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Difenoconazole/Fludioxonil/Metalaxyl-M/Cyclobutrifluram

Difenoconazole/Fludioxonil/Metalaxyl-M/Cyclobutrifluram FS (A23793B) - Acute Eye Irritation Study in Rabbits

Final Report

TEST GUIDELINE(S):	OECD 405 (2021) EPA 870.2400 (1998) EC 2017/735, B.5 (2017)
AUTHOR(S):	Ivett Orosz, M.Sc.
COMPLETION DATE:	29 March 2022
PERFORMING LABORATORY:	Charles River Laboratories Hungary Kft. H-8200 Veszprém, Szabadságpuszta, hrsz. 028/1., Hungary
LABORATORY PROJECT ID:	Report Number: 21/245-005N Study Number: 21/245-005N Task Number: TK0653804
SPONSOR(S):	Syngenta Ltd. Jealott's Hill International Research Centre Bracknell, Berkshire, RG42 6EY, United Kingdom

STATEMENT OF DATA CONFIDENTIALITY CLAIMS

The Following Statement Applies To The United States of America:

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS
UNDER SPECIFIED FIFRA PROVISIONS

No claim of confidentiality, on any basis whatsoever, is made for any information contained in this document. I acknowledge that information not designated as within the scope of FIFRA sec. 10(d)(1)(A), (B), or (C) and which pertains to a registered or previously registered pesticide is not entitled to confidential treatment and may be released to the public, subject to the provisions regarding disclosure to multinational entities under FIFRA 10(g).

Company: Syngenta Crop Protection, LLC
410 Swing Road
Post Office Box 18300
Greensboro, NC 27419-8300 USA

Submitter: _____ Date: _____

Syngenta is the owner of this information and data. Syngenta has submitted this material to the United States Environmental Protection Agency specifically under the provisions contained in FIFRA as amended and, hereby, consents to use and disclosure of this material by EPA according to FIFRA. In submitting this material to EPA according to method and format requirements contained in PR Notice 2011-3, we do not waive any protection or right involving this material that would have been claimed by the company if this material had not been submitted to the EPA, nor do we waive any protection or right provided under FIFRA Section 3 (concerning data exclusivity and data compensation) or FIFRA Section 10(g) (prohibiting disclosure to foreign and multinational pesticide companies or their agents).

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study has been performed in accordance with the Principles of Good Laboratory Practice (Hungarian GLP Regulations: 42/2014. (VIII. 19.) EMMI decree of the Ministry of Human Capacities which corresponds to the OECD GLP, ENV/MC/CHEM (98) 17.).

This study was conducted in accordance with a written Study Plan, authorized by the Sponsor and Charles River Laboratories Hungary Kft. Management, and followed applicable Standard Operating Procedures.

I, the undersigned, declare that this report constitutes a true record of the actions undertaken and the results obtained in the course of this study. By virtue of my dated signature, I accept the responsibility for the validity of the data.

Signature: Orosz Ivett Date: 29 March 2022

Ivett Orosz, M.Sc.
Study Director

Performing Laboratory: Charles River Laboratories Hungary Kft.
H-8200 Veszprém, Szabadságpuszta, hrsz. 028/1.,
Hungary

To be completed for USA EPA submission only:
Representative of Submitter/Sponsor:

_____ Date: _____

Submitter/Sponsor: Syngenta Crop Protection, LLC
410 Swing Road
Post Office Box 18300
Greensboro, NC 27419-8300 USA

FLAGGING STATEMENT

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QUALITY ASSURANCE STATEMENT

Study Number: 21/245-005N

Study Title: Difenoconazole/Fludioxonil/Metalaxyl-M/Cyclobutrifluram FS (A23793B) - Acute Eye Irritation Study in Rabbits

This Study has been audited by Quality Assurance in accordance with the applicable Good Laboratory Practice regulations. Audit reports were submitted in accordance with SOPs as follows:

Date of Inspection	Phase(s) Inspected/Audited	Date of report to	
		Management	Study Director
20 January 2022	Study Plan	24 January 2022	24 January 2022
18 January 2022	Treatment	18 January 2022	18 January 2022
17 March 2022	Draft Report	22 March 2022	22 March 2022
23 March 2022	Clinical observation	23 March 2022	23 March 2022
29 March 2022	Final Report	29 March 2022	29 March 2022

In addition to the above-mentioned audits, (which may include study specific inspections and/or relevant process based inspections) routine facility inspections were also conducted. The Final Report reflects the raw data and accurately and completely describes the methods and procedures of the study.

Signature: Ivett Schleicher, Ph.D.
On Behalf of QA

Date: 29 March 2022

MANAGEMENT STATEMENT

According to the conditions of the research and development agreement between Syngenta Ltd. (as Sponsor) and Charles River Laboratories Hungary Kft. (as Test Facility) the study titled "Difenoconazole/Fludioxonil/Metalaxyl-M/Cyclobutrifluram FS (A23793B) - Acute Eye Irritation Study in Rabbits" was performed, in compliance with the Principles of Good Laboratory Practice.

Signature: Bale Tóth Date: 29 March 2022

Balázs Tóth, Ph.D.
General Manager

GENERAL INFORMATION

Contributors
The following contributed to this report in the capacities indicated*:

Name	Function
Ivett Orosz, M.Sc.	Study Director
Nikoletta Szalóki, Ph.D.	Assistant Scientist
Ivett Schleicher, Ph.D.	Quality Assurance
László Székelyhidi, D.V.M.	Veterinary Care
Tamás Mészáros, Ph.D.	Pharmacy
Carolina Vaccari, Ph.D.	Syngenta Study Manager

**Other trained, competent personnel worked on the study as required.*

Study dates

Study initiation date:	17 January 2022
Experimental starting date:	25 January 2022
Experimental completion date:	04 February 2022
Receipt of animals:	12 January 2022
Acclimatisation:	12 – 25 January 2022, 12 – 31 January 2022
Treatment:	26 January 2022 (animal no. 2899)
	01 February 2022 (animal no. 2886)
	01 February 2022 (animal no. 2888)
Observation:	26 January – 29 January 2022 (animal no. 2899)
	01 February – 04 February 2022 (animal no. 2886)
	01 February – 04 February 2022 (animal no. 2888)
Draft Report:	22 March 2022
Final Report:	29 March 2022

Deviations from the guidelines and the Study Plan
Due to technical error, temperature value (maximum of 24.0°C) outside the expected range of 17-23°C were recorded occasionally in the animal rooms during the study.

This deviation has no effect on the outcome of the study.

Performing laboratory test substance reference number
210563

Other
The study documents and samples:
- Study Plan,
- all raw data,
- sample of the test item,

- original Study Report and any amendments,
- correspondence

will be archived according to the Hungarian GLP regulations and to applicable SOPs in the archives of Charles River Laboratories Hungary Kft., H-8200 Veszprém, Szabadságpuszta, hrsz. 028/1., Hungary.

After the retention time of 15 years has elapsed, all the archived materials listed above will be returned to the Sponsor or retained for a further period if agreed by a contract. Otherwise the materials will be discarded.

TABLE OF CONTENTS

1.0 EXECUTIVE SUMMARY

1.1 Study Design

The primary eye irritation effect of the test item, difenoconazole/fludioxonil/metalaxyl-m/cyclobutrifluram FS (A23793B), was investigated using three young adult male New Zealand White rabbits. The test item was administered as an installation of a single dose of 0.1 ml into the conjunctival sac of the left eye with the untreated right eyes serving as the control. Scoring of irritation effects was performed approximately 1, 24, 48, 72 hours after test material installation. Observations with fluorescein staining were made approximately 24 hours before treatment and then 24 hours after treatment. Rabbits were treated with analgesic and anaesthetic as per the regulatory guideline. Results obtained from these three animals were used to classify the test item for irritation potential. The animals were exposed consecutively.

1.2 Results

No Initial Pain Reaction (IPR) and no Pain Reaction (PR) were observed at treatment or during the experimental period.

Eye irritation results with difenoconazole/fludioxonil/metalaxyl-m/cyclobutrifluram FS (A23793B):

Animal No.		Scores after treatment *				Mean scores (24-72 h)	Reversible (day)
		1 h	24 h	48 h	72 h		
2899	Corneal opacity	1	0	0	0	0.00	1
	Iritis	0	0	0	0	0.00	-
	Redness conjunctivae	2	0	0	0	0.00	1
	Chemosis conjunctivae	1	0	0	0	0.00	1
	Discharge	2	0	0	0	0.00	1
2886	Corneal opacity	0	0	0	0	0.00	-
	Iritis	0	0	0	0	0.00	-
	Redness conjunctivae	2	0	0	0	0.00	1
	Chemosis conjunctivae	0	0	0	0	0.00	-
	Discharge	1	0	0	0	0.00	1
2888	Corneal opacity	0	0	0	0	0.00	-
	Iritis	0	0	0	0	0.00	-
	Redness conjunctivae	2	0	0	0	0.00	1
	Chemosis conjunctivae	0	0	0	0	0.00	-
	Discharge	1	0	0	0	0.00	1

*according to the Draize scheme (Draize, 1977)
h = hour(s)

All animals became symptom-free 1 day after treatment, therefore, the study was terminated 3 days after the treatment of the third rabbit.

Fluorescein staining was negative at 24 hours after treatment in the case of all animals.

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 bodyweights of all rabbits were considered to be within the normal range of variability.

1.3 Conclusion

The test item difenoconazole/fludioxonil/metalaxyl-m/cyclobutrifluram FS (A23793B) was graded as a minimal irritant (Class 3 on a 1 to 8 scale) to the rabbit eye according to the modified Kay and Calandra classification system.

2.0 INTRODUCTION

2.1 Purpose

The purpose of this eye irritation study was to assess the irritancy potential of difenoconazole/fludioxonil/metalaxyl-m/cyclobutrifluram FS (A23793B), following a single application to the rabbit eye.

The albino rabbit has been shown to be a suitable model for this type of study and is recommended in the test method. The results of the study are believed to be of value in predicting the likely eye irritancy potential of the test material to man. An *in vitro* eye irritation study conducted on Isolated Chicken Eyes with difenoconazole/fludioxonil/metalaxyl-m/cyclobutrifluram FS (A23793B) (Charles River Laboratories Hungary Kft. study code: 21/245-038CS) concluded that the test item is not classified as a severe irritant and not classified as non-irritant. This study was required for regulatory purposes.

2.2 Guidelines

The study followed the procedures indicated by the following internationally accepted guidelines and recommendations:

- OECD Guidelines for the Testing of Chemicals, Section 4, Number 405 "Acute Eye Irritation/Corrosion", adopted 2021.

- United States Environmental Protection Agency, Health Effects Test Guidelines, OPPTS 870.2400, Acute Eye Irritation EPA 712-C-98-195, 1998.

- Commission Regulation (EU) No 2017/735, B.5 (L 112, 2017) amending Regulation (EC) No 440/2008.

2.3 Test Facility

This study was performed in an AAALAC-accredited laboratory. The Institutional Animal Care and Use Committee (IACUC) of Charles River Laboratories Hungary Kft. reviewed the Study Plan and authorised the conduct of the study.

3.0 MATERIALS AND METHODS

3.1 Test Substance

The following information was provided by the Sponsor.

Name:	Difenoconazole/Fludioxonil/Metalaxyl-M/Cyclobutrifluram FS (A23793B)
Batch number:	1200767
Other name:	Difenoconazole/Fludioxonil/Metalaxyl-M/Cyclobutrifluram FS (062.51/049.93/050.05/250.08)
Active ingredient content*:	Difenoconazole 5.45 % w/w 64.0 g/L, fludioxonil 4.37 % w/w 51.3 g/L, metalaxyl-M 4.31 % w/w 50.6 g/L, cyclobutrifluram 21.0 % w/w 247 g/L
Density:	1.174 g/cm³
Appearance:	Red liquid
Recertification date:	31 August 2024
Storage conditions:	Room temperature (<30°C)
Safety precautions:	Routine safety precautions (gloves, goggles, face mask, lab coat) for unknown materials were applied to ensure personnel health and safety.

*Note: No adjustment for active ingredient content of the test item was applied as agreed by the Sponsor.

A Certificate of Analysis supplied by the Sponsor is given in Appendix 1. The integrity of supplied data relating to the identity, purity and stability of the test material is the responsibility of the Sponsor.

3.2 Other Materials

For washing and fluorescein control:

Name:	Saline (0.9% NaCl)
Lot No.:	94922Y05-1
Expiry Date:	30 November 2022
Produced by:	B. Braun Pharmaceuticals SA

Name:	Fluorescein 100 mg/mL
Batch No.:	1037A
Expiry Date:	28 February 2022
Produced by:	Alcon Pharma GmbH

Fluorescein (100 mg/mL) (Batch number: 1037A, Expiry date: 28 February 2022) was mixed with physiological saline solution (Batch number: 94922Y05-1, Expiry date: 30 November

2022) to achieve the final concentration of 2% (v/v). The final solution was stored at room temperature (Dispensary code: S43243, Expiry date: 18 February 2022).

Systemic opiate analgesic:

Name:	Bupredine Multidose (0.3 mg/mL buprenorphine)
Batch No.:	21B162
Expiry Date:	31 July 2023
Produced by:	Le Vet Beheer B.V., The Netherlands

Topical ocular anaesthetic:

Name:	Oftacain (4 mg/mL oxybuprocaine-hydrochloride)
Batch No.:	2000841 / 2008951
Expiry Date:	31 January 2022 / 31 May 2022
Produced by:	S.C. Rompharm Company S.R.L., Romania

Non-steroidal anti-inflammatory drug:

Name:	Melovem (5 mg/mL meloxicam)
Batch No.:	20C30-01C4
Expiry Date:	31 March 2022
Produced by:	Dopharma Research B.V.

Note: Other batches of the used materials (e.g. syringe) in the study are not reported, but archived with the raw data.

3.3 Experimental Design

3.3.1 Animals

Species and strain:	New Zealand white rabbit
Source:	S&K-LAP Kft. 2173 Kartal, Császár út 135, Hungary
Justification of strain:	The New Zealand White rabbit is one of the standard strains used for acute irritation toxicity studies.
Number of animals:	3 animals
Sex:	Male
Age of animals at dosing:	11 weeks
Body weight range at dosing:	3171 – 3428 g
Body weight range at termination:	3242 – 3494 g
Identification:	The animals were identified by engraved ear tags. The cages were marked with individual identity cards with information about study number, sex, cage number, dose and individual animal number.
Acclimatisation time:	14/20 days

3.3.2 Husbandry

Animal health:	Only healthy animals were used for the study, as certified by the veterinarian.
Housing:	Animals were housed individually in AAALAC approved metal wire rabbit cages.
Enrichment:	Cages are of an open wire structure, and cages are placed together to allow some social interaction with rabbit(s) in adjoining cages. Dumbbell Enrichment Devices (produced by Bio-Serv Inc., United States) and stainless steel rattles were available for animals during the study. Copies of the Certificates of Analyses of enrichment devices are retained in the archive at Charles River Laboratories Hungary Kft.
Light:	12 hours daily from 6.00 a.m. to 6.00 p.m. (and during the analgesic/anaesthetic treatment)
Temperature:	18.2 – 24.0°C
Relative humidity:	31 – 58%
Ventilation:	15-20 air exchanges/hour.

The temperature and relative humidity values were measured continuously. The measured range was checked regularly during the acclimatisation and experimental phases.

3.3.3 Food and feeding

The animals received UNI diet for rabbits (produced by Cargill Takarmány Zrt., Hungary), *ad libitum*. The food was considered not to contain any contaminants that could reasonably be expected to affect the purpose or integrity of the study.

The batch numbers of the lots used in the study are:

- Batch no.: 0008019204, expiry date: 06 April 2022

A detailed description of the contents of the lots used is archived with the raw data at Charles River Laboratories Hungary Kft.

3.3.4 Water supply and quality control

The animals received tap water, fit for human consumption, *ad libitum*, from the automatic system supplied by the communal water network. The water was considered not to contain any contaminants that could reasonably be expected to affect the purpose or integrity of the study.
The quality control analysis is performed at least once every 3 months and microbiological assessment is performed monthly by Local Public Health Laboratories (H-8200 Veszprém,

József Attila u. 36., Hungary). The quality control results are retained in the archive at Charles River Laboratories Hungary Kft.

3.4 Pre-Study and Analgesic and Anaesthetic Treatment Procedures

3.4.1 *In vitro* study results

An *in vitro* eye irritation study was performed prior to treatment on any animal. The results from the *in vitro* eye irritation study (Charles River Laboratories Hungary Kft. study code: 21/245-038CS) in the Isolated Chicken Eye model with difenoconazole/fludioxonil/metalaxyl-m/cyclobutrifluram FS (A23793B) (Batch number: 1200767), in accordance with the guidance from the OECD No. 438 for this method, concluded that the test item is not classified as a severe irritant and not classified as non-irritant. It was concluded that further information is required for classification.

3.4.2 Identification of pH

The pH of the test item was measured as pH 6.53, which is within the acceptable range given in the OECD guideline, permitting the test item to be used in the animal studies.

3.4.3 Pre-study examination

Before the start of the test, both eyes of the provisionally selected test rabbits were examined for evidence of ocular irritation or defect. Additionally, to assess the presence of corneal damage, fluorescein staining was employed at least approximately 24 hours prior to instillation, using a hand-held slit-lamp. Only animals free of ocular damage were used.

3.4.4 Chronology of animal use

Initially only one rabbit was treated with test item. The animal (2899) showed scores of above zero at 1-hour observation point after treatment, and the fluorescein staining was negative at 24 observation points after treatment. The first animal became symptom-free after 1 day, therefore the second and third rabbits (2886, 2888) were treated 5 days after treatment of the first rabbit.

3.4.5 Analgesic and anaesthetic treatment

Sixty minutes (60 ±10 min) prior to test substance application, a systemic opiate analgesic was administered by subcutaneous injection under direct Veterinary supervision.

Five minutes (5 ±1.5 min) prior to test substance application, a topical ocular anaesthetic was applied to each eye (including the control eye) to ensure direct comparison of any ocular observations.

Eight hours (8 to 9 h) after test substance application, a systemic opiate analgesic and a non-steroidal anti-inflammatory drug (NSAID) were administered by subcutaneous injection under direct Veterinary supervision. The systemic opiate analgesic was again injected ~12 hours after the post-treatment analgesic and then every 12 hours, until eye scores were zero. The NSAID was again injected every 24 hours, until the ocular lesions were resolved and eye scores were zero.

Systemic opiate analgesic: 0.01 mg/kg of buprenorphine
Topical ocular anaesthetic: one-two drops/eye oxybuprocaine-hydrochloride
Non-steroidal anti-inflammatory drug: 0.5 mg/kg of meloxicam

3.5 Administration of the Test Item

3.5.1 Dosage

A single volume of 0.1 ml of difenoconazole/fludioxonil/metalaxyl-m/cyclobutrifluram FS (A23793B) was administered to the left eye of each animal.

3.5.2 Application of the test item

The test substance was placed in the conjunctival sac of the left eye of the animal after gently pulling the lower lid away from the eyeball. The lids were then gently held together for at least one second in order to prevent loss of the material.

The untreated contralateral eye served as the control.

3.5.3 Duration of exposure

Both eyes of the test animal were rinsed with physiological saline solution following fluorescein control at approximately 24 (±30 min) after treatment for each animal.

3.6 Observations and Scoring

3.6.1 Clinical observations and evaluation of ocular irritation

Scoring of irritation effects was performed approximately 1, 24, 48, 72 hours after test material installation.

Observations with fluorescein staining were made approximately 24 hours before treatment and then 24 after treatment.

The duration of the observation period was sufficient to identify reversibility or irreversibility of changes. Any clinical signs of toxicity or signs of ill-health during the study were recorded. All rabbits were examined for distress at least twice daily, with observations at least 6 hours apart. Clinical observations or signs of ill-health were recorded.

3.6.2 Scoring and assessment of local reaction

The eye irritation scores were evaluated according to the scoring system by Draize (1977), and OECD 405 (2021) shown in Appendix 3.

3.6.3 Classification of the test item

The numerical values corresponding to each animal, tissue and observation time were recorded. The data relating to the conjunctivae were designated by the letters A (redness), B (chemosis) and C (discharge), those relating to the iris designated by the letter D and those relating to the cornea by the letters E (degree of opacity) and F (area of cornea involved). For each tissue the score was calculated as follows:

Score for conjunctivae	=	(A + B + C) x 2
Score for iris	=	D x 5
Score for cornea	=	(E x F) x 5

Using the numerical data obtained, a modified version of the system described by Kay, J. H. and Calandra, J. C. (1962), J. Soc. Cosmet. Chem. 13, 281 289 (see Appendix 4) was used to classify the ocular irritancy potential of the test material. This was achieved by adding together the scores for the cornea, iris and conjunctivae for each time point for each rabbit. The group means of the total scores for each observation were calculated. The highest of these group means (the maximum group mean score) together with the persistence of the reactions enabled classification of the eye irritancy potential of the test material.

3.6.4 Measurement of body weight

Individual body weight was recorded on the day of treatment and before euthanasia (Table 5).

3.7 *Post Mortem* Investigations

At the end of the observation period, animals were euthanised by intravenous sodium pentobarbital (Euthanimal) anaesthesia. Following confirmation of death, the carcasses were discarded without further examination. Details of the material employed for euthanasia are retained in the raw data and detailed in Section 3.7.1.

3.7.1 Material used for euthanasia

Name:	Euthanimal 40% (400 mg/ mL sodium pentobarbital)
Lot No.:	2001004-06
Expiry Date:	31 January 2023
Produced by:	Alfasan Nederland BV, The Netherlands

4.0 RESULTS AND DISCUSSION

4.1 Ocular Reactions

Individual ocular reactions and individual total scores results are presented in Table 1, 2, 3.

No Initial Pain Reaction (IPR) and no Pain Reaction (PR) was observed in any animal during the study.

One hour after the application, redness (score 2), chemosis (score 1 or 0), discharge (score 2 or 1) and corneal opacity (score 1 or 0, area 2 or 0) were noted in all animals.

At 24, 48 and 72 hours after the treatment, no conjunctival or corneal effects were observed in this animal. No fluorescein staining was observed at 24 hours after treatment.

Fluorescein staining is presented in Table 4.

The study was terminated 3 days after the treatment of the second and third rabbit.

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

4.2 Bodyweight

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

Individual bodyweights and bodyweight changes are given in Table 5.

4.3 Clinical Signs

No clinical signs of systemic toxicity were observed in any animal in this study (Table 6).

4.4 Mortality

No mortality occurred during the study.

5.0 CONCLUSIONS

The test item difenoconazole/fludioxonil/metalaxyl-m/cyclobutrifluram FS (A23793B) was graded as a minimal irritant (Class 3 on a 1 to 8 scale) to the rabbit eye according to the modified Kay and Calandra classification system.

6.0 REFERENCES

Literature references listed are available upon request.

External references

OECD Guidelines for the Testing of Chemicals, 405 (2021)

Directive 2004/73/EC B.5 (L 152 2004 29 April) Commission Regulation (EU) No 2017/735, B.5 (L 112, 14 February 2017) amending Regulation (EC) No 440/2008

OPPTS 870.2400 (EPA 712-C-98-195) August 1998

Draize J. H. (1977): Dermal and eye toxicity tests. In: Principles and procedures for evaluating the toxicity of household substances. National Academy of Sciences, 31–2.

Kay J. H., Calandra J. C. (1962): Interpretation of eye irritation tests. J Soc Cosmet Chem 13:281–289.

Internal references

Balázs Orovecz, B.Sc. (2021): Difenoconazole/Fludioxonil/Metalaxyl-M/Cyclobutrifluram FS (A23793B) - *In Vitro* Eye Irritation Test in Isolated Chicken Eyes. Charles River Laboratories Hungary Kft. H-8200 Veszprém, Szabadságpuszta, hrsz. 028/1., Hungary. Study code: 21/245-038CS.

TABLES SECTION

TABLE 1 **Individual Draize Scores and Individual Total Scores* for Ocular Irritation**

**Based on Kay J H and Calandra J C (1962)*

Rabbit number and sex	**2899, male**				**2886, male**				**2888, male**			
IPR	0				0				0			
PR	0	0	0	0	0	0	0	0	0	0	0	0
Time after treatment	1 Hr	24 Hr	48 Hr	72 Hr	1 Hr	24 Hr	48 Hr	72 Hr	1 Hr	24 Hr	48 Hr	72 Hr
CORNEA												
E = Degree of Opacity	1	0	0	0	0	0	0	0	0	0	0	0
F = Area of Cornea involved	2	0	0	0	0	0	0	0	0	0	0	0
* Score (E x F) x 5	10	0	0	0	0	0	0	0	0	0	0	0
IRIS												
D	0	0	0	0	0	0	0	0	0	0	0	0
* Score (D x 5)	0	0	0	0	0	0	0	0	0	0	0	0
CONJUNCTIVAE												
A = Redness	2	0	0	0	2	0	0	0	2	0	0	0
B = Chemosis	1	0	0	0	0	0	0	0	0	0	0	0
C = Discharge	2	0	0	0	1	0	0	0	1	0	0	0
* Score (A+B+C) x 2	10	0	0	0	6	0	0	0	6	0	0	0
* Total Score	20	0	0	0	6	0	0	0	6	0	0	0

IPR: Initial pain reaction
PR: Pain reaction
Hr: Hour(s)
W: Week(s)

TABLE 2 **Eye Irritation Scores - Mean Values after 1, 24, 48 and 72 Hours**

Animal Number	Sex	Corneal opacity	Iritis	Conjunctival redness	Conjunctival oedema (chemosis)
2899	male	0.25	0	0.50	0.25
2886	male	0.00	0	0.50	0.00
2888	male	0.00	0	0.50	0.00

TABLE 3 **Individual Total Scores and Group Mean Scores for Ocular Irritation Calculated from the Draize Scores**

Rabbit Number and Sex	* Individual Total Scores At:			
	1 Hour	24 Hours	48 Hours	72 Hours
2899, Male	20	0	0	0
2886, Male	6	0	0	0
2888, Male	6	0	0	0
* Group Total	32	0	0	0
* Group Mean Score	10.67	0.00	0.00	0.00

*: Kay J H and Calandra J C (1962)

TABLE 4 Individual Fluorescein Staining

Rabbit Number and Sex	Fluorescein Staining (treated eye) at times after treatment	
	-24 Hours	24 Hours
2899 Male	-	-
2866 Male	-	-
2888 Male	-	-

Remarks:
- : Absence of Fluorescein Stain
+ : Presence of Fluorescein Stain

TABLE 5 Individual Bodyweights and Bodyweight Change

Rabbit Number and Sex	Individual Bodyweight (g)		Bodyweight Change (g)
	Before treatment	At termination	
2899, Male	3171	3242	71
2886, Male	3428	3494	66
2888, Male	3320	3418	98

TABLE 6 Individual Clinical Signs

Rabbit Number and Sex	Day 0	Day 1	Day 2	Day 3
2899, Male	N	N	N	N
2886, Male	N	N	N	N
2888, Male	N	N	N	N

Y: Present
N: Absent

APPENDICES SECTION

APPENDIX 1 Certificate of Analysis

syngenta

Syngenta Crop Protection, LLC
Analytical and Product Chemistry
Greensboro, NC 27409

Certificate of Analysis

A23793B

Batch ID 1200767 (GP210610)

Test Substance Name:	CGA169374/CGA173506/CGA329351/SYN549522 FS (062.51/049.93/050.05/250.08)
Common Name:	Difenoconazole/Fludioxonil/Metalaxyl-M/Cyclobutrifluram FS (062.51/049.93/050.05/250.08)
Material ID:	A23793B
Batch ID:	1200767
Other ID:	GP210610
Source:	Syngenta Crop Protection LLC., 410 Swing Road, Greensboro, NC 27409, US

Chemical Analysis

AI	% w/w	g/L
Difenoconazole	5.45	64.0
Fludioxonil	4.37	51.3
Metalaxyl-M	4.31	50.6
Cyclobutrifluram	21.0	247

Identity of the Active Ingredients: Confirmed

Methodology Used for Characterization: LC, mass spectrometry, oscillating density meter.

The Active Ingredient(s) content is within the FAO limits.

Isomer Assay

Analyte	Isomer	% w/w
CGA329351	D-alanine, N-(2,6-dimethylphenyl)-N-(methoxyacetyl)-, Methyl Ester	4.15
CGA351920	L-alanine, N-(2,6-dimethylphenyl)-N-(methoxyacetyl)-, Methyl Ester	0.15

COA Number: USGR210208

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Physical Analysis

Analyte	Value	Units
Density	1.174	g/cm3

Appearance: red liquid

Storage Temperature: <30°C

Re-certification Date:End of Aug/2024

If stored under the conditions given above, this test substance can be considered stable until the recertification date is reached.

The stability of this test substance will be determined concurrently through reanalysis of material held in inventory under GLP conditions at Syngenta Crop Protection, LLC, Greensboro, NC.

This Certificate of Analysis is summarizing data from a study that has been performed in compliance with Good Laboratory Practices per 40 CFR Part 160. Raw data, documentation, protocols, any amendments to study protocols and reports pertaining to this study are maintained in the Syngenta Crop Protection Archives in Greensboro, NC.

Study Number: USGR210208

Authorization: Sherry Perine

Sherry C Perine

Sherry Perine

Analytical and Product Chemistry Department

Aug 24, 2021

Date

COA Number: USGR210208

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APPENDIX 2 Pain Reaction

When the test material is instilled in the eye there may be an initial local pain reaction (IPR) and local pain reaction (PR). The reaction was graded as follows:

IPR/PR Score	Reaction by Animal	Descriptive Rating
0	No response	No pain
1	A few blinks only, normal within one or two minutes	Practically no pain
2	Rabbit blinks and tries to open eye, but reflex closes it	Slight pain
3	Rabbit holds eye shut and puts pressure on lids, may rub eye with paw	Moderate pain
4	Rabbit holds eye shut vigorously, may squeal	Severe pain
5	Rabbit holds eye shut vigorously, may squeal, claw at eye, jump and try to escape	Very severe pain

NOTE: if an IPR/PR score of 4 or 5 is observed, or if more than transient score 3 is observed, then the rabbit is treated with "rescue analgesia".

APPENDIX 3 Draize Scale for Scoring Ocular Irritation

1. 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
Diffuse beefy red	3

(B) Chemosis

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

(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 a considerable area around the eye	3

* **THE TOTAL SCORE = (A + B + C) x 2** **MAXIMUM TOTAL = 20**

2. IRIS

(D) 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
No reaction to light, haemorrhage, gross destruction (any or all of these)	2

* **THE TOTAL SCORE = D x 5** **MAXIMUM TOTAL = 10**

3. CORNEA

(E) Degree of Opacity (most dense area used)

No opacity	0
Scattered or diffuse areas, details of iris clearly visible	1
Easily discernible translucent areas, details of iris slightly obscured	2
Opalescent areas, no details of iris visible, size of pupil barely discernible	3
Opaque, iris not discernible through the opacity	4

(F) 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

* **THE TOTAL SCORE = (E x F) x 5** **MAXIMUM TOTAL = 80**

* **MAXIMUM TOTAL SCORE POSSIBLE = 110**

*: Total scores according to Kay and Calandra system (1962)

APPENDIX 4 Modified Kay and Calandra Interpretation of Eye Irritation Test

MAXIMUM MEAN SCORE		PERSISTENCE OF SCORE	DESCRIPTION RATING (AND CLASS)	
0.0 to 0.5	Group mean total score at 24 hours = 0		Non-irritant	(1)
	Group mean total score at 24 hours > 0		Practically non-irritant	(2)
0.5 to 2.5	Group mean total score at 24 hours = 0		Practically non-irritant	(2)
	Group mean total score at 24 hours > 0		Minimal irritant	(3)
2.5 to 15	Group mean total score at 48 hours = 0		Minimal irritant	(3)
	Group mean total score at 48 hours > 0		Mild irritant	(4)
15 to 25	Group mean total score at 72 hours = 0		Mild irritant	(4)
	Group mean total score at 72 hours > 0		Moderate irritant	(5)
25 to 50	Group mean total score at 7 days 20 or less	More than half of the individual total scores at 7 days 10 or less	Moderate irritant	(5)
		More than half of the individual total scores at 7 days > 10 but no individual total score at 7 days > 30	Moderate irritant	(5)
		More than half of the individual total scores at 7 days > 10 and any individual score at 7 days > 30	Severe irritant	(6)
	Group mean total score at 7 days > 20		Severe irritant	(6)
50 to 80	Group mean total score at 7 days 40 or less	More than half of the individual total scores at 7 days 30 or less	Severe irritant	(6)
		More than half of the individual total scores at 7 days > 30 but no individual total scores at 7 days > 60	Severe irritant	(6)
		More than half of the individual total scores at 7 days > 30 and individual total score at 7 days > 60	Very severe irritant	(7)
	Group mean total score at 7 days > 40		Very severe irritant	(7)
80 to 100	Group mean total score at 7 days 80 or less	More than half of the individual total scores at 7 days 60 or less	Very severe irritant	(7)
		More than half of the individual total scores at 7 days > 60 but no individual total score at 7 days > 100	Very severe irritant	(7)
		More than half of the individual total scores at 7 days > 60 and individual total score at 7 days > 100	Extremely severe irritant	(8)
	Group mean total score at 7 days > 80		Extremely severe irritant	(8)
100 to 110	Group mean total score at 7 days 80 or less		Very severe irritant	(7)
	Group mean total score at 7 days > 80		Extremely severe irritant	(8)

APPENDIX 5 GLP Certificate

OGYÉI Országos Gyógyszerészeti és Élelmezés-egészségügyi Intézet
Hatósági Ellenőrzési Főosztály

1051 Budapest, Zrínyi utca 3.
Levélcím: 1372 Postafiók 450
Tel.: +36 1 886 9300, Fax: +36 1 886 9460
E-mail: ogyei@ogyei.gov.hu
Web: www.ogyei.gov.hu

Ref. no: OGYÉI/-29520-2/2021
Admin.: Dr. Szaller Zoltán

GOOD LABORATORY PRACTICE (GLP) CERTIFICATE

It is hereby certified that the test facility

Charles River Laboratories Hungary Kft.

H-8200 Veszprém, Szabadságpuszta

is able to carry out

physico-chemical testing, toxicity studies, mutagenicity studies, environmental toxicity studies on aquatic or terrestrial organisms, studies on behaviour in water, soil and air; bio-accumulation, analytical and clinical chemistry, pathology studies, preparation of microscopic tissue sections, reproduction toxicology, in vitro studies, inhalation toxicology, and contract archiving

in compliance with the Principles of GLP (Good Laboratory Practice) and also complies with the corresponding OECD/European Community requirements.

Date of the inspection: **07-11 May 2018.**

This certificate is valid up to 11th of May, 2022.

Dr. Lukács Ferenc József
Digitálisan aláírta: Dr. Lukács Ferenc József
Dátum: 2021.05.06 13:04:14 +02'00'

Dr. Ferenc Lukács
Head of Inspectorate

Note: Translation of the text of the certificate in the header: ("Országos Gyógyszerészeti és Élelmezés-egészségügyi Intézet") - ("National Institute of Pharmacy and Nutrition"); (“Hatósági Ellenőrzési Főosztály”) - (Inspectorate Division) and at the signature: (“Digitálisan aláírta”) - (Digitally signed); (“Dátum”) - (“Date”).