

Thiamethoxam

**Thiamethoxam SL (A23943A) -
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:	22 June 2022
PERFORMING LABORATORY:	Charles River Laboratories Hungary Kft. H-8200 Veszprém, Szabadságpuszta, hrsz. 028/1., Hungary
LABORATORY PROJECT ID:	Report Number: 21/309-005N Study Number: 21/309-005N Task Number: TK0544291
SPONSOR(S):	Syngenta Ltd. Jealott's Hill International Research Centre Bracknell, Berkshire, RG42 6EY, United Kingdom

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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: Ivett Orosz Date: 22 June 2022
Ivett Orosz, M.Sc.
Study Director

Performing Laboratory: Charles River Laboratories Hungary Kft.
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To be completed for USA EPA submission only:
Representative of Submitter/Sponsor:

_____ Date: _____

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FLAGGING STATEMENT

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QUALITY ASSURANCE STATEMENT

Study Number: 21/309-005N

Study Title: Thiamethoxam SL (A23943A) - 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
11 March 2022	Study Plan	24 March 2022	24 March 2022
18 March 2022	Treatment	18 March 2022	18 March 2022
25 March 2022	Body weight measurement	25 March 2022	25 March 2022
23 March 2022	Clinical observation	31 March 2022	31 March 2022
08 June 2022	Draft Report	08 June 2022	08 June 2022
22 June 2022	Final Report	22 June 2022	22 June 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: Wyihai Ildikó
Ildikó Nyitrai, M.Sc.
On Behalf of QA

Date: 22 June 2022

MANAGEMENT STATEMENT

According to the conditions of the research and development agreement between Syngenta Ltd. (as Sponsor) and Charles River Laboratories Hungary Kft. (as Test Facility) the study titled "Thiamethoxam SL (A23943A) - Acute Eye Irritation Study in Rabbits" was performed, in compliance with the Principles of Good Laboratory Practice.

Signature: Balász Tóth Date: 22 June 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
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Carolina Vaccari, Ph.D.	Syngenta Study Manager

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

Study dates

Study initiation date:	08 March 2022
Experimental starting date:	15 March 2022
Experimental completion date:	17 May 2022
Receipt of animals:	09 March 2022
Acclimatisation:	09 – 15 March 2022 (animal no. 5507) 09 – 30 March 2022 (animal no. 5505) 09 March – 25 April 2022 (animal no. 5501)
Treatment:	16 March 2022 (animal no. 5507) 31 March 2022 (animal no. 5505) 26 April 2022 (animal no. 5501)
Observation:	16 – 30 March 2022 (animal no. 5507) 31 March – 21 April 2022 (animal no. 5505) 26 April – 17 May 2022 (animal no. 5501)
Draft Report:	08 June 2022
Final Report:	22 June 2022

Deviations from the guidelines and the Study Plan

Due to technical reasons, humidity values (minimum of 25 °C, maximum of 78 °C) outside the expected range of 30-70 °C were recorded occasionally in the animal rooms during the study.

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

Performing laboratory test substance reference number

210612

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.

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1.0 EXECUTIVE SUMMARY

1.1 Study Design

The primary eye irritation effect of the test item, thiamethoxam SL (A23943A), 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. In case of the first treated animal (ID 5507) 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. Scoring of irritation effects was performed approximately 1, 24, 48, 72 hours, 1 week and 2 weeks after test material installation in case of the first animal, 1, 24, 48, 72 hours, 1 week, 2 and 3 weeks after test material installation in case of the second animal, 1, 24, 48, 72, 96, 120 hours, 1 week, 2 and 3 weeks after test material installation in case of the third animal. Observations with fluorescein staining were made approximately 24 hours before treatment and then 24, 48, 72 hours, 1 week and 2 weeks after test material installation in case of the first animal, 24, 48, 72 hours, 1 week, 2 and 3 weeks after test material installation in case of the second animal, 24, 48, 72, 96, 120 hours*, 1 week, 2 and 3 weeks after test material instillation in case of the third 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.

*Note: Increasing in score of discharge was observed at the 72-hour observation time point, therefore, 96- and 120-hour post-treatment scores were made.

1.2 Results

Initial Pain Reaction (IPR) (score 2 or 1) was observed in all animals.

Pain Reaction (PR) (score 1) was observed in the first and second animal at 1-hour observation time point.

Eye irritation results with thiamethoxam SL (A23943A):

Animal No.		Scores after treatment *				Mean scores (24-72 h)	Reversible (day)
		1 h	24 h	48 h	72 h		
5507	Corneal opacity	1	1	1	1	1.00	7
	Iritis	0	0	0	0	0.00	-
	Redness conjunctivae	2	2	2	2	2.00	14
	Chemosis conjunctivae	3	3	3	2	2.67	7
	Discharge	2	3	2	2	2.33	14
5505	Corneal opacity	1	1	1	1	1.00	21
	Iritis	0	0	0	0	0.00	-
	Redness conjunctivae	2	2	2	2	2.00	14
	Chemosis conjunctivae	2	2	2	2	2.00	7
	Discharge	2	3	3	3	3.00	7
5501	Corneal opacity	1	1	1	1	1.00	>21
	Iritis	0	0	0	0	0.00	-
	Redness conjunctivae	2	2	2	2	2.00	>21
	Chemosis conjunctivae	2	2	2	2	2.00	7
	Discharge	2	1	1	3	1.67	7

*according to the Draize scheme (Draize, 1977);

h = hour(s)

The first treated animal became symptom-free at 2 weeks after treatment, the second animal became symptom-free at 3 weeks after treatment; the third animal had only slight symptoms at 3 weeks after treatment, therefore (in agreement with the Sponsor) the study was terminated 3 weeks after the treatment of the third rabbit.

Fluorescein staining was positive in case of the first animal at 24, 48, 72 hours and 1 week after treatment, in case of the second animal at 24, 48, 72 hours, 1 week and 2 weeks after treatment, and in case of the third animal at 24, 48, 72, 96, 120, 1 week, 2 and 3 weeks after treatment.

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 thiamethoxam SL (A23943A) applied to the rabbit's eye mucosa, caused conjunctival and corneal effects which were not fully reversible within 21 days in a single rabbit (1/3). Therefore, the test item is considered to be a severe eye irritant.

The test item thiamethoxam SL (A23943A) 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 thiamethoxam SL (A23943A), 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 thiamethoxam SL (A23943A) (Charles River Laboratories Hungary Kft. study code: 21/309-038CS) concluded that the test item is not classified as a severe irritant and not classified as non-irritant. This study was required for regulatory purposes.

2.2 Guidelines

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

- OECD Guidelines for the Testing of Chemicals, Section 4, Number 405 "Acute Eye Irritation/Corrosion", adopted 2021.
- United States Environmental Protection Agency, Health Effects Test Guidelines, OPPTS 870.2400, Acute Eye Irritation EPA 712-C-98-195, 1998.
- Commission Regulation (EU) No 2017/735, B.5 (L 112, 2017) amending Regulation (EC) No 440/2008.

2.3 Test Facility

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

3.0 MATERIALS AND METHODS

3.1 Test Substance

The following information was provided by the Sponsor.

Name:	Thiamethoxam SL (A23943A)
Other name:	TMX 75 SL
Batch number:	NSI001-085-017
Design code:	A23943A
Active ingredient content*:	Content of thiamethoxam 6.63 % w/w, corresponding to 75.42 g/L
Density:	1.1378 g/mL
Appearance:	Dark orange homogeneous and translucent liquid
Recertification date:	10 September 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 5.0.

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.:	10651Y05-2 / 94922Y05-1
Expiry Date:	31 January 2024 / 30 November 2022
Produced by:	B. Braun Pharmaceuticals SA

Name:	Fluorescein 100 mg/mL
Batch No.:	10CAF
Expiry Date:	31 August 2022
Produced by:	Alcon Pharma GmbH

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; Batch number: 10651Y05-2, Expiry date: 31 January 2024) to achieve the final

concentration of 2% (v/v). The final solution was stored at room temperature (Dispensary code: S43253, Expiry date: 17 June 2022; Dispensary code: S43249, Expiry date: 14 May 2022; Dispensary code: S43247, Expiry date: 17 April 2022; Dispensary code: S43245, Expiry date: 18 March 2022).

Systemic opiate analgesic:

Name: Bupredine Multidose (0.3 mg/mL buprenorphine)
Batch No.: 21E264
Expiry Date: 30 April 2023
Produced by: Le Vet Beheer B.V., The Netherlands

Topical ocular anaesthetic:

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

Non-steroidal anti-inflammatory drug:

Name: Meloxidyl (5 mg/mL meloxicam)
Batch No.: M2109275
Expiry Date: 31 May 2024
Produced by: Ceva Santé Animale, France

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

3.3 Experimental Design

3.3.1 Animals

Species and strain: New Zealand white rabbit
Source: S&K-LAP Kft.
2173 Kartal, Császár út 135, Hungary
Justification of strain: The New Zealand White rabbit is one of the standard strains used for acute irritation toxicity studies.
Number of animals: 3 animals
Sex: Male
Age of animals at dosing: ~10 / 12 / 16 weeks
Body weight range at dosing: 2859 – 3572 g
Body weight range at termination: 3186 – 3903 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: 7 / 22 / 48 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: 17.5 – 22.1 °C
Relative humidity: 25 – 78%
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.: 0008038307, expiry date: 13 April 2022
- Batch no.: 0008211304, expiry date: 22 June 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: 21/309-038CS) in the Isolated Chicken Eye model with thiamethoxam SL (A23943A) (Batch number: NSI001-085-017), in accordance with the guidance from the OECD No. 438 for this method, concluded that the test item is not classified as a severe irritant and not classified as non-irritant. It was concluded that further information is required for classification.

3.4.2 Identification of pH

The pH of the test item was measured as pH 5.0, 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 (5507) showed scores of above zero at 1, 24, 48, 72-hour, 1-week 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 2 weeks, therefore the second rabbit (5505) 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 and 2-week observation points after treatment, the fluorescein staining was positive at 24, 48, 72-hour, 1-week and 2-week observation points after treatment. The second rabbit became symptom-free after 3 weeks, therefore the third rabbit (5501) was treated after the 3-week observation of the second rabbit.

The third animal showed scores of above zero at 1, 24, 48, 72, 96, 120-hour*, 1-week, 2-week and 3-week observation points after treatment, the fluorescein staining was positive at 24, 48, 72, 96, 120-hour, 1-week, 2-week and 3-week observation points after treatment. The animal had only slight symptoms at the 3-week observation point; thus, the study was terminated after the 3-week observation of the third animal.

*Note: Increasing in score of discharge was observed at the 72-hour observation time point, therefore, 96- and 120-hour post-treatment scores were made.

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 thiamethoxam SL (A23943A) 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 first treated animal (ID 5507) 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 as part of the fluorescein observation process: 24, 48, 72 hours, 1 week and 2 weeks after test item application in case of the first animal, 24, 48, 72 hours, 1 week, 2 and 3 weeks after test item application in case of the second animal and 24, 48, 72, 96, 120 hours, 1 week, 2 and 3 weeks after test item application in case of the third animal.

3.6 Observations and Scoring

3.6.1 Clinical observations and evaluation of ocular irritation

Scoring of irritation effects was performed approximately 1 hour, 24, 48, 72 hours, 1 week and 2 weeks after test material installation in case of the first animal, 1 hour, 24, 48, 72 hours, 1 week, 2 and 3 weeks after test material installation in case of the second animal and 1 hour, 24, 48, 72, 96, 120 hours, 1 week, 2 and 3 weeks after test material installation in case of the third animal.

Observations with fluorescein staining were made approximately 24 hours before treatment and then 24, 48, 72 hours, 1 week and 2 weeks after test item application in case of the first animal, 24, 48, 72 hours, 1 week, 2 and 3 weeks after test item application in case of the second animal and 24, 48, 72, 96, 120 hours, 1 week, 2 and 3 weeks after test item application in case of the third animal.

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 and/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 or 1) was observed in all animals.

Pain Reaction (PR) (score 1) was observed in the first and second animal at 1-hour observation time point.

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

At 72 hours after the treatment, redness (score 2), chemosis (score 2), discharge (score 3 or 2) and corneal opacity (score 1, area 4) was noted in all animals. The rabbits showed positive reaction to fluorescein staining.

At 96 hours after the treatment, redness (score 2), chemosis (score 2), discharge (score 1) and corneal opacity (score 1, area 4) was noted in the third animal. The rabbit showed positive reaction to fluorescein staining.

At 120 hours after the treatment, redness (score 2), chemosis (score 1), discharge (score 1) and corneal opacity (score 1, area 4) was noted in the third animal. The rabbit showed positive reaction to fluorescein staining.

At 1 week after the treatment, redness (score 1) was noted in all animals. Corneal opacity (score 1, area 4) was noted in the second and third animals. Discharge (score 2) was observed in the first animal. Fluorescein staining was positive in all animals.

At 2 weeks after the treatment, corneal opacity (score 1, area 4) was noted in the second and third animal. Redness (score 1) was noted in the third animal. Fluorescein staining was positive in the second and third animal.

At 3 weeks after the treatment, redness (score 1), corneal opacity (score 1, area 4) was noted in the third animal. Fluorescein staining was positive in the third animal.

Fluorescein staining is presented in Table 4.

The study was terminated 3 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 thiamethoxam SL (A23943A) applied to the rabbit's eye mucosa, caused conjunctival and corneal effects at 1 hour, which were not fully reversible within 21 days in a single rabbit. Therefore, the test item is considered to be a severe eye irritant.

The test item thiamethoxam SL (A23943A) 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. (2022): Thiamethoxam SL (A23943A) - *In Vitro* Eye Irritation Test in Isolated Chicken Eyes. Charles River Laboratories Hungary Kft. H-8200 Veszprém, Szabadságpuszta, hrsz. 028/1., Hungary. Study code: 21/309-038CS.

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	5507, male	5505, male	5501, male
IPR	2	2	1
PR	1 0 0 0 0 0 NA	1 0 0 0 0 0 0	0 0 0 0 0 0 0 0 0
Time after treatment	1 24 48 72 1 2 3 Hr Hr Hr Hr W W W	1 24 48 72 1 2 3 Hr Hr Hr Hr W W W	1 24 48 72 96 120 1 2 3 Hr Hr Hr Hr HR Hr W W W
CORNEA E = Degree of Opacity F = Area of Cornea involved	1 1 1 1 0 0 NA 4 4 4 4 0 0 NA	1 1 1 1 1 1 0 4 4 4 4 4 4 0	1 1 1 1 1 1 1 1 4 4 4 4 4 4 4 4
* Score (E x F) x 5	20 20 20 20 0 0 NA	20 20 20 20 20 20 0	20 20 20 20 20 20 20 20
IRIS			
D	0 0 0 0 0 0 NA	0 0 0 0 0 0 0	0 0 0 0 0 0 0 0
* Score (D x 5)	0 0 0 0 0 0 NA	0 0 0 0 0 0 0	0 0 0 0 0 0 0 0
CONJUNCTIVAE A = Redness B = Chemosis C = Discharge	2 2 2 2 1 0 NA 3 3 3 2 0 0 NA 2 3 2 2 2 0 NA	2 2 2 2 1 0 0 2 2 2 2 0 0 0 2 3 3 3 0 0 0	2 2 2 2 2 2 1 1 1 2 2 2 2 2 1 0 0 0 2 1 1 3 1 1 0 0 0
* Score (A+B+C) x 2	14 16 14 12 6 0 NA	12 14 14 14 2 0 0	12 10 10 14 10 8 2 2 2
* Total Score	34 36 34 32 6 0 NA	32 34 34 34 22 20 0	32 30 30 34 30 28 22 22 22

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)
5507	male	1.00	0	2.00	2.75
5505	male	1.00	0	2.00	2.00
5501	male	1.00	0	2.00	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	24	48	72	96	120	1	2	3
	Hour	Hours	Hours	Hours	Hours	Hours	Week	Weeks	Weeks
5507, Male	34	36	34	32	NA	NA	6	0	NA
5505, Male	32	34	34	34	NA	NA	22	20	0
5501, Male	32	30	30	34	30	28	22	22	22
* Group Total	98	100	98	100	30	28	50	42	22
* Group Mean Score	32.67	33.33	32.67	33.33	30.00	28.00	16.67	14.00	11.00

*: 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	96 Hours	120 Hours	1 Week	2 Weeks	3 Weeks
5507 Male	-	+	+	+	NA	NA	+	-	NA
5505 Male	-	+	+	+	NA	NA	+	+	-
5501 Male	-	+	+	+	+	+	+	+	+

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	
5507, Male	2859	3186	327
5505, Male	3053	3308	255
5501, Male	3572	3903	331

TABLE 6 Individual Clinical Signs

Rabbit Number and Sex	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	1 week	2 weeks	3 weeks
5507, Male	N	N	N	N	NA	NA	N	N	NA
5505, Male	N	N	N	N	NA	NA	N	N	N
5501, Male	N	N	N	N	N	N	N	N	N

N: Absent

NA: Not applicable

APPENDICES SECTION



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SYNGENTA PROTEÇÃO DE CULTIVOS Ltda.
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CEP 04730-000 – Bairro Várzea de Baixo
São Paulo-SP – Brazil

Certificate of Analysis

A23943A
Thiamethoxam SL (075)
NSI001-085-017

Batch Identification	NSI001-085-017
Product Code	A23943A
Other Product Code(s)	A23943A; EXF23867A; CGA293343 SL (075); Thiamethoxam SL (075)
EUP number:	739/2021 Expiry date: 26/04/2024
Received on:	28 September 2021
Source	Syngenta Proteção de Cultivos Ltda. 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 Thiamethoxam *** **6.63 % w/w corresponding to 75.42 g/L**

The Active Ingredient content is within the FAO limits.
Methodology used for Characterization: HPLC (SF-1151/1)

Physical Analysis

- Appearance	Homogeneous and translucent
- Color	6/12 – 5YR (Dark Orange)
- Physical state	Liquid
- Density *	1.1378 g/cm ³

Stability:

- Storage Temperature	<30°C
- Recertification Date	10 September 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: 26785/2021CF and 26787/2021CC

Authorization: 10 November 2021

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

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		More than half of the individual total scores at 7 days 10 or less	Moderate irritant (5)
	Group mean total score at 7 days 20 or less	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		More than half of the individual total scores at 7 days 30 or less	Severe irritant (6)
	Group mean total score at 7 days 40 or less	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		More than half of the individual total scores at 7 days 60 or less	Very severe irritant (7)
	Group mean total score at 7 days 80 or less	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: OGYÉI/28510-6/2022

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 till 11th of August, 2022.

Dr. Lukács
Ferenc
József

Digitálisan aláírta:
Dr. Lukács Ferenc
József
Dátum: 2022.06.21
12:42:42 +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").