Product Safety Labs

Isopyrazam/Difenconazole SC (A21295D)

Isopyrazam/Difenconazole SC A21295D - Local Lymph Node Assay (LLNA) in Mice

Final Report

DATA REQUIREMENT(S): OECD 429
EPA 870.2600
EC Directive No. 440/2008, B.42 (2008)
Guidance Document on Toxicology for Registration of pesticides in India (2014).

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COMPLETION DATE: December 21, 2017

PERFORMING LABORATORY: Product Safety Labs
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LABORATORY PROJECT ID: Report Number: 45770
Study Number: 45770
Task Number: TK0296436

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 OECD Principles of GLP (as revised in 1997): ENV/MC/CHEM(98)17, OECD, Paris, 1998; U.S. EPA GLP (FIFRA): 40 CFR Part 160, 1989; 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 with the following exception: The stability, uniformity of mixture and verification of concentration of alpha-Hexylcinnamaldehyde (HCA), ≥ 95%, in its carriers were not determined.

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

12/12/2017
Date

Performing Laboratory: Product Safety Labs
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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	A. Adamiec; A. Villagran	Apr 3, 2017[1]; Jul 14, 2017	Apr 3, 2017; Jul 18, 2017
In-process inspection: *Lymph node assessment on Day 7*	A. Villagran	Jun 27, 2017	Jun 27, 2017
Raw data audit	A. Villagran	Jul 14, 17, and 18, 2017	Jul 18, 2017
Draft report review	A. Villagran	Jul 14, 17, and 18, 2017	Jul 18, 2017

Final report reviewed by:

Alicia Villagran

Alicia Villagran, AS, RQAP-GLP
Quality Assurance Auditor
Product Safety Labs

DEC 21, 2017
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
William Masinja, MSc	Syngenta Study Monitor
Matthew Sorber, BS	Scientist
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Katherine Sibley, BS	Scientist
Shannon Stevens, BS, CVT	Scientist

Study dates

Study initiation date: May 31, 2017
Experimental start date: June 1, 2017
Experimental termination date: June 27, 2017

Deviations from the Guidelines

None

Amendment to Final Protocol

At the request of the Sponsor, the Test Procedure Guideline was updated to add the following test procedure guideline for India:

Guidance Document on Toxicology for Registration of Pesticides in India (2014). Ministry of Agriculture Department of Agriculture & co-operation Central Insecticides Board & Registration Committee Directorate of Plant Protection Quarantine & Storage. NH-IV, Faridabad.

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 (P327 SYN) and all raw data generated at PSL, is maintained in the PSL Archives in Notebook No. 45770: pages 1-70. 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 request continued archiving by PSL.

Performing laboratory test substance reference number

170501-3H

TABLE OF CONTENTS

1.0 EXECUTIVE SUMMARY

1.1 Study Design

A local lymph node assay (LLNA) was conducted with mice to examine the dermal sentization potential for Isopyrazam/Difenconazole SC A21295D.

Two concentrations (25% and 50%) of the test substance in 1% Pluronic® L92 Surfactant w/w in distilled water (1% Pluronic® L92) and the neat test substance (100%) were topically applied to twelve healthy test mice (4 mice/group) for three consecutive days. Three days after the last application, 250 µL of sterile phosphate buffered saline (PBS) containing 20 µCi of ^{3}H-methyl thymidine was injected intravenously via the tail vein of each mouse. Approximately five hours later, all animals were euthanized via an overdose of inhaled Isoflurane and the draining (auricular) lymph nodes were harvested and prepared for analysis in a scintillation counter. The results are presented in disintegrations per minute per mouse (dpm/mouse). Each animal's ears were also evaluated for erythema and edema prior to each application and again on Day 6, prior to the IV injection.

A vehicle control group (four animals) was maintained under the same environmental conditions and treated in the same manner as the test animals. The vehicle control animals were treated with 1% Pluronic® L92. In an effort to reduce the total number of animals used, this study was run concurrently with another study to utilize a common vehicle control group.

The sensitivity of the procedure was validated using recent historical positive control data (Study 44857). A positive control group (four animals) was maintained under the same environmental conditions and treated in the same manner as the test and vehicle control animals. The positive control group animals were treated with a 25% (w/w) mixture of alpha-hexylcinnamaldehyde (HCA), purity ≥ 95%, in 1% Pluronic® L92.

1.2 Results

A table summarizing the sensitization results noted is found below:

	Mean DPM	Stimulation Index[1]
Group 1 - Vehicle Control	2044.55	-
Group 2 - 25% Test Substance	2576.64	1.26
Group 3 - 50% Test Substance	2350.48	1.15
Group 4 - 100% Test Substance	1386.76	0.68

[1] The stimulation index is derived by dividing the dpm of each experimental group by the dpm of the vehicle control group. A stimulation index of greater than or equal to 3.0 generally indicates a positive response.

1.3 Conclusion

Based on the results of this study, Isopyrazam/Difenconazole SC A21295D is not considered to be a contact dermal sensitizer in the LLNA. Proper conduct of the LLNA was confirmed via a positive response with 25% alpha-Hexylcinnamaldehyde, ≥ 95% (HCA), a moderate contact sensitizer.

2.0 INTRODUCTION

2.1 Purpose

This study was conducted to determine the potential for Isopyrazam/Difenconazole SC A21295D to elicit a dermal sensitization reaction.

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, Test No. 429 (2010)
- U.S. EPA Health Effects Test Guidelines, OPPTS 870.2600 (2003)
- Commission Regulation (EC) No 640/2012, B.42 (2012) amending Regulation (EC) No 440/2008 (2008)
- Guidance Document on Toxicology for Registration of Pesticides in India (2014). Ministry of Agriculture Department of Agriculture & co-operation Central Insecticides Board & Registration Committee Directorate of Plant Protection Quarantine & Storage. NH-IV, Faridabad (see Amendment in Amendment to Final Protocol)

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: Isopyrazam/Difenconazole SC
A21295D
Batch ID JHU002-109-004

It was received on May 1, 2017, and was further identified with PSL Reference Number 170501-3H. 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: Difenoconazole (129 g/L), 11.9% w/w
Isopyrazam (130 g/L), 11.9% w/w

Physical Description: Off-white liquid

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

Recertification Date: End of November 2018

3.2 ^{3}H-methyl Thymidine

^{3}H-methyl Thymidine, Lot No. 201706, was received on June 6 and 22, 2017 and stored refrigerated. Documentation of the methods of synthesis, fabrication, or derivation is retained by PerkinElmer, Inc., Boston, MA.

The following information related to the characterization of the radioisotope was provided on the Technical Data Sheet:

Specific Activity: 20 Ci/mmol

Molecular Weight: 242

Radioactive Concentration: 37 MBq/mL; 1.0 mCi/mL

Radiochemical Purity: > 97% (HPLC)

Thymine Content: < 0.5%

Expiration Date: July 6 and 22, 2017

3.3 Experimental Design

3.3.1 Animals

Species/Strain: Mouse, CBA/J

Number of Animals: 17

Number of Groups: 5

Number of Animals per Group:
Preliminary Irritation: 1
Test (3 groups): 4 per group
Vehicle (Negative) Control: 4

Sex: Female, nulliparous and non-pregnant.

Age: Preliminary Animals: Young adult (9-10 weeks)

Age/Body Weight: Test and Control Animals: Young adult (9-10 weeks)/17.7-23.8 grams at experimental start.

Source: Received from Envigo RMS Inc. on May 31, 2017 (Preliminary Irritation Animals) and on June 14, 2017 (Test Control Group and Test Group Animals).

3.3.2 Husbandry

Housing: The animals were individually housed in plastic solid bottom cages during the dosing and resting phase of the study. After final weighing until sacrifice, animals were housed in their respective dose groups in plastic cages with bedding. Enrichment (e.g., nesting material) was placed in each cage. Bedding in the plastic, solid bottom cages was changed at least once per week. All caging conformed to the size recommendations in the most recent *Guide for the Care and Use of Laboratory Animals* (Natl. Res. Council, 2011).

Animal Room Temperature: 19-22ºC

Animal Room Relative Humidity: 45-58%

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: 7 days

3.3.3 Food and feeding

Food: Envigo Teklad Global 16% Protein Rodent Diet® #2016. The diet was available *ad libitum*.

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.

3.3.4 Identification

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

Animal: Each animal was marked with a color code and given a sequential animal number assigned to study 45770, which constituted unique identification. Only the sequential animal number is presented in this report.

3.4 Preparation of Test Substance

The test substance as received (neat) was mixed well prior to use. Solubility testing conducted by PSL indicated that the test substance was soluble in 1% Pluronic® L92. All preparations were mixed well.

3.5 Preliminary Toxicity Testing

One mouse was treated with the test substance at the maximum concentration suitable for application (100%). The ears of the mouse were evaluated for erythema and edema immediately prior to dosing on Days 1, 2, 3, and on Day 6 according to the scoring system described in Table 15. Body weight measurements were taken on Days 1 and 6. Ear thickness measurements were taken on Day 1 (pre-dose), Day 3 and Day 6.

Twenty-five µL of the test substance was applied to the dorsum of both ears of the mouse once per day for three consecutive days. Application was done using an appropriate size micropipette to accurately deliver 25 µL. The dose was gently spread as evenly as possible over the dorsal surface of the ear using the disposable pipette tip. No treatment was made on Days 4 and 5. On Day 6, each site was evaluated for local reactions (erythema & edema).

The animal was observed daily for signs of toxicity. The Study Director used this data in conjunction with any pre-existing data to select the three concentrations to be tested. The test substance at 25% and 50% (w/w) mixtures in 1% Pluronic® L92 and the test substance at 100% were selected for test.

3.6 Selection of Animals/Dose Levels

Prior to dosing, the animals were weighed and the ears were checked for any abnormalities or clinical signs of diseases or injury. Sixteen healthy, naive female mice without pre-existing ear irritation were selected and distributed (four mice per group) into the following groups:

Group #	Purpose	Concentration %
1	Vehicle Control	0
2	Test Substance	25
3	Test Substance	50
4	Test Substance	100

Concentrations were selected based on toxicity, solubility, irritancy, and viscosity.

3.7 Sample Preparation

Concentrations of 25%, 50% and 100% were selected for the main test based on results of the preliminary screening test. Dilutions of the test substance were prepared as w/w mixtures in 1% Pluronic® L92. The vehicle control, 1% Pluronic® L92 was also prepared. All dosage preparations were freshly prepared on the day of application and mixed well.

3.8 Test Substance Application

Beginning on Day 1, a quantity of 25 µL of the appropriate test substance concentration or the vehicle alone was applied to the dorsum of both ears of each mouse once per day for three consecutive days (Days 1, 2, and 3) using a micropipette. During application, the material was gently spread as evenly as possible over the dorsal surface of the ear using the micro-pipette tip.

3.9 Dermal Scoring

Prior to each application (Days 1, 2, and 3) and on Day 6, the ears were evaluated for erythema and edema according to the modified Draize scoring system (Draize, Woodard, & Calvary, 1944; see Table 15).

3.10 Ear Thickness Measurements

Duplicate measurements of the preliminary animal's ears were made using a micrometer. The measurements were made at the apex of the pinna. Measurements were taken on the preliminary screen animal on Day 1 (pre-dose), Day 3 and Day 6. The % ear swelling was calculated for each ear using the following equation:

$$\% \text{ Ear swelling} = \frac{(B - A)}{A} \times 100\% \text{ where:}$$

A = ear thickness measurement on Day 1 (mm x 10^{-2})

B = ear thickness measurement on Day 3 or 6 (mm x 10^{-2})

3.11 ^{3}H- Methyl Thymidine Injections

On Day 6 of the study (three days after the final topical application) 250 µL of sterile phosphate buffered saline (PBS) containing 20 µCi of ^{3}H-methyl thymidine was injected intravenously via the tail vein of each mouse.

3.12 Lymph Node Assessment

Approximately five hours after the injection, all test and control mice were euthanized via overdose of inhaled Isoflurane and the draining auricular lymph nodes from all animals were

excised. The lymph nodes were evaluated for each individual mouse. A single cell suspension of lymph node cells (LNC) was prepared in PBS by gently massaging the lymph nodes between the frosted ends of two microscope slides over a collection vessel. The slides were then rinsed briefly with PBS into the vessel. The contents of the vessel were transferred to a centrifuge tube and washed with an excess of PBS and centrifuged for approximately 10 minutes at 1800 rpm, with an RCF[1] of 489G. This process was carried out twice. In both cases, the supernatant was decanted and discarded following each centrifugation. After the second wash, 5 mL of the 5% trichloroacetic acid (TCA) in distilled water was then added to the sediment and the tube was vortexed briefly. The DNA was then precipitated in the 5% TCA in distilled water at approximately 4°C overnight (approximately 18 hours).

Following the overnight precipitation of the DNA, the tubes were centrifuged again for approximately 10 minutes at 1800 rpm and the supernatant was discarded. The resulting precipitate was re-suspended using 1 mL of the 5% TCA in distilled water and transferred to 10 mL of scintillation fluid. Incorporation of ^{3}H-methyl thymidine was measured by β-scintillation counting and expressed as disintegrations per minute, minus background dpm.

3.13 Clinical Observations

All test, control and preliminary mice were observed for signs of mortality, gross toxicity, and/or behavioral changes daily. All test and control mice were euthanized via overdose of Isoflurane (an anesthetic) by inhalation on Day 6.

3.14 Body Weights

Individual body weights of the preliminary animal were recorded on Day 1 (initial) shortly before test substance application and prior to sacrifice on Day 6. Individual body weights of test and control animals were recorded on Day 1 (initial) shortly before test substance application and prior to IV injections of ^{3}H-methyl thymidine on test Day 6.

3.15 Evaluation

The mean and standard deviation of the dpm values were calculated for each dose group. A stimulation index (SI) was derived for each experimental group by dividing the mean dpm of each experimental group by the mean dpm of the vehicle control group. Any test substance that produces an SI $\geq$ 3 in the LLNA is normally considered "positive" for dermal sensitization potential (Kimber et al., 1994).

The EC3 value was not calculated since all dose levels induced a stimulation index of less than 3.0.

[1] Relative Centrifugal Force.

3.16 Historical Positive Control Validation Study

The procedures used in this study were validated using alpha-hexylcinnamaldehyde, purity ≥ 95% (HCA) as the positive control substance, namely 25% (w/w) mixture of HCA in 1% Pluronic® L92. The most recent validation, PSL Study #44857, was performed by PSL between February 15 and 21, 2017. A copy of the signed report, together with the protocol and all raw data generated at PSL, are maintained in the PSL Archives in Notebook No. 44857: pages 1-48. This test was conducted at the Dayton Facility with CBA/J mice from Envigo RMS following procedures similar to those described in Sections 3.9 through 3.15. The results obtained from this testing are presented below.

Historical Vehicle Control Group – 1% Pluronic® L92: No dermal irritation was observed for any of the historical vehicle control group sites.

Historical Positive Control Group – 25% (w/w) Test Substance in 1% Pluronic® L92: Very slight erythema (score of 1) was evident at one historical positive control site on Day 2, seven sites on Day 3 and four sites on Day 6. Slight edema (score of 1) was present at one site on Day 3. Desquamation was present at all sites on Day 6.

Number of positive control sites with dermal irritation

Day	Erythema				Edema	
	Very slight (score of 1)	Well-defined (score of 2)	Moderate to Severe (score of 3)	Severe (score of 4)	Slight (score of 1)	Marked (score of 2)
2	1/8	0/8	0/8	0/8	0/8	0/8
3	7/8	0/8	0/8	0/8	1/8	0/8
6	4/8	0/8	0/8	0/8	0/8	0/8

3.17 Statistical Analysis

Statistical analysis was performed. Significance was judged at $p < 0.05$. The treated groups and negative vehicle control group were compared using a One-Way Analysis of Variance (ANOVA), followed by comparison of the treated groups to control by Dunnett's t-test for multiple comparisons (INSTAT Biostatistics, Graph Pad Software, San Diego, CA). Outlier analysis was conducted using Grubbs' test (Grubbs, 1969).

4.0 RESULTS AND DISCUSSION

Preliminary group irritation body weights, testing scores, ear thickness and individual cage-side and necropsy observations are presented in Tables 1-3. Individual body weights for vehicle, test, and historical positive control animals are presented in Tables 5-6. Individual

dermal irritation scores are presented in Tables 7-8. Individual cage-side observations are presented in Tables 9-10. Individual and mean dpm values are presented in Table 11-12. A summary of results for vehicle control, test, and historical positive control animals is presented in Tables 13-14. The Draize Primary Skin Irritation Scoring System is presented in Table 15. The Certificate of Analysis is presented in Appendix 1.

All animals appeared active and healthy throughout the study. Three mice from the vehicle control and two mice in the test substance groups lost or failed to gain body weight; however, all other animals gained body weight during the study.

Group 1 (Vehicle Control – 1% Pluronic® L92): No dermal irritation was observed for any of the vehicle control group sites.

Group 2 (25% Test Substance in 1% Pluronic® L92): No dermal irritation was observed for any of the test group sites.

Group 3 (50% Test Substance in 1% Pluronic® L92): No dermal irritation was observed for any of the test group sites.

Group 4 (100% Test Substance): No dermal irritation was observed for any of the test group sites.

Treatment of mice with 25%, 50% and 100% of Isopyrazam/Difenconazole SC A21295D resulted in stimulation index values of 1.26, 1.15 and 0.68, respectively. As a stimulation index (SI) of less than 3.0 was observed in all the treatment groups, the test substance was not considered positive for a dermal sensitization potential. The positive control (HCA) at 25% produced a dermal sensitization response in mice (SI = 3.44). Therefore, the LLNA test system was valid for this study with Isopyrazam/Difenconazole SC A21295D.

5.0 CONCLUSIONS

Based on these findings and on the evaluation system used, Isopyrazam/Difenconazole SC A21295D is not considered to be a contact dermal sensitizer in the LLNA.

The positive response observed in the historical positive control validation study with 25% alpha-Hexylcinnamaldehyde (HCA), ≥ 95%, validated the test system used in this study (see Section 3.16).

6.0 REFERENCES

Boverhof, D. R., Wiescinski, C. M., Botham, P., Lees, D., Debruyne, E., Repetto-Larsay, M., Ladics, G., Hoban, D., Gamer, A., Remmele, M., Wang-Fan, W., Ullmann, L. G., Mehta, J., Billington, R. & Woolhiser, M. R. (2008). Interlaboratory validation of 1% Pluronic® L92 surfactant as a suitable, aqueous vehicle for testing pesticide formulations using the murine local lymph node assay. *Toxicol. Sci*. 105(1), 79-85.

Draize, J.H., Woodward, 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.

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

Ryan, C.A., Cruse. L.W., Skinner, R.A., Dearman, R.J., Kimber, I., & Gerberick, G.F. (2002). Examination of a vehicle for use with water soluble materials in the murine local lymph node assay. *Food Chem. Toxicol.,* 40, 1719-1725.

TABLES SECTION

TABLE 1 Preliminary Irritation Group Body Weights

Animal No.	Sex	Body Weight (g)	
		Day 1	Day 6
Group 1P – 100%[1]			
3680	F	17.6	17.7

F – Female

[1] 25 µL of the test substance was applied as received to each ear (50 µL total).

TABLE 2 Preliminary Irritation Group Testing Scores

Erythema/Edema

Animal No.	Sex	Day							
		1		2		3		6	
		Left	Right	Left	Right	Left	Right	Left	Right
Group 1P - 0%[1]									
3680	F	0/0	0/0	0/0[2]	0/0[2]	0/0[2]	0/0[2]	1/0[2]	0/0[2]

[1] 25 µL of 1% Pluronic® L92 was applied to each ear (50 µL total).
[2] Test substance residue at the dose site.

TABLE 3 Preliminary Group: Ear Thickness Measurements (mm)

Preliminary Animal (Left Ear)

Dose Level	Group No.	Animal No.	Day 1 1st	Day 1 2nd	Mean Thickness Day 1	Day 3 1st	Day 3 2nd	Mean Thickness Day 3	% Change Days 1-3	Day 6 1st	Day 6 2nd	Mean Thickness Day 6	% Change Days 1-6
100% Test Substance	1P	3680	0.23	0.23	0.23	0.25	0.24	0.25	8.70	0.26	0.25	0.26	13.04

Preliminary Animal (Right Ear)

Dose Level	Group No.	Animal No.	Day 1 1st	Day 1 2nd	Mean Thickness Day 1	Day 3 1st	Day 3 2nd	Mean Thickness Day 3	% Change Days 1-3	Day 6 1st	Day 6 2nd	Mean Thickness Day 6	% Change Days 1-6
100% Test Substance	1P	3680	0.23	0.25	0.24	0.25	0.23	0.24	0.00	0.24	0.23	0.24	0.00

TABLE 4 Preliminary Irritation Group Individual Cage-Side Observations

Animal Number	Animal Sex	Group	Dose Conc. (%)	Observation	Day of Observation (x=observation is present)					
					1	2	3	4	5	6
3680	F	1P	100	Active and healthy	x	x	x	x	x	x

TABLE 5 Individual Body Weights

Animal No.	Group	Sex	Day 1 (g)	Day 6 (g)
3601	1 Vehicle Control (1% Pluronic® L92)	F	22.3	22.3
3602		F	18.0	19.3
3603		F	19.8	19.6
3604		F	23.4	23.3
3605	2 25% Test Substance in 1% Pluronic® L92	F	17.7	18.0
3606		F	20.2	20.9
3607		F	19.4	21.0
3608		F	21.2	22.6
3609	3 50% Test Substance in 1% Pluronic® L92	F	21.1	21.2
3610		F	18.1	18.6
3611		F	20.9	21.4
3612		F	21.8	21.3
3613	4 100% Test Substance	F	21.2	22.3
3614		F	23.8	23.4
3615		F	18.7	19.7
3616		F	22.1	22.5

TABLE 6 Individual Body Weights Historical Positive Control Validation Study[1]

Animal No.	Group	Sex	Day 1 (g)	Day 6 (g)
3701	1 Vehicle Control (1% Pluronic® L92)	F	18.6	19.6
3702		F	18.7	18.8
3703		F	19.2	20.2
3704		F	19.0	19.9
3705	2 Positive Control (25% HCA in 1% Pluronic® L92)	F	16.9	18.2
3706		F	18.8	18.8
3707		F	16.9	17.1
3708		F	18.5	19.3

[1] PSL Study # 44857, testing was performed by PSL between February 15 and 21, 2017.

TABLE 7 Individual Dermal Irritation Scores

Group 1 – Vehicle Control[1]

Erythema/Edema

Animal No.	Sex	Days							
		1		2		3		6	
		Left	Right	Left	Right	Left	Right	Left	Right
3601	F	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
3602	F	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
3603	F	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
3604	F	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0

Group 2 – 25% Test Substance[2]

Erythema/Edema

Animal No.	Sex	Days							
		1		2		3		6	
		Left	Right	Left	Right	Left	Right	Left	Right
3605	F	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
3606	F	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
3607	F	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
3608	F	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0

[1] 25 µL of 1% Pluronic® L92 was applied to each ear (50 µL total).

[2] 25 µL of the test substance was applied as a w/w mixture in 1% Pluronic® L92 to each ear (50 µL total).

TABLE 7 Individual Dermal Irritation Scores (Continued)

Group 3 – 50% Test Substance[1]

Erythema/Edema

Animal No.	Sex	Days							
		1		2		3		6	
		Left	Right	Left	Right	Left	Right	Left	Right
3609	F	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
3610	F	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
3611	F	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
3612	F	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0

Group 4 – 100% Test Substance[2]

Erythema/Edema

Animal No.	Sex	Days							
		1		2		3		6	
		Left	Right	Left[3]	Right[3]	Left[3]	Right[3]	Left[3]	Right[3]
3613	F	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
3614	F	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
3615	F	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
3616	F	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0

[1] 25 µL of the test substance was applied as a w/w mixture in 1% Pluronic® L92 to each ear (50 µL total).
[2] 25 µL of the test substance was applied as received to each ear (50 µL total).
[3] Test substance residue at the dose site(s).

TABLE 8 Individual Dermal Irritation Scores Historical Positive Control Validation Study[1]

Group 1 – Vehicle Control[2]

Erythema/Edema

Animal No.	Sex	Days							
		1		2		3		6	
		Left	Right	Left	Right	Left	Right	Left	Right
3701	F	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
3702	F	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
3703	F	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
3704	F	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0

Group 2 – Positive Control [3]

Erythema/Edema

Animal No.	Sex	Days							
		1		2		3		6	
		Left	Right	Left	Right	Left	Right	Left[4]	Right[4]
3705	F	0/0	0/0	0/0	1/0	1/0	1/0	0/0	0/0
3706	F	0/0	0/0	0/0	0/0	1/0	1/0	1/0	0/0
3707	F	0/0	0/0	0/0	0/0	0/0	1/0	1/0	1/0
3708	F	0/0	0/0	0/0	0/0	1/0	1/1	1/0	0/0

[1] PSL Study # 44857, testing was performed by PSL between February 15 and 21, 2017.
[2] 25 µL of 1% Pluronic® L92 was applied to each ear (50 µL total).
[3] 25 µL of a 25% HCA was applied as a w/w mixture in 1% Pluronic® L92 to each ear (50 µL total).
[4] Desquamation at all dose sites.

TABLE 9 Individual Cage-Side Observations

Animal Number	Animal Sex	Group	Dose Conc. (%)	Observation	Day of Observation (x=observation is present)					
					1	2	3	4	5	6
3601	F	1	Vehicle Control (1% Pluronic® L92)	Active and healthy	x	x	x	x	x	x
3602	F	1	Vehicle Control (1% Pluronic® L92)	Active and healthy	x	x	x	x	x	x
3603	F	1	Vehicle Control (1% Pluronic® L92)	Active and healthy	x	x	x	x	x	x
3604	F	1	Vehicle Control (1% Pluronic® L92)	Active and healthy	x	x	x	x	x	x

TABLE 9 (cont.) **Individual Cage-Side Observations**

Animal Number	Animal Sex	Group	Dose Conc. (%)	Observation	Day of Observation (x=observation is present)					
					1	2	3	4	5	6
3605	F	2	25% Test Substance in 1% Pluronic® L92	Active and healthy	x	x	x	x	x	x
3606	F	2	25% Test Substance in 1% Pluronic® L92	Active and healthy	x	x	x	x	x	x
3607	F	2	25% Test Substance in 1% Pluronic® L92	Active and healthy	x	x	x	x	x	x
3608	F	2	25% Test Substance in 1% Pluronic® L92	Active and healthy	x	x	x	x	x	x

TABLE 9 (cont.) **Individual Cage-Side Observations**

Animal Number	Animal Sex	Group	Dose Conc. (%)	Observation	Day of Observation (x=observation is present)					
					1	2	3	4	5	6
3609	F	3	50% Test Substance in 1% Pluronic® L92	Active and healthy	x	x	x	x	x	x
3610	F	3	50% Test Substance in 1% Pluronic® L92	Active and healthy	x	x	x	x	x	x
3611	F	3	50% Test Substance in 1% Pluronic® L92	Active and healthy	x	x	x	x	x	x
3612	F	3	50% Test Substance in 1% Pluronic® L92	Active and healthy	x	x	x	x	x	x

TABLE 9 (cont.) **Individual Cage-Side Observations**

Animal Number	Animal Sex	Group	Dose Conc. (%)	Observation	Day of Observation (x=observation is present)					
					1	2	3	4	5	6
3613	F	4	100% Test Substance	Active and healthy	x	x	x	x	x	x
3614	F	4	100% Test Substance	Active and healthy	x	x	x	x	x	x
3615	F	4	100% Test Substance	Active and healthy	x	x	x	x	x	x
3616	F	4	100% Test Substance	Active and healthy	x	x	x	x	x	x

TABLE 10 Individual Cage-Side Observations Historical Positive Control Validation Study[1]

Animal Number	Animal Sex	Group	Dose Conc. (%)	Observation	Day of Observation (x=observation is present)					
					1	2	3	4	5	6
3701	F	1	Vehicle Control (1% Pluronic® L92)	Active and healthy	x	x	x	x	x	x
3702	F	1	Vehicle Control (1% Pluronic® L92)	Active and healthy	x	x	x	x	x	x
3703	F	1	Vehicle Control (1% Pluronic® L92)	Active and healthy	x	x	x	x	x	x
3704	F	1	Vehicle Control (1% Pluronic® L92)	Active and healthy	x	x	x	x	x	x
3705	F	2	Positive Control (25% HCA in 1% Pluronic® L92)	Active and healthy	x	x	x	x	x	x
3706	F	2	Positive Control (25% HCA in 1% Pluronic® L92)	Active and healthy	x	x	x	x	x	x
3707	F	2	Positive Control (25% HCA in 1% Pluronic® L92)	Active and healthy	x	x	x	x	x	x
3708	F	2	Positive Control (25% HCA in 1% Pluronic® L92)	Active and healthy	x	x	x	x	x	x

[1] PSL Study # 44857, testing was performed by PSL between February 15 and 21, 2017.

TABLE 11 Individual Dpm[1] Values

Background:	55.28						
Group	Animal #	dpm	dpm minus background[2]	Group Mean DPM	Std. Dev	SI[3]	SI ≥3
1 Vehicle Control (1% Pluronic® L92)	3601	2410.12	2354.84	2044.55	339.69	-	-
	3602	2275.42	2220.14				
	3603	1632.15	1576.87				
	3604	2081.64	2026.36				
2 25% Test Substance in 1% Pluronic® L92	3605	2665.85	2610.57	2576.64	713.41	1.26	No
	3606	2376.59	2321.31				
	3607	1895.26	1839.98				
	3608	3589.96	3534.68				
3 50% Test Substance in 1% Pluronic® L92	3609	1790.02	1734.74	2350.48	438.87	1.15	No
	3610	2414.12	2358.84				
	3611	2625.34	2570.06				
	3612	2793.56	2738.28				
4 100% Test Substance	3613	1704.11	1648.83	1386.76	340.69	0.68	No
	3614	1238.62	1183.34				
	3615	1756.54	1701.26				
	3616	1068.90	1013.62				

[1] Disintegrations per minute.
[2] Values analyzed for outliers, Grubbs, 1969.
[3] Stimulation Index = Average dpm of Test Substance/Average dpm of Vehicle.

TABLE 12 Individual Dpm[1] Values Historical Positive Control Validation Study[2]

Background:	51.83						
Group	Animal #	dpm	dpm minus background[3]	Group Mean DPM	Std. Dev	SI[4]	SI ≥3
1 Vehicle Control (1% Pluronic® L92)	3701	1747.86	1696.03	1386.71	683.19	-	-
	3702	1468.04	1416.21				
	3703	479.73	427.90				
	3704	2058.54	2006.71				
2 Positive Control (25% HCA in 1% Pluronic® L92)	3705	6648.24	6596.41	4775.21	1882.72	3.44	Yes
	3706	2816.47	2764.64				
	3707	3645.63	3593.80				
	3708	6197.82	6145.99				

[1] Disintegrations per minute.
[2] PSL Study # 44857, testing was performed by PSL between February 15 and 21, 2017.
[3] Values analyzed for outliers, Grubbs, 1969.
[4] Stimulation Index = Average dpm of Test Substance/Average dpm of Vehicle.

TABLE 13 Stimulation Index

Group		Group Mean DPM	SI	Sensitization Response
Vehicle Control	1	2044.55	-	N/A
25% Test Substance	2	2576.64	1.26	Not a Sensitizer
50% Test Substance	3	2350.48	1.15	Not a Sensitizer
100% Test Substance	4	1386.76	0.68	Not a Sensitizer

N/A= Not Applicable

TABLE 14 Stimulation Index Historical Positive Control Validation Study[1]

Group		Group Mean DPM	SI	Sensitization Response
Vehicle Control	1	1386.71	-	N/A
Positive Control (25% HCA)	2	4775.21	3.44	Positive – valid study

[1] PSL Study # 44857, testing was performed by PSL between February 15 and 21, 2017.

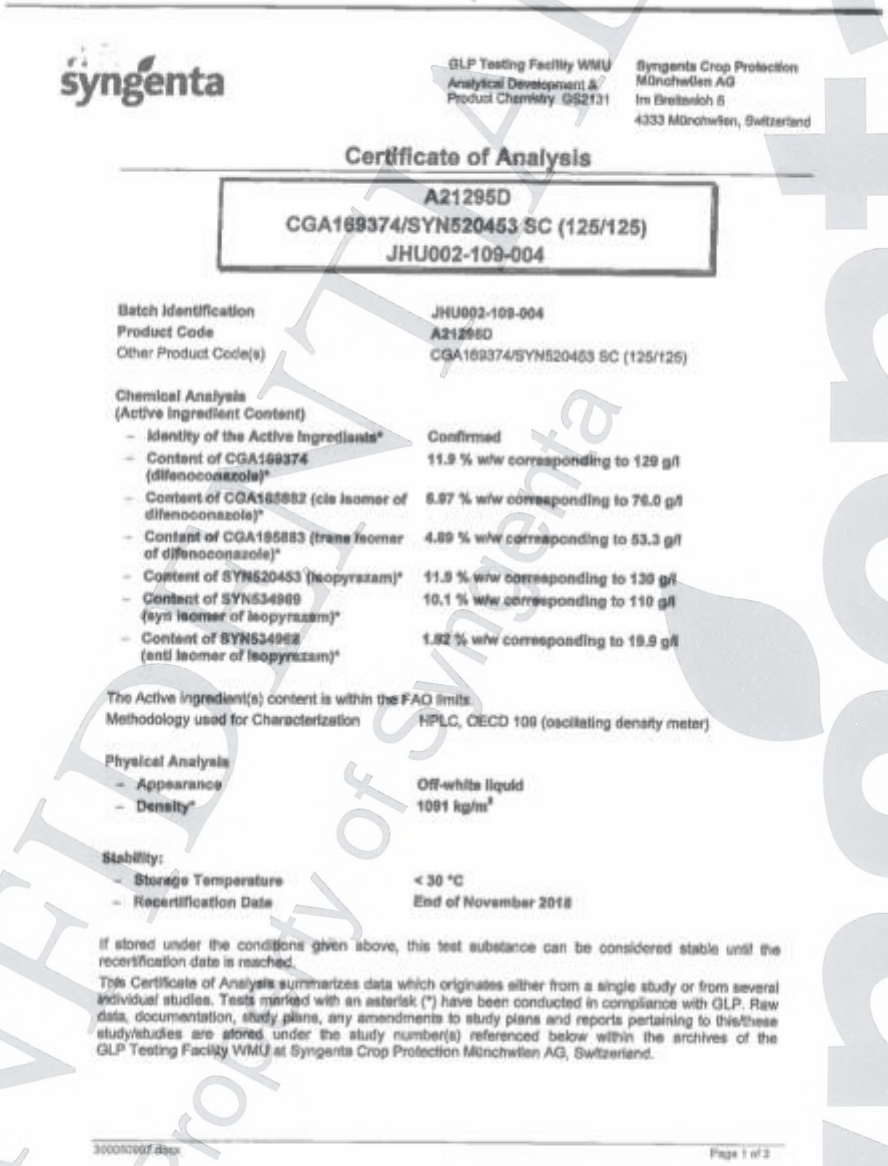
TABLE 15 Primary Skin Irritation Scoring System[1]

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
Slight edema (barely perceptible)	1
Marked edema (swelling is obvious)	2

[1] Modified from a published method (Draize, et al., 1944).

APPENDICES SECTION

APPENDIX 1 Certificate of Analysis



syngenta

GLP Testing Facility WMU
Analytical Development & Product Chemistry GS2131

Syngenta Crop Protection Münchwilen AG
Im Breitenloh 5
4333 Münchwilen, Switzerland

Certificate of Analysis

A21295D
CGA169374/SYN520453 SC (125/125)
JHU002-109-004

Batch Identification	**JHU002-109-004**
Product Code	**A21295D**
Other Product Code(s)	CGA169374/SYN520453 SC (125/125)

Chemical Analysis
(Active Ingredient Content)

– **Identity of the Active Ingredients***	**Confirmed**
– **Content of CGA169374 (difenoconazole)***	**11.9 % w/w corresponding to 129 g/l**
– **Content of CGA185882 (cis isomer of difenoconazole)***	**6.97 % w/w corresponding to 76.0 g/l**
– **Content of CGA185883 (trans isomer of difenoconazole)***	**4.89 % w/w corresponding to 53.3 g/l**
– **Content of SYN520453 (isopyrazam)***	**11.9 % w/w corresponding to 130 g/l**
– **Content of SYN534969 (syn isomer of isopyrazam)***	**10.1 % w/w corresponding to 110 g/l**
– **Content of SYN534962 (anti isomer of isopyrazam)***	**1.82 % w/w corresponding to 19.9 g/l**

The Active ingredient(s) content is within the FAO limits

Methodology used for Characterization	HPLC, OECD 109 (oscillating density meter)

Physical Analysis

– **Appearance**	**Off-white liquid**
– **Density***	**1091 kg/m³**

Stability:

– **Storage Temperature**	**< 30 °C**
– **Recertification Date**	**End of November 2018**

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 Münchwilen AG, Switzerland.

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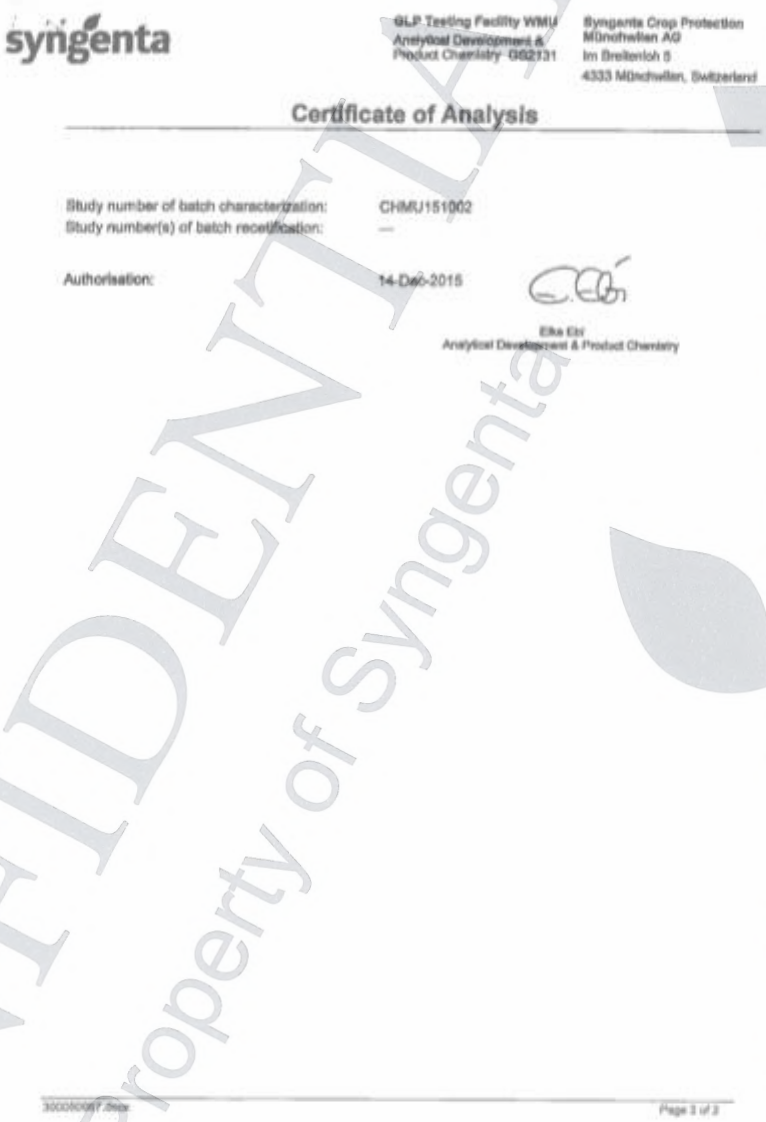

syngenta

GLP Testing Facility WMU
Analytical Development &
Product Chemistry G02131

Syngenta Crop Protection
Münchwilen AG
Im Breitenloh 5
4333 Münchwilen, Switzerland

Certificate of Analysis

Study number of batch characterization: CHMU151002
Study number(s) of batch recertification: —

Authorisation: 14-Dec-2015

Elke Ebi
Analytical Development & Product Chemistry

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