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Isocycloseram/Emamectin Benzoate

Isocycloseram/Emamectin Benzoate SC (A23220A) - Acute Eye Irritation Study in Rabbits

Final Report

TEST GUIDELINE(S): OECD 405 (2017)
EPA 870.2400 (1998)
EC No 2017/735, B.5 (2017)

AUTHOR(S): Ivett Orosz, M.Sc.

COMPLETION DATE: 23 July 2020

PERFORMING LABORATORY: Charles River Laboratories Hungary Kft.
(formerly Citoxlab Hungary Ltd.)
H-8200 Veszprém, Szabadságpuszta, hrsz. 028/1.,
Hungary

LABORATORY PROJECT ID: Report Number: 20/080-005N
Study Number: 20/080-005N
Task Number: TK0539399

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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UNDER SPECIFIED FIFRA PROVISIONS

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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: 23 July 2020

Ivett Orosz, M.Sc.
Study Director

Performing Laboratory: Charles River Laboratories Hungary Kft.
(formerly Citoxlab Hungary Ltd.)
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

This page is intentionally left blank. It will be replaced by an appropriate Flagging statement by the Sponsor.

QUALITY ASSURANCE STATEMENT

Study Number: 20/080-005N

Study Title: Isocycloseram/Emamectin Benzoate SC (A23220A) - Acute Eye Irritation Study in Rabbits

Test Item: Isocycloseram/Emamectin Benzoate SC (A23220A)

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
11 June 2020	Study Plan	11 June 2020	11 June 2020
23 June 2020	Treatment	23 June 2020	23 June 2020
09 July 2020	Draft Report	09 July 2020	09 July 2020
22 July 2020	Final Report	22 July 2020	22 July 2020

Signature: Merazga Leila Date: 23 July 2020

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 Charles River Laboratories Hungary Kft. (as Test Facility) the study titled "Isocycloseram/Emamectin Benzoate SC (A23220A) - Acute Eye Irritation Study in Rabbits" was performed, in compliance with the Principles of Good Laboratory Practice.

Signature: _____ Date: 23 July 2020

Balázs Tóth, Ph.D.
General Manager

GENERAL INFORMATION

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

Name	Function or Department
Ivett Orosz, M.Sc.	Study Director
Zsolt Tarcai, M.Sc.	Assistant Scientist
Leila Merazga, M.Sc.	Quality Assurance
László Székelyhidi, D.V.M.	Veterinary Care
Tamás Mészáros, Ph.D.	Pharmacy
Carolina Vaccari	Syngenta Study Manager

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

Study dates

Study Initiation Date:	15 June 2020
Experimental Starting Date:	23 June 2020
Experimental Completion Date:	28 June 2020
Receipt of animals:	06 May 2020
Acclimatisation:	06 May - 22 / 24 June 2020
Treatment:	23 June 2020 (animal no. 0190) 25 June 2020 (animal no. 0185, 0189)
Observation:	23 - 26 June 2020 (animal no. 0190) 25 - 28 June 2020 (animal no. 0185, 0189)
Draft Report:	10 July 2020
Final Report:	23 July 2020

Performing laboratory test substance reference number
200164

Deviation from the guideline
There was no deviation from the guideline.

Deviations from the Study Plan
Due to technical oversight, additional fluorescein staining was performed at the 24, 48 and 72-hour observation point in case of all animals. This fact had no impact on the study on the results or integrity of the study.

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. 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.

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1.0 EXECUTIVE SUMMARY

1.1 Study Design

The primary eye irritation effect of the test item isocycloseram/emamectin benzoate SC (A23220A) 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, 48, 72 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.

1.2 Results

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

Eye irritation results with isocycloseram/emamectin benzoate SC (A23220A) were as follows:

Animal No.	Observations	Scores after treatment *				Mean scores (24-72 h)	Reversible (day)
		1 h	24 h	48 h	72 h		
0190	Corneal opacity	0	0	0	0	0.00	-
	Iritis	0	0	0	0	0.00	-
	Redness conjunctivae	1	0	0	0	0.00	1
	Chemosis conjunctivae	0	0	0	0	0.00	-
	Discharge	0	0	0	0	0.00	-
0185	Corneal opacity	0	0	0	0	0.00	-
	Iritis	0	0	0	0	0.00	-
	Redness conjunctivae	1	0	0	0	0.00	1
	Chemosis conjunctivae	0	0	0	0	0.00	-
	Discharge	0	0	0	0	0.00	-
0189	Corneal opacity	0	0	0	0	0.00	-
	Iritis	0	0	0	0	0.00	-
	Redness conjunctivae	1	0	0	0	0.00	1
	Chemosis conjunctivae	0	0	0	0	0.00	-
	Discharge	0	0	0	0	0.00	-

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

The second and third animal was symptom-free 2 days after treatment, therefore the study was terminated after the 3-day observation period (72 hours).

Fluorescein staining was negative in the test item treated eyes during the study.

The control eye of each animal was symptom-free during the study, fluorescein staining was negative during the study.

No mortality occurred during the study and no clinical signs of systemic toxicity were observed in any animal in this study.

The bodyweights were considered to be within the normal range of variability in the animals.

1.3 Conclusion

The test item isocycloseram/emamectin benzoate SC (A23220A) was graded as practically non-irritant (Class 2 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 test item isocycloseram/emamectin benzoate SC (A23220A), 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 isocycloseram/emamectin benzoate SC (A23220A) (Charles River Laboratories code: 20/080-038CS) concluded that the test item is non-irritant. As the test item could not be identified as causing serious damage or as a product beyond the limits of classification for eye irritation and severe eye damage based on the *in vitro* test, it was concluded that an *in vivo* study is required for regional registration 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 09 October 2017.

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

- Commission Regulation (EU) No 2017/735, B.5 (L 112, 14 February 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:	Isocycloseram/Emamectin Benzoate SC (A23220A)
Batch number:	TSC002-041-001
Design code:	A23220A
Appearance:	Brown liquid
Active ingredient content:	Isocycloseram: 17.5 % w/w corresponding to 201 g/L emamectin benzoate: 4.18 % w/w corresponding to 48.1 g/L
Expiry date:	31 January 2023
Storage conditions:	Room temperature (<30°C)
Safety precautions:	Enhanced safety precautions (half mask at least with P3 filter cartridge, nitrile gloves, lab coat, safety glasses) were applied considering the supplied safety datasheet to assure personnel health and safety.

Note: No adjustment for active ingredient content was applied.

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 and treatment:

Name:	Saline (0.9% NaCl)
Lot No.:	91133Y05-1 / 91134Y05-1
Expiry Date:	28 February 2022
Produced by:	B. Braun Pharmaceuticals SA

Name:	Fluorescein 10%
Batch No.:	320687F
Expiry Date:	31 October 2021
Produced by:	Alcon Pharma GmbH

This material was mixed with physiological saline solution (Batch number: 91134Y05-1, Expiry date: 28 February 2022) to achieve the final concentration of 2% (w/v). The final solution was stored at room temperature (Dispensary code: S43196, Expiry date: 19 July 2020).

Systemic opiate analgesic:

Name:	Bupaq Multidose (0.3 mg/mL buprenorphine)
Batch No.:	0719659AE
Expiry Date:	30 June 2022
Produced by:	Richter Pharma AG, Austria

Topical ocular anaesthetic:

Name:	Oftacain (4 mg/mL oxybuprocaine chloride)
Batch No.:	1909281
Expiry Date:	30 June 2021
Produced by:	S.C. Rompharm Company S.R.L., Romania

Non-steroidal anti-inflammatory drug:

Name:	Melovem® (5 mg/mL meloxicam)
Batch No.:	19E27-01C7
Expiry Date:	31 May 2021
Produced by:	Dopharma Research B.V., The Netherlands

Note: Other batches of the used materials (e.g. syringe) in the study are not reported.

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:	~16 weeks
Body weight range at dosing:	3947 – 4298 g
Body weight range at termination:	4110 – 4389 g
Identification:	The animals were identified by an ear tag. The cages were marked with individual identity cards with information about study number, sex, cage number, dose and individual animal number.
Acclimatisation time:	48 / 50 days

3.3.2 Husbandry

Animal health:	Only healthy animals were used for the study, as certified by the veterinarian.
Room:	033 and 030 (during treatment)
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. Additional enrichment Bunny Blocks and Dumbbell Devices were also provided for the rabbits.
Light:	12 hours daily from 6.00 a.m. to 6.00 p.m. (and during the analgesic/anaesthetic treatment)
Temperature:	19.3 – 21.8°C
Relative humidity:	45 – 66%
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., H-5300 Karcag, Madarasi út, 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 number of the lot used in the study was:

- Batch no.: 0006371944, expiry date: 27 May 2020.
- Batch no.: 0006461678, expiry date: 07 July 2020.

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 Veszprém County Institute of State Public Health and Medical Officer Service (ÁNTSZ, 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 code: 20/080-038CS) in the Isolated Chicken Eye model with isocycloseram/emamectin benzoate SC (A23220A) in accordance with the guidance from the OECD 438 for this method, concluded that the test item is non-irritant.

As the test item could not be identified as causing serious damage or as a product beyond the limits of classification for eye irritation and severe eye damage, the test item was permitted to use in *in vivo* study. The Sponsor confirmed this statement.

3.4.2 Identification of pH

The pH of the test item was determined at the Pharmacy of Charles River Laboratories Hungary Kft. and found to be 8.78. This is within the acceptable range given by the OECD guideline (pH is 2 or less or 11.5 or greater), therefore the test item was permitted to use in this study.

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 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. As no irritation scores were noted at 24 hours, the second and third rabbits were treated 2 days after the first animal. No further animals were used in the study.

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.

Systemic opiate analgesic: 0.01 mg/kg of buprenorphine
Topical ocular anaesthetic: one-two drops/eye oxybuprocaine chloride
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 liquid isocycloseram/emamectin benzoate SC (A23220A) 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

The eyes were not washed for following instillation.

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, 48 and 72 hours 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 (09 October 2017) 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 euthanized by intravenous sodium pentobarbital 40% (Euthanimal) 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 Material used for euthanasia

Name:	Euthanimal 40% (400 mg/ mL sodium pentobarbital)
Lot No.:	1811347-03
Expiry Date:	31 December 2021
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/Pain reaction (IPR/PR) was observed in the animals.

First animal (no. 0190)

At 1-hour after the application, conjunctivae redness (score 1) was observed.
At 24, 48 and 72-hour after application, no conjunctival or corneal effects were observed in this animal. No fluorescein staining was observed.

Second animal (no. 0185)

At 1-hour after the application, conjunctivae redness (score 1) was observed.
At 24, 48 and 72-hour after application, no conjunctival or corneal effects were observed in this animal. No fluorescein staining was observed.

Third animal (no. 0189)

At 1-hour after the application, conjunctivae redness (score 1) was observed.
At 24, 48 and 72-hour after application, no conjunctival or corneal effects were observed in this animal. No fluorescein staining was observed.

Fluorescein staining is presented in Table 4.

The study was terminated 72 hours after the second and third rabbit treatment.

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

4.2 Bodyweight

The bodyweights were considered to be within the normal range of variability in the animals. Individual bodyweights and bodyweight changes are given in Table 5.

4.3 Clinical Signs

No clinical signs of systemic toxicity were observed in the animals in this study. Summary of the clinical signs are summarised in Table 6.

4.4 Mortality

No mortality occurred during the study.

5.0 CONCLUSIONS

The test item isocycloseram/emamectin benzoate SC (A23220A) was graded as practically non-irritant (Class 2 on a 1 to 8 scale) to the rabbit eye according to the modified Kay and Calandra classification system.

6.0 REFERENCES

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.

Balázs Orovecz, B.Sc. (2020): Isocycloseram/Emamectin Benzoate SC (A23220A) – *In Vitro* Eye Irritation Test in Isolated Chicken Eyes. Charles River Laboratories Hungary Kft. H-8200 Veszprém, Szabadságpuszta, hrsz. 028/1., Hungary. 20/080-038CS.

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	**0190, Male**							**0185, Male**							**0189, Male**						
IPR	0							0							0						
PR	0	0	0	0	NA	NA	NA	0	0	0	0	NA	NA	NA	0	0	0	0	NA	NA	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	0	0	0	0	NA	NA	NA	0	0	0	0	NA	NA	NA	0	0	0	0	NA	NA	NA
F = Area of Cornea involved	0	0	0	0	NA	NA	NA	0	0	0	0	NA	NA	NA	0	0	0	0	NA	NA	NA
* Score (E x F) x 5	0	0	0	0	NA	NA	NA	0	0	0	0	NA	NA	NA	0	0	0	0	NA	NA	NA
IRIS																					
D	0	0	0	0	NA	NA	NA	0	0	0	0	NA	NA	NA	0	0	0	0	NA	NA	NA
* Score (D x 5)	0	0	0	0	NA	NA	NA	0	0	0	0	NA	NA	NA	0	0	0	0	NA	NA	NA
CONJUNCTIVAE																					
A = Redness	1	0	0	0	NA	NA	NA	1	0	0	0	NA	NA	NA	1	0	0	0	NA	NA	NA
B = Chemosis	0	0	0	0	NA	NA	NA	0	0	0	0	NA	NA	NA	0	0	0	0	NA	NA	NA
C = Discharge	0	0	0	0	NA	NA	NA	0	0	0	0	NA	NA	NA	0	0	0	0	NA	NA	NA
* Score (A+B+C) x 2	2	0	0	0	NA	NA	NA	2	0	0	0	NA	NA	NA	2	0	0	0	NA	NA	NA
* Total Score	2	0	0	0	NA	NA	NA	2	0	0	0	NA	NA	NA	2	0	0	0	NA	NA	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)
0190	male	0.00	0.00	0.25	0.00
0185	male	0.00	0.00	0.25	0.00
0189	male	0.00	0.00	0.25	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	1 Week	2 Weeks	3 Weeks
0190, Male	2	0	0	0	NA	NA	NA
0185, Male	2	0	0	0	NA	NA	NA
0189, Male	2	0	0	0	NA	NA	NA
* Group Total	6	0	0	0	NA	NA	NA
* Group Mean Score	2.00	0.00	0.00	0.00	NA	NA	NA

*: 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
0190 Male	-	-	-	-	NA	NA	NA
0185 Male	-	-	-	-	NA	NA	NA
0189 Male	-	-	-	-	NA	NA	NA

Remarks:
-: Absence of Fluorescein Stain
+: Presence of Fluorescein Staining
NA: Not applicable

TABLE 5 Individual Bodyweights and Bodyweight Change

Rabbit Number and Sex	Individual Bodyweight (g)		Bodyweight Change (g)
	*Before treatment	At termination	
0190, Male	4298	4316	18
0185, Male	4135	4389	254
0189, Male	3947	4110	163

*: Considering treatment day and termination day

TABLE 6 Individual Clinical Signs

Rabbit Number and Sex	Day 0	Day 1	Day 2	Day 3	1 week	2 weeks	3 weeks
0190, Male	N	N	N	N	NA	NA	NA
0185, Male	N	N	N	N	NA	NA	NA
0189, Male	N	N	N	N	NA	NA	NA

Remarks:
N = Symptom-free
NA = Not Applicable

APPENDICES SECTION

APPENDIX 1 Certificate of Analysis

syngenta

Syngenta Crop Protection AG
GLP Testing Facility WMU
Analytical Development & Product Chemistry
Breitenloh 5
4333 Münchwilen, Switzerland

Certificate of Analysis

A23220A

isocycloseram/emamectin benzoate SC (200/050)

TSC002-041-001

Batch Identification	**TSC002-041-001**
Other Batch ID	1122866
Product Code	**A23220A**
Other Product Code(s)	isocycloseram/emamectin benzoate SC (200/050)
Chemical Analysis (Active Ingredient content)	
- **Identity of the Active Ingredient(s)***	**confirmed**
- **Content of isocycloseram***	**17.5 % w/w corresponding to 201 g/l**
- **Content of emamectin benzoate***	**4.18 % w/w corresponding to 48.1 g/l**
	The Active Ingredient(s) content is within the FAO limits.
Methodology used for Characterization / Recertification	LC. chiral LC, oscillating density meter
Physical Analysis	
- **Appearance**	**brown liquid**
- **Density***	**1150 kg/m³**
Stability:	
- **Storage Temperature**	**< 30°C**
- **Recertification Date**	**End of January 2023**

If stored under the conditions given above, this test substance can be considered stable until the recertification date is reached.
This Certificate of Analysis summarizes data which originates either from a single study or from several individual studies. Tests marked with an asterisk (*) have been conducted in compliance with GLP.
Raw data, documentation, study plans, any amendments to study plans and reports pertaining to this/these study/studies are stored under the study number(s) referenced below within the archives of the GLP Testing Facility WMU at Syngenta Crop Protection AG, Switzerland.

Study number of batch characterization: CHMU200180
Study number(s) of batch recertification:

Authorization: 19-Feb-2020

Dr. Karine Heintz
Analytical Development & Product Chemistry

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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 Structured Study Summary

Structured Study Summary Table

Test substance design code	Isocycloseram/Emamectin Benzoate SC (A23220A)
Test substance batch code	TSC002-041-001
Test substance purity (% w/w)	Isocycloseram: 17.5 % w/w corresponding to 201 g/L emamectin benzoate: 4.18 % w/w corresponding to 48.1 g/L
Study number	20/080-005N
Study type	EYE IRRITATION (DRAIZE)
Lab Reference	Charles River Laboratories Hungary Kft.
Study guidelines	OECD 405 (2017), OPPTS 870.2400 (1998), EC No 2017/735, B.5 (2017)
Nonstandard elements	
Species	Rabbit
Strain	New Zealand White

Structured Study Results Table

Animal number	Clinical Observations	Mortality	Time when first rinsed (if earlier than 24hrs)	Time units
0190	-	No	-	hour
0185	-	No	-	hour
0189	-	No	-	hour

APPENDIX 6 GLP Certificate

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Ref. no: OGYÉI/22762-5/2018
Admin.: Dr. Juhász Uzonka
Date: 03 August 2018

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, 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.**

Tarjáni Ibolya
Head of Inspectorate

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