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**Profenofos**

**Profenofos EC (A8591B) -**
**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:** 07 April 2022

**PERFORMING LABORATORY:** Charles River Laboratories Hungary Kft.
H-8200 Veszprém, Szabadságpuszta, hrsz. 028/1.,
Hungary

**LABORATORY PROJECT ID:** Report Number: 21/201-005N
Study Number: 21/201-005N
Task Number: TK0655770

**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:**

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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: 07 April 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: 21/201-005N

Study Title: Profenofos EC (A8591B) - 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 |
| 24 January 2022 | Study Plan | 24 January 2022 | 24 January 2022 |
| 18 January 2022 | Treatment | 18 January 2022 | 18 January 2022 |
| 25 March 2022 | Body weight measurement | 25 March 2022 | 25 March 2022 |
| 23 March 2022 | Clinical observation | 23 March 2022 | 23 March 2022 |
| 06 April 2022 | Draft Report | 06 April 2022 | 06 April 2022 |
| 06 April 2022 | Final Report | 06 April 2022 | 06 April 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: Nyitrai Ildikó Date: 07. april 2022

Ildikó Nyitrai, M.Sc.
On Behalf of QA

# 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 "Profenofos EC (A8591B) – Acute Eye Irritation Study in Rabbits" was performed, in compliance with the Principles of Good Laboratory Practice.

Signature: _____ Date: 07 April 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 |
| Ágnes Rédl, M.Sc. | Quality Assurance |
| Ildikó Nyitrai, M.Sc. | 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: | 24 January 2022 |
| Experimental completion date: | 03 March 2022 |
| Receipt of animals: | 12 January 2022 |
| Acclimatisation: | 12 – 24 January, 12 January – 08 February, 12 January – 16 February 2022 |
| Treatment: | 25 January 2022 (animal no. 2885)<br>09 February 2022 (animal no. 2895)<br>17 February 2022 (animal no. 2898) |
| Observation: | 25 January – 15 February 2022 (animal no. 2885)<br>09 February – 02 March 2022 (animal no. 2895)<br>17 February – 03 March 2022 (animal no. 2898) |
| Draft Report (non-QA audited): | 05 April 2022 |
| Draft Report (QA audited): | 06 April 2022 |
| Final Report: | 07 April 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 19-23°C were recorded occasionally in the animal rooms during the study.

Due to technical error, the 24-hours clinical observation of the second animal was slightly out (+ 14 minutes) of the recommended range, 24 hour (± 5 minutes), as indicated in the Study Plan.

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

**Performing laboratory test substance reference number**

**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, profenofos EC (A8591B), 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, 1 week, 2 weeks and 3 weeks after test material installation in case of the first and second treated animals and 1, 24, 48, 72 hours, 1 week and 2 weeks after test material installation in case of the third treated animal. Observations with fluorescein staining were made approximately 24 hours before treatment and then 24, 48, 72 hours, 1 week, 2 weeks and 3 weeks after treatment in case of the first and second treated animals and 1, 24, 48, 72 hours, 1 week and 2 weeks after test material installation in case of the third treated animal. 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

Initial Pain Reaction (IPR) (score 2) was observed in the second and third animal.

No Pain Reaction (PR) was observed in any animal during the study.

Eye irritation results with profenofos EC (A8591B):

| Animal No. | | Scores after treatment * | | | | Mean scores (24-72 h) | Reversible (day) |
|---|---|---|---|---|---|---|---|
| | | 1 h | 24 h | 48 h | 72 h | | |
| 2885 | Corneal opacity | 1 | 1 | 1 | 1 | 1.00 | 7 |
| | Iritis | 0 | 0 | 0 | 0 | 0.00 | - |
| | Redness conjunctivae | 2 | 2 | 2 | 1 | 1.67 | 21 |
| | Chemosis conjunctivae | 2 | 2 | 2 | 1 | 1.67 | 14 |
| | Discharge | 3 | 3 | 1 | 0 | 1.33 | 3 |
| 2895 | Corneal opacity | 1 | 1 | 0 | 0 | 0.33 | 2 |
| | Iritis | 0 | 0 | 0 | 0 | 0.00 | - |
| | Redness conjunctivae | 2 | 2 | 2 | 1 | 1.67 | 14 |
| | Chemosis conjunctivae | 3 | 1 | 1 | 1 | 1.00 | 7 |
| | Discharge | 2 | 1 | 0 | 0 | 0.33 | 2 |
| 2898 | Corneal opacity | 1 | 1 | 1 | 1 | 1.00 | 7 |
| | Iritis | 0 | 0 | 0 | 0 | 0.00 | - |
| | Redness conjunctivae | 2 | 2 | 2 | 1 | 1.67 | 14 |
| | Chemosis conjunctivae | 3 | 3 | 1 | 1 | 1.67 | 7 |
| | Discharge | 2 | 1 | 1 | 1 | 1.00 | 7 |

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

The first treated animal became symptom-free at 3 weeks after treatment, the second animal became symptom-free at 2 weeks after treatment (however the fluorescein staining was positive at 2, 3 weeks after treatment), the third animal became symptom-free at 2 weeks after treatment therefore, the study was terminated 2 weeks after the treatment of the third rabbit.

Fluorescein staining was positive at 24, 48, 72 hours and at 1 week after treatment in the case of the first animal, at 24, 48, 72 hours and at 2, 3 weeks after treatment in the case of the second animal and at 24, 48, 72 hours after treatment in the case of the third animal.

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 within the normal range of variability.

## 1.3 Conclusion

The test item profenofos EC (A8591B) was graded as a moderate irritant (Class 5 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 profenofos EC (A8591B), 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 profenofos EC (A8591B) (Charles River Laboratories Hungary Kft. study code: 21/201-038CS) concluded that the test item is 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: | Profenofos EC (A8591B) |
| Other name: | CGA15324 EC (960) |
| Batch number: | RAN001-099-019 |
| Design code: | A8591B |
| Active ingredient content*: | Content of profenofos 73.12 % w/w corresponding to 969.03 g/L |
| Density: | 1.3253 g/cm³ |
| Appearance: | Light yellow liquid |
| Recertification date: | 06 May 2023 |
| Storage conditions: | Room temperature (< 30°C) |
| Safety precautions: | Enhanced safety precautions (nitrile gloves, goggles, face mask (ABEK-P3-filter), lab coat) for unknown materials were applied to assure personnel health and safety). |

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

The pH of the test item was measured and was found to be 6.02.

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 / 10651Y05-2 |
| Expiry Date: | 30 November 2022 / 31 January 2024 |
| Produced by: | B. Braun Pharmaceuticals SA |

| | |
|---|---|
| Name: | Fluorescein 100 mg/mL |
| Batch No.: | 1037A / 10CAF |
| Expiry Date: | 28 February 2022 / 31 August 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).
Fluorescein (100 mg/mL) (Batch number: 10CAF, Expiry date: 31 August 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: S43245, Expiry date: 18 March 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., The Netherlands |

*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.<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: | ~11/13/14 weeks |
| Body weight range at dosing: | 3224 – 3759 g |
| Body weight range at termination: | 3921 – 4191 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: | 13 / 28 / 36 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: | 17.9 – 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
- Batch no.: 0008038307, expiry date: 13 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: 21/201-038CS) in the Isolated Chicken Eye model with profenofos EC (A8591B) (Batch number: RAN001-099-019), in accordance with the guidance from the OECD No. 438 for this method, concluded that the test item is non-irritant.

### 3.4.2 Identification of pH

The pH of the test item was measured as pH 6.02, 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 (2885) showed scores of above zero at 1, 24, 48, 72-hour, 1-week and 2-weeks observation points after treatment, and the fluorescein staining was positive at 24, 48, 72-hour, 1-week observation points after treatment. The first animal became symptom-free after 3 weeks, therefore the second rabbit (2895) was treated after the 2-week observation of the first rabbit.

The second animal showed scores of above zero at 1, 24, 48, 72-hour, 1-week observation points after treatment, the fluorescein staining was positive at 24, 48, 72-hour, 2-week and 3-week observation points after treatment. The fluorescein staining was negative at 1-week

observation points after treatment, therefore the third rabbit (2898) was treated after the 1-week observation of the second rabbit.

The third animal showed scores of above zero at 1, 24, 48, 72-hour, 1-week observation points after treatment, the fluorescein staining was positive at 24, 48, 72-hour observation points after treatment.

### 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 profenofos EC (A8591B) 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

In case of the second and third treated animal (ID 2895, ID 2898) at the 1-hour observation time point, conjunctival chemosis (score 3) was observed, therefore washing of the eye with physiological saline solution was necessary at 1 hour.

Both eyes of the test animals were rinsed with physiological saline solution following fluorescein control: 24, 48, 72 hours, 1 week, 2 weeks and/or 3 weeks 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, 72 hours, 1 week, 2 weeks and/or 3 weeks after test material installation.

Observations with fluorescein staining were made approximately 24 hours before treatment and then 24, 48, 72 hours, 1 week, 2 weeks and/or 3 weeks 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 then 1 week, 2 weeks or 3 weeks (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.

Initial Pain Reaction (IPR) (score 2) was observed in the second and third animal.

No Pain Reaction (PR) was observed in any animal during the study.

**One hour after the application**, redness (score 2), chemosis (score 3 or 2), discharge (score 3 or 2) and corneal opacity (score 1, area 4) were noted in all animals.

**At 24 hours after the treatment**, redness (score 2), chemosis (score 3 or 2 or 1), discharge (score 3 or 1) and corneal opacity (score 1, area 4) were noted in all animals. The rabbits showed positive reaction to fluorescein staining.

**At 48 hours after the treatment**, redness (score 2), chemosis (score 2 or 1) were noted in all animals. Discharge (score 1) and corneal opacity (score 1, area 4) were noted in the first and third animal. The rabbits showed positive reaction to fluorescein staining.

**At 72 hours after the treatment**, redness (score 1) and chemosis (score 1) were noted in all animals. Discharge (score 1) was noted in the third animal. Corneal opacity (score 1, area 4) was noted in the first and third animal. The rabbits showed positive reaction to fluorescein staining.

**At 1 week after the treatment**, redness (score 1) was noted in all animals. Chemosis (score 1) was noted in the first animal. Fluorescein staining was positive in the first animal.

**At 2 weeks after the treatment**, redness (score 1) was noted in the first animal. Fluorescein staining was positive in the second animal.

**At 3 weeks after the treatment**, no conjunctival or corneal effects were observed in the first and second animal. Fluorescein staining was positive in the second animal.

Fluorescein staining is presented in Table 4.

The study was terminated 2 weeks after the treatment of the third rabbit.

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

### 4.2 Bodyweight

The bodyweights of all rabbits were 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 profenofos EC (A8591B) was graded as a moderate irritant (Class 5 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): Profenofos EC (A8591B) - *In Vitro* Eye Irritation Test in Isolated Chicken Eyes. Charles River Laboratories Hungary Kft. H-8200 Veszprém, Szabadságpuszta, hrsz. 028/1., Hungary. Study code: 21/201-038CS.

## 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** | **2885, male** | | | | | | | **2895, male** | | | | | | | **2898, male** | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **IPR** | 0 | | | | | | | 2 | | | | | | | 2 | | | | | | |
| **PR** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | NA |
| **Time after treatment** | 1 Hr | 24 Hr | 48 Hr | 72 Hr | 1 W | 2 W | 3 W | 1 Hr | 24 Hr | 48 Hr | 72 Hr | 1 W | 2 W | 3 W | 1 Hr | 24 Hr | 48 Hr | 72 Hr | 1 W | 2 W | 3 W |
| **CORNEA** | | | | | | | | | | | | | | | | | | | | | |
| E = Degree of Opacity | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | NA |
| F = Area of Cornea involved | 4 | 4 | 4 | 4 | 0 | 0 | 0 | 4 | 4 | 0 | 0 | 0 | 0 | 0 | 4 | 4 | 4 | 4 | 0 | 0 | NA |
| * Score (E x F) x 5 | 20 | 20 | 20 | 20 | 0 | 0 | 0 | 20 | 20 | 0 | 0 | 0 | 0 | 0 | 20 | 20 | 20 | 20 | 0 | 0 | NA |
| **IRIS** | | | | | | | | | | | | | | | | | | | | | |
| D | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | NA |
| * Score (D x 5) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | NA |
| **CONJUNCTIVAE** | | | | | | | | | | | | | | | | | | | | | |
| A = Redness | 2 | 2 | 2 | 1 | 1 | 1 | 0 | 2 | 2 | 2 | 1 | 1 | 0 | 0 | 2 | 2 | 2 | 1 | 1 | 0 | NA |
| B = Chemosis | 2 | 2 | 2 | 1 | 1 | 0 | 0 | 3 | 1 | 1 | 1 | 0 | 0 | 0 | 3 | 3 | 1 | 1 | 0 | 0 | NA |
| C = Discharge | 3 | 3 | 1 | 0 | 0 | 0 | 0 | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 2 | 1 | 1 | 1 | 0 | 0 | NA |
| * Score (A+B+C) x 2 | 14 | 14 | 10 | 4 | 4 | 2 | 0 | 14 | 8 | 6 | 4 | 2 | 0 | 0 | 14 | 12 | 8 | 6 | 2 | 0 | NA |
| * Total Score | 34 | 34 | 30 | 24 | 4 | 2 | 0 | 34 | 28 | 6 | 4 | 2 | 0 | 0 | 34 | 32 | 28 | 26 | 2 | 0 | NA |

IPR: Initial pain reaction
PR: Pain reaction
Hr: Hour(s)
W: Week(s)
NA: Not Applicable

**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) |
|---|---|---|---|---|---|
| 2885 | male | 1.00 | 0 | 1.75 | 1.75 |
| 2895 | male | 0.50 | 0 | 1.75 | 1.50 |
| 2898 | male | 1.00 | 0 | 1.75 | 2.00 |

**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 | 1 Week | 2 Weeks | 3 Weeks |
| 2885, Male | 34 | 34 | 30 | 24 | 4 | 2 | 0 |
| 2895, Male | 34 | 28 | 6 | 4 | 2 | 0 | 0 |
| 2898, Male | 34 | 32 | 28 | 26 | 2 | 0 | NA |
| * Group Total | 102 | 94 | 64 | 54 | 8 | 2 | 0 |
| * Group Mean Score | 34.00 | 31.33 | 21.33 | 18.00 | 2.67 | 0.67 | 0.00 |

*: Kay J H and Calandra J C (1962)
NA: Not Applicable

## TABLE 4 Individual Fluorescein Staining

| Rabbit Number and Sex | Fluorescein Staining (treated eye) at times after treatment | | | | | | |
|---|---|---|---|---|---|---|---|
| | -24 Hours | 24 Hours | 48 Hours | 72 Hours | 1 Week | 2 Weeks | 3 Weeks |
| 2885, Male | - | + | + | + | + | - | - |
| 2895, Male | - | + | + | + | - | + | + |
| 2898, Male | - | + | + | + | - | - | NA |

Remarks:
- : Absence of Fluorescein Stain
+ : Presence of Fluorescein Stain
NA: Not Applicable

## TABLE 5 Individual Bodyweights and Bodyweight Change

| Rabbit Number and Sex | Individual Bodyweight (g) | | Bodyweight Change (g) |
|---|---|---|---|
| | Before treatment | At termination* | |
| 2885, Male | 3224 | 3998 | 774 |
| 2895, Male | 3759 | 4191 | 432 |
| 2898, Male | 3634 | 3921 | 287 |

*: The first and second animal was terminated at 3 weeks after treatment, the third animal was terminated after 2 weeks.

## TABLE 6 Individual Clinical Signs

| Rabbit Number and Sex | Day 0 | Day 1 | Day 2 | Day 3 | 1 week | 2 weeks | 3 weeks |
|---|---|---|---|---|---|---|---|
| 2885, Male | N | N | N | N | N | N | N |
| 2895, Male | N | N | N | N | N | N | N |
| 2898, Male | N | N | N | N | N | N | NA |

Y: Present
N: Absent
NA: Not Applicable

# APPENDICES SECTION

## APPENDIX 1 Certificate of Analysis

**ALS Laboratórios LS Ltda.**
Rua Fábia, 59 – CEP: 05051-030
São Paulo, SP - Brazil

**SYNGENTA PROTEÇÃO DE CULTIVOS Ltda.**
Rua Doutor Rubens Gomes Bueno nº 691,
11º andar, Torre Sigma
CEP 04730-000 – Bairro Várzea de Baixo
São Paulo-SP – Brazil

### Certificate of Analysis

**A8591B**
**Profenofos EC (960)**
**RAN001-099-019**

| | |
|---|---|
| **Batch Identification** | RAN001-099-019 |
| **Product Code** | A8591B |
| **Other Product Code(s)** | A8591; CGA15324 EC (960); EXF23490E |
| **EUP number** | 514/2020 Expiry date: 26/02/2023 |
| **Received on** | 12 May 2021 |
| **Source** | Syngenta Proteção de Cultivos Ltda.<br>Rodovia Professor Zeferino Vaz, SP 332, s/nº, km 127,5 – Bairro Santa Terezinha, CEP 13148-915 – Paulínia – SP – Brasil |

**Chemical Analysis**
**(Active Ingredients Content)**

| | |
|---|---|
| – **Content of Profenofos *** | **73.12 % w/w corresponding to 969.03 g/L** |

The Active Ingredient content is within the FAO limits.
Methodology used for Characterization: CG-FID (SF-1135/1)

**Physical Analysis**

| | |
|---|---|
| – **Density *** | 1.3253 g/cm³ |

**Stability:**

| | |
|---|---|
| – **Storage Temperature** | **<30°C** |
| – **Recertification Date** | **06 May 2023** |

If stored under the conditions given above, this test item 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. All original raw data, including any storage medium for electronically recorded data, documentation, the signed study plan, the protocol amendments, the final report and a sample of the test item will be retained in the GLP Archives at ALS Laboratórios LS Ltda.

Study number of batch characterization: 25926/2021CC

Authorization: 26 May 2021

Victor F.G. da Silva
*Victor Ferreira Gomes da Silva*
ALS Laboratórios LS Ltda.



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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”).