**Isopyrazam/Difenoconazole**

**Isopyrazam/Difenoconazole SC (A21295D) -**
**Acute Eye Irritation Study in Rabbits**

**Final Report**

**DATA REQUIREMENT(S):** OECD 405 (2012)
EPA OPPTS 870.2400 (1998)
EC No 440/2008, B.5 (2008)
Directive 2004/73/EC B.5 (L 152 2004)

**AUTHOR(S):** Éva Váliczkó, M.Sc.

**COMPLETION DATE:** 23 May 2016

**PERFORMING LABORATORY:** CiToxLAB Hungary Ltd.
H-8200 Veszprém, Szabadságpuszta,
Hungary

**LABORATORY PROJECT ID:** Report Number: 15/419-005N
Study Number: 15/419-005N
Task Number: TK0283676

**SPONSOR(S):** Syngenta Ltd
Jealott's Hill International Research Centre,
Bracknell, Berkshire, RG42 6EY, United Kingdom

**VOLUME 1 OF 1 OF STUDY**

## STATEMENT OF DATA CONFIDENTIALITY CLAIMS

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# GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study has been performed in accordance with the study plan, and 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.).

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.

Signature: _____ Date: 23 May 2016

Éva Váliczkó, M.Sc.
Study Director

Performing Laboratory: CiToxLAB Hungary Ltd.
H-8200 Veszprém, Szabadságpuszta,
Hungary

## FLAGGING STATEMENT

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# QUALITY ASSURANCE STATEMENT

Study Number: 15/419-005N

Study Title: Isopyrazam/Difenoconazole SC (A21295D) - Acute Eye Irritation Study in Rabbits

Test Item: Isopyrazam/Difenoconazole SC (A21295D)

This study has been inspected, and this report audited by the Quality Assurance Unit in compliance with the Principles of Good Laboratory Practice. As far as it can be reasonably established the methods described and the results incorporated in this report accurately reflect the raw data produced during this study.

All inspections, data reviews and the report audit were reported in written form to the study director and to management. The dates of such inspections and of the report audit are given below:

| Date of Inspection | Phase(s) Inspected/Audited | Date of report to | |
|---|---|---|---|
| | | Management | Study Director |
| 25 January 2016 | Study Plan | 25 January 2016 | 25 January 2016 |
| 27 January 2016 | Treatment | 27 January 2016 | 27 January 2016 |
| 06 April 2016 | Draft Report | 06 April 2016 | 06 April 2016 |
| 20 May 2016 | Final Report | 20 May 2016 | 20 May 2016 |

Signature: Meraiga Leila Date: 25 May 2016

Leila Merazga, M.Sc.
On Behalf of QA

## MANAGEMENT STATEMENT

According to the conditions of the research and development agreement between Syngenta Ltd. (as Sponsor) and CiToxLAB Hungary Ltd. (as Test Facility) the study titled "Isopyrazam/Difenoconazole SC (A21295D) - Acute Eye Irritation Study in Rabbits" was performed, in compliance with the Principles of Good Laboratory Practice.

Signature: _____ Date: 13 May 2016

Szabolcs Gáty, M.Sc.
Senior Director of Operations

# GENERAL INFORMATION

## Contributors

The following contributed to this report in the capacities indicated:

| Name | Function |
|---|---|
| Éva Váliczkó, M.Sc. | Study Director |
| Zsolt Tarcai, M.Sc. | Assistant Scientist |
| Leila Merazga, M.Sc. | Quality Assurance Unit |
| István Pásztor, DVM | Veterinary Control |
| Edina Röber, DVM | Veterinary Control |
| Claire Elliott | Syngenta Study Manager |

## Study dates

| | |
|---|---|
| Study Initiation Date: | 26 January 2016 |
| Experimental Starting Date: | 27 January 2016 |
| Experimental Completion Date: | 01 February 2016 |
| Acclimatization: | 21 - 26/28 January 2016 |
| Treatment: | 27/29 January 2016 |
| Draft Report: | 08 April 2016 |

## Deviations from the guidelines

The actual relative humidity range was 24-48 % instead of 30-70 % as it was indicated in the Study Plan. This deviation is considered to have no impact on the results or integrity of the study.

## Retention of samples

See in other below.

## Performing laboratory test substance reference number

150407

**Other**

The study documents and samples:
- study plan,
- all raw data,
- sample of the test item,
- final study report and any amendments,
- correspondence

will be archived according to the Hungarian GLP and applicable SOP's in the archives of CiToxLAB Hungary Ltd. 8200 Veszprém, Szabadságpuszta, 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 isopyrazam/difenoconazole SC (A21295D) was investigated using 3 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 eye serving as control. Scoring of irritation effects was performed approximately 1, 24, 48 and 72 hours after test material installation in all animals. Observations with fluorescein staining were made approximately 24 hours before treatment and then 24, 48 and 72 hours after the treatment in all animals. 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.

## 1.2 Results

No Initial Pain Reaction/Pain reaction (IPR/PR) was observed.

Conjunctival redness (score 1), chemosis (score 1) and discharge (score 1) were seen in all rabbits at 1 hour after treatment. At 24, 48 and 72 hours after the application, no clinical signs and no corneal effects were observed in any animal.

Fluorescein staining was negative in all animals during the experiment.

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

No mortality occurred during the study and bodyweights of all rabbits were considered to be within the normal range of variability.

## 1.3 Conclusion

The test item isopyrazam/difenoconazole SC (A21295D) 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 the test item, 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* study (CiToxLAB code: 15/419-038CS) indicated that the test item was non-irritant *in vitro*.

## 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 02 October 2012.
- Directive 2004/73/EC B.5 (L 152 2004 29$^{th}$ April).
- United States Environmental Protection Agency, Health Effects Test Guidelines, OPPTS 870.2400, Acute Eye Irritation EPA 712-C-98-195, August 1998.
- Commission Regulation (EC) No 440/2008, B.5 (L 142, 30 May 2008).

## 2.3 Test Facility

This study was performed in an AAALAC-accredited laboratory. The Institutional Animal Care and Use Committee (IACUC) of CiToxLAB Hungary Ltd. reviewed the study plan and authorized the conduct of the study.

# 3.0 MATERIALS AND METHODS

## 3.1 Test Substance

The following information was provided by the Sponsor.

| | |
|---|---|
| Name: | Isopyrazam/difenoconazole SC (A21295D) |
| Batch number: | JHU002-109-004 |
| Product code: | A21295D |
| Other Product Code: | CGA169374/SYN520453 SC (125/125) |
| Appearance: | Off-white liquid |
| Active Ingredient Content: | CGA169374: 11.9 % w/w corresponding to 129 g/l<br>CGA185882: 6.97 % w/w corresponding to 76.0 g/l<br>CGA185883: 4.89 % w/w corresponding to 53.3 g/l<br>SYN520453: 11.9 % w/w corresponding to 130 g/l<br>SYN534969: 10.1 % w/w corresponding to 110 g/l<br>SYN534968: 1.82 % w/w corresponding to 19.9g/l |
| Density: | 1091 kg/$m^3$ |
| Recertification date: | 30 November 2018 |
| Storage conditions: | Room temperature (<30$^o$C) |
| Safety precautions: | Routine safety precautions (lab coat, gloves, safety glasses and face mask) for unknown materials were applied to assure personnel health and safety. Causes serious eye irritation, may cause an allergic skin reaction. Suspected of damaging the unborn child. Very toxic to aquatic life. |

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: | Disposable Syringe Luer Solo, 20 mL |
| Lot No.: | 1G11048 |
| Expiry Date: | July 2016 |
| Supplier: | Braun |

| | |
|---|---|
| Name: | Saline (NaCl (0.9%)) |
| Lot No.: | 51642Y05-1 |
| Expiry Date: | 31 March 2018 |
| Produced by: | B.Braun Melsungen AG |

Name: SOLOGARD Syringe, 1 mL
Lot No.: 2014 10
Expiry Date: February 2019
Manufactured by: SAFEGARD MEDICAL (Hungary) KFT.

Name: Fluorescein 100 mg/mL
Batch No.: 13221042
Expiry Date: 30 April 2016
Produced by: Delpharm Huningue SAS

Systemic opiate analgesic:

Name: Bupaq 0.3 mg/mL
Batch No.: 0115034AD
Expiry Date: December 2017
Produced by: Richterpharma AG

Topical ocular anaesthetic:

Name: Humacain 4 mg/mL
Batch No.: 7360914
Expiry Date: September 2017
Produced by: Teva zRt.

Nonsteroidal anti-inflammatory drug:

Name: Metacam 5 mg/mL
Batch No.: G20806C-05
Expiry Date: June 2017
Produced by: Boehringer Ingelheim

## 3.3 Experimental Design

### 3.3.1 Animals

| | |
|---|---|
| Species and strain: | New Zealand white rabbit |
| Source: | S&K-LAP Kft.<br>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: | 11-12 weeks |
| Body weight range at dosing: | 2955 – 3131 g |
| Body weight range at termination: | 3060 – 3237 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. |
| Acclimatization time: | at least 5 days |

### 3.3.2 Husbandry

| | |
|---|---|
| Animal health: | Only healthy animals were used for the study, as certified by the veterinarian. |
| Room: | 609 |
| Housing/Enrichment: | Rabbits were individually housed in AAALAC approved metal wire rabbit cages. Cages were of an open wire structure and cages were placed together to allow some social interaction with rabbit(s) in adjoining cages. |
| Light: | 12 hours daily from 6.00 a.m. to 6.00 p.m. (and during the analgesic/anaesthetic treatment) |
| Temperature: | 18.5 – 21.5 °C |
| Relative humidity: | 24 – 48 % |
| Ventilation: | 15-20 air exchanges/hour. |

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

### 3.3.3 Food and feeding

The animals received UNI diet for rabbits produced by Cargill Takarmány ZRT., H-5300 Karcag, Madarasi út, Hungary, *ad libitum*. The batch numbers of the lots used in the study were: Lot number: 0002882754, Expiry date: 26 January 2016 and Lot number: 0003003494, Expiry date: 17 March 2016. A detailed description of the contents of the lots used is archived with the raw data at CiToxLAB Hungary Ltd.

### 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 once every 3 months and microbiological assessment is performed monthly, by Veszprém County Institute of State Public Health and Medical Officer Service (ÁNTSZ, H-8201 Veszprém, József A.u.36., Hungary). The quality control results are retained in the archive at CiToxLAB Hungary Ltd.

## 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. According to the results from the *in vitro* eye irritation study (CiToxLAB code: 15/419-038CS) in the Isolated Chicken Eye model with test item, in accordance with the guidance from the OECD 438 for this method, suggests that the test item is a non-irritant.

### 3.4.2 Identification of pH

The pH of the test item was measured as pH 5.0, 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 local effects were non-irritating (all scores were zero) at 24 hours, therefore two further rabbits were treated with test item.

### 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. Repeat injections were given on the first day as appropriate to maintain an adequate level of analgesia.

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 hr) after test substance application, a systemic opiate analgesic and a nonsteroidal 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.

**Systemic opiate analgesic:** Buprenorphine 0.01 mg/kg.
**Topical ocular anaesthetic:** Humacain (oxybuprocaine) one-two drops per eye.
**Nonsteroidal anti-inflammatory drug**: Meloxicam 0.5 mg/kg.

## 3.5 Administration of the Test Item

### 3.5.1 Dosage

A single volume of the 0.1 ml of isopyrazam/difenoconazole SC (A21295D) 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: 24, 48 and 72 hours after test item application as part of the fluorescein observation process.

## 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 and 72 hours in all animals after test material installation. Observations with fluorescein staining were made approximately 24 hours before treatment and then 24, 48 and 72 hours after treatment in all animals.

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 (02 October 2012) 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 4).

## 3.7 *Post Mortem* Investigations

At the end of the observation period, animals were euthanised by intramuscular injections of ketamin 10% (Ketamidor) and xylazin 2% (Primazin) followed by intravenous pentobarbital sodium (Euthanimal 40%) anaesthesia. Following confirmation of death, the carcasses were discarded without further examination. Details of materials employed for euthanasia are retained in the raw data and detailed in Section 3.7.1.

### 3.7.1 Materials used for euthanasia

| | |
|---|---|
| Name: | Ketamidor |
| Batch No.: | 0914489AG |
| Expiry Date: | August 2017 |
| Produced by: | Richterpharma ag, 4600 Wels, Austria |
| | |
| Name: | Primazin |
| Batch No.: | 1404117-01 |
| Expiry Date: | May 2016 |
| Produced by: | Alfasan International B.V., Kuipersweg 9, 3449 JA Woerden, The Netherlands |

Name: Euthanimal 40%
Lot No.: 1409236-06
Expiry Date: September 2017
Produced by: Alfasan International B.V., Kuipersweg 9, 3449 JA Woerden, The Netherlands

# 4.0 RESULTS AND DISCUSSION

## 4.1 Ocular Reactions

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

No Initial Pain Reaction/Pain reaction (IPR/PR) was observed.

Conjunctival redness (score 1), chemosis (score 1) and discharge (score 1) were seen in all rabbits at 1 hour after treatment. At 24, 48 and 72 hours after the application, no clinical signs and no corneal effects were observed in any animal.

Fluorescein staining was negative in all animals during the experiment. (Table 3)

The control eyes were symptom-free during the study.

## 4.2 Body Weight

The bodyweights of all rabbits were considered to be within the normal range of variability. Individual bodyweights and bodyweight changes are given in Table 4.

## 4.3 Clinical Signs

No clinical signs of systemic toxicity were observed in any animal in this study (Table 5).

## 4.4 Mortality

No mortality occurred during this study.

# 5.0 CONCLUSIONS

The test item isopyrazam/difenoconazole SC (A21295D) 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.

## TABLES SECTION

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

| Rabbit number and sex | 668 Male | | | | 671 Male | | | | 675 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 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| F = Area of Cornea involved | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| * Score (E x F) x 5 | 0 | 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 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| B = Chemosis | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| C = Discharge | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| * Score (A+B+C) x 2 | 6 | 0 | 0 | 0 | 6 | 0 | 0 | 0 | 6 | 0 | 0 | 0 |
| * Total Score | 6 | 0 | 0 | 0 | 6 | 0 | 0 | 0 | 6 | 0 | 0 | 0 |

*IPR: Initial pain reaction*
*PR: Pain reaction*
*Hr: Hour(s)*
** Kay J H and Calandra J C (1962)*

**TABLE 2 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 |
| 668 Male | 6 | 0 | 0 | 0 |
| 671 Male | 6 | 0 | 0 | 0 |
| 675 Male | 6 | 0 | 0 | 0 |
| * Group Total | 18 | 0 | 0 | 0 |
| * Group Mean Score | 6.00 | 0.00 | 0.00 | 0.00 |

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

**TABLE 3 Individual Fluorescein Staining**

| Rabbit Number and sex | Fluorescein Staining (treated eye) | | | |
|---|---|---|---|---|
| | 24 Hours Prior to Instillation | 24 Hours After Instillation | 48 Hours After Instillation | 72 Hours After Instillation |
| 668 Male | - | - | - | - |
| 671 Male | - | - | - | - |
| 675 Male | - | - | - | - |

- : Absence of Fluorescein Stain

## TABLE 4 Individual Bodyweights and Bodyweight Change

| Rabbit Number and Sex | Individual Bodyweight (g) | | Bodyweight Change (g) |
|---|---|---|---|
| | Before treatment | At termination | |
| 668 Male | 3131 | 3183 | 52 |
| 671 Male | 2955 | 3060 | 105 |
| 675 Male | 3081 | 3237 | 156 |

## TABLE 5 Individual Clinical Signs

| Rabbit Number and Sex | Day 0 | Day 1 | Day 2 | Day 3 |
|---|---|---|---|---|
| 668 Male | N | N | N | N |
| 671 Male | N | N | N | N |
| 675 Male | N | N | N | N |

N: Symptom-free

## 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 SYN534968 (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/m$^3$** |

**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.

syngenta

**GLP Testing Facility WMU**
Analytical Development &
Product Chemistry GS2131

**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 recetification: | --- |
| Authorisation: | 14-Dec-2015 |

Elke Ebi
Analytical Development & Product Chemistry

## 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

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**Ref. no: OGYI/19440-7/2015**
**Admin.**: Szatmári Andrea
**Date:** 22 September, 2015

### GOOD LABORATORY PRACTICE (GLP) CERTIFICATE

It is hereby certified that the test facility

**CiToxLAB Hungary Ltd.**

**H-8200 Veszprém, Szabadságpuszta**

is able to carry out

***physico-chemical testing, toxicity studies, in vitro studies and mutagenicity studies, environmental toxicity studies on aquatic or terrestrial organisms, studies on behaviour in water, soil and air; bio-accumulation, reproduction toxicology, inhalation toxicology, analytical chemistry 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: **02-04. June 2015.**

Dr. József Reiter
Deputy Director-General

Note: Translation of the Stamp on the official document ("Országos Gyógyszerészeti és Élelmezés-egészségügyi Intézet"): ("National Institute of Pharmacy and Nutrition")