, adverse clinical effects, or abnormal behavior.

One hour after test substance instillation, conjunctival redness (score of 1), chemosis (score of 1 or 2), discharge (score of 1 or 2) were noted in all animals. At 24 hours after application, conjunctival redness (score 1) and chemosis (score 1), were noted. There was no corneal opacity or iritis observed in any treated eye during this study. The overall incidence and severity of irritation decreased with time. All animals were free of ocular irritation by 48 hours.

The control eye of each animal was symptom-free during the study.
The Maximum Mean Total Score of SYN549522 FS (A22417C) is 7.3.

5.0 CONCLUSIONS

The test item, SYN549522 FS (A22417C) was graded as minimally irritating to the rabbit eye according to the modified Kay & Calandra classification system.

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.

Kay, J.H., & Calandra, J.C. (1962). Interpretation of eye irritation tests. *J. Soc. Cos. Chem.*, *13*, 281-289.

National Research Council. (2011) *Guide for the Care and Use of Laboratory Animals*: Eighth Edition. Washington, D.C.: The National Academies Press.

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
3401	F	2364	2527
3402	F	2550	2630
3403	F	1947	2264

F - Female

TABLE 2 Individual In-life Observations

Animal Number	Animal Sex	Observation	Day of Observation (x=observation is present)			
			0	1	2	3
3401	F	Active and healthy	x	x	x	x
3402	F	Active and healthy	x	x	x	x
3403	F	Active and healthy	x	x	x	x

apryse

TABLE 3 Summary of Severity of Irritation

Time Post Instillation	Severity of Irritation – Mean Score
1 hour	7.3
24 hours	3.3
48 hours	0.0
72 hours	0.0

TABLE 4 Individual Scores for Ocular Irritation

	Rabbit No.: 3401 (Female)			
	Hour			
	1[1]	24[1]	48[1]	72[1]
I. Cornea				
A. Opacity	0	0[2]	0	0
B. Area	4	4	4	4
(AxB)x5	0	0	0	0
II. Iris				
A. Values	0	0	0	0
Ax5	0	0	0	0
III. Conjunctivae				
A. Redness	1	1	0	0
B. Chemosis	2	1	0	0
C. Discharge	2	0	0	0
(A+B+C)x2	10	4	0	0
Total	10	4	0	0

[1] Red staining on fur around eye.
[2] Ophthalmic fluorescein sodium dye was used to verify the absence of corneal opacity.

TABLE 4 Individual Scores for Ocular Irritation (Continued)

	Rabbit No.: 3402 (Female)			
	Hour			
	1[1]	24[1]	48[1]	72[1]
I. Cornea				
A. Opacity	0	0[2]	0	0
B. Area	4	4	4	4
(AxB)x5	0	0	0	0
II. Iris				
A. Values	0	0	0	0
Ax5	0	0	0	0
III. Conjunctivae				
A. Redness	1	1	0	0
B. Chemosis	1	1	0	0
C. Discharge	1	0	0	0
(A+B+C)x2	6	4	0	0
Total	6	4	0	0

[1] Red staining on fur around eye.
[2] Ophthalmic fluorescein sodium dye was used to verify the absence of corneal opacity.

TABLE 4 Individual Scores for Ocular Irritation (Continued)

	Rabbit No.: 3403 (Female)			
	Hour			
	1[1]	24[1]	48[1]	72[1]
I. Cornea				
A. Opacity	0	0[2]	0	0
B. Area	4	4	4	4
(AxB)x5	0	0	0	0
II. Iris				
A. Values	0	0	0	0
Ax5	0	0	0	0
III. Conjunctivae				
A. Redness	1	1	0	0
B. Chemosis	1	0	0	0
C. Discharge	1	0	0	0
(A+B+C)x2	6	2	0	0
Total	6	2	0	0

[1] Red staining on fur around eye.
[2] Ophthalmic fluorescein sodium dye was used to verify the absence of corneal opacity.

TABLE 5 Scale for Scoring Ocular Lesions[1]

1. Cornea

A. Opacity-degree of density (area most dense taken for reading)

No Opacity 0
Scattered or diffuse area, details of iris clearly visible ..... 1[2]
Easily discernible translucent areas, details of iris slightly obscured ..... 2[2]
Opalescent areas, no details of iris visible, size of pupil barely discernible ..... 3[2]
Opaque, iris invisible ..... 4[2]

B. Area of cornea involved

One quarter (or less) but not zero 1
Greater than one quarter, but less than half 2
Greater than half, but less than three quarters 3
Greater than three quarters, up to whole area 4

A X B X 5 Total Maximum = 80

2. Iris

A. Values

Normal 0
Folds above normal, congestion, swelling, circumcorneal injection (any or all of these or combination of any thereof) iris still reacting to light (sluggish reaction is positive) ..... 1[2]
No reaction to light, hemorrhage, gross destruction (any or all of these) ..... 2[2]

A X 5 Total Maximum = 10

3. Conjunctivae

A. Redness (refers to palpebral and bulbar conjunctivae excluding cornea and iris)

Vessels normal 0
Vessels definitely injected above normal 1
More diffuse, deeper crimson red, individual vessels not easily discernible ..... 2[2]
Diffuse beefy red ..... 3[2]

B. Chemosis

No swelling 0
Any swelling above normal (includes nictitating membrane) 1
Obvious swelling with partial eversion of lids ..... 2[2]
Swelling with lids about half-closed ..... 3[2]
Swelling with lids about half-closed to completely closed ..... 4[2]

C. Discharge

No discharge 0
Any amount different from normal (does not include small amounts observed in inner canthus of normal animals) 1
Discharge with moistening of the lids and hairs just adjacent to lids 2
Discharge with moistening of the lids and hairs, and considerable area around the eye 3

Score (A + B + C) X 2 Total Maximum = 20

Total Maximum Score: 110 represents the sum of all scores obtained for the cornea, iris and conjunctivae.

[1] Draize, et al, 1944.
[2] These scores represent a positive response.

TABLE 6 Classification of Eye Irritation Scores

MMTS	Irritation Classification	Requirement For Maintenance of Classification[1]
0.0 - 0.5	non	Up to 0.5 at 1 hour with zeros at 24 hours; otherwise, increase one level
0.6 - 2.5	practically non	with zeros at 24 hours; otherwise, increase one level
2.6 - 15.0	minimally	with zeros at 48 hours; otherwise, increase one level
15.1 - 25.0	mildly	with zeros at 96 hours; otherwise, increase one level
25.1 - 50.0	moderately	with 7 day mean $\leq$ 20 and individual total scores $\leq$ 10 in at least 60% of the rabbits with no total score >30; otherwise, increase one level
50.1 - 80.0	severely	with 7 day mean $\leq$ 40 and individual total scores $\leq$ 30 in at least 60% of the rabbits with no total score > 60; otherwise, increase one level
80.1 - 100.0	extremely	with 7 day mean $\leq$ 80 and individual total scores $\leq$ 60 in at least 60% of the rabbits with no total score >050; otherwise, increase one level
100.1 - 110	maximally	with 7 day mean > 80 and individual total scores > 60 in at least 60% of the rabbits; otherwise, decrease one level

[1] Kay, J.H., & Calandra, J.C., 1962.

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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sample