citoxlab
Safety and Health Research

SYN549522

SYN549522 SC (A22011B) - Acute Eye Irritation Study in Rabbits

Final Report

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

AUTHOR(S): Máté Weisz, M.Sc.

COMPLETION DATE: 16 January 2019

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

LABORATORY PROJECT ID: Report Number: 18/212-005N
Study Number: 18/212-005N
Task Number: TK0317167

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

VOLUME 1 OF 1 OF STUDY

STATEMENT OF DATA CONFIDENTIALITY CLAIMS

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GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

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

This study was conducted in accordance with a written Study Plan, authorized by the Sponsor and Citoxlab Hungary Ltd. 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.

Signature: _____ Date: 16 January 2019

Máté Weisz, M.Sc.
Study Director

Performing Laboratory:

Citoxlab Hungary Ltd.
H-8200 Veszprém, Szabadságpuszta
Hungary

FLAGGING STATEMENT

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QUALITY ASSURANCE STATEMENT

Study Number: 18/212-005N

Study Title: SYN549522 SC (A22011B) - Acute Eye Irritation Study in Rabbits

Test Item: SYN549522 SC (A22011B)

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
10 October 2018	Study Plan	10 October 2018	10 October 2018
16 October 2018	Treatment	16 October 2018	16 October 2018
07 December 2018	Draft Report	07 December 2018	07 December 2018
16 January 2019	Final Report	16 January 2019	16 January 2019

Signature: Merazga Leila Date: 16 January 2019

Leila Merazga, M.Sc.
On Behalf of QA

MANAGEMENT STATEMENT

According to the conditions of the research and development agreement between Syngenta Ltd. (as Sponsor) and Citoxlab Hungary Ltd. (as Test Facility) the study titled "SYN549522 SC (A22011B) - Acute Eye Irritation Study in Rabbits" was performed, in compliance with the Principles of Good Laboratory Practice.

Signature: _____ Date: 16 Jan 2019

Alyson Leyshon, M.Sc.
Managing Director

GENERAL INFORMATION

Contributors

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

Name	Function
Máté Weisz, M.Sc.	Study Director
Ádám Appl, M.Sc.	Assistant Scientist
Szabolcs Gáty, M.Sc.	Senior Director of Quality Assurance Unit
Leila Merazga, M.Sc.	Deputy Head of Quality Assurance Unit
László Székelyhidi, DVM	Veterinary Care
Tamás Mészáros, Ph.D.	Pharmacy
Monique Trevisan Inforzato	Syngenta Study Manager

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

Study dates

Study initiation date:	11 October 2018
Experimental starting date:	16 October 2018
Experimental completion date:	01 November 2018
Receipt of animals:	03 October 2018
Acclimatisation:	03 – 15/23/28 October 2018
Treatment:	16 October 2018 (animal no. 2427) 24 October 2018 (animal no. 2839) 29 October 2018 (animal no. 2838)
Observation:	16 – 19 October 2018 (animal no. 2427) 24 – 27 October 2018 (animal no. 2839) 29 October – 01 November 2018 (animal no. 2838)

Deviations from the guidelines

There was no deviation during the study.

Performing laboratory test substance reference number

180275

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 Citoxlab Hungary Ltd. 8200 Veszprém, Szabadságpuszta, Hungary.

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

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1.0 EXECUTIVE SUMMARY

1.1 Study Design

The primary eye irritation effect of the test item SYN549522 SC (A22011B) 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 and 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. 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.

Eye irritation results with SYN549522 SC (A22011B):

Animal No.		Scores after treatment *				Mean scores (24-72 h)	Reversible (day)
		1 h	24 h	48 h	72 h		
2427	Corneal opacity	0	0	0	0	0	-
	Iritis	0	0	0	0	0	-
	Redness conjunctivae	2	2	2	0	1.33	3
	Chemosis conjunctivae	2	1	1	0	0.67	3
	Discharge	3	0	0	0	0	1
2839	Corneal opacity	1	0	0	0	0	1
	Iritis	0	0	0	0	0	-
	Redness conjunctivae	2	2	2	0	1.33	3
	Chemosis conjunctivae	2	1	1	0	0.67	3
	Discharge	3	0	0	0	0	1
2838	Corneal opacity	0	0	0	0	0	-
	Iritis	0	0	0	0	0	-
	Redness conjunctivae	2	1	1	0	0.67	3
	Chemosis conjunctivae	1	0	0	0	0	1
	Discharge	1	0	0	0	0	1

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

All animals became symptom free after 72 hours.

Fluorescein staining was positive at 24 and 48 hours in case of the first animal (ID 2427) and at 24 hours only in case of the second animal (ID 2839).

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

No clinical signs of systemic toxicity were observed in any animal in this study.

No mortality occurred during the study.

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

1.3 Conclusion

The test item SYN549522 SC (A22011B) was graded as a mild irritant (Class 4 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 SYN549522 SC (A22011B), 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 SYN549522 SC (A22011B) (Orosz, 2018; Citoxlab code: 18/212-038CS) suggests that the test item is not classified as a severe irritant and not classified as non-irritant, therefore it was concluded that an *in vivo* study is required for classification.

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 Citoxlab Hungary Ltd. 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:	SYN549522 SC (A22011B)
Batch number:	SMU7JP001
Active ingredient content*:	SYN549522 (a mixture of SYN547386 and SYN548941), 38.1% w/w corresponding to 448 g/L SYN547386, 34.3 % w/w corresponding to 403 g/L SYN548941, 3.83 % w/w corresponding to 45.0 g/L
Density:	1175 kg/m^3
Appearance:	Beige liquid
Recertification date:	30 November 2020
Storage conditions:	Room temperature (<30°C)
Safety precautions:	Routine safety precautions (lab coat, gloves, safety glasses, face mask) for unknown materials were applied to assure personnel health and safety.

**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:

Name:	Injekt® (Disposable Syringe Luer Solo, 20 mL)
Lot No.:	3M09048
Expiry Date:	31 December 2018
Supplier:	B.Braun Melsungen AG

Name:	Saline (0.9% NaCl)
Lot No.:	72522Y05-2
Expiry Date:	31 May 2020
Produced by:	B. Braun Pharmaceuticals SA

Name: Omnifix®-F (Disposable syringe, 1 mL)
Lot No.: 18C26C8
Expiry Date: 01 March 2023
Manufactured by: B. Braun Melsungen AG

Name: Fluorescein 100 mg/mL
Batch No.: 271594F / 293600F
Expiry Date: 31 October 2018 / 31 January 2020
Produced by: Alcon Pharma GmbH

Name: Saline (0.9% NaCl)
Batch No.: 81172Y05-2
Expiry date: 28 February 2021
Produced by: B. Braun Pharmaceuticals SA

Systemic opiate analgesic:

Name: Bupredine Multidose (0.3 mg/mL buprenorphine)
Batch No.: 17K032
Expiry Date: 30 April 2020
Produced by: Le Vet. Beheer B.V.

Topical ocular anaesthetic:

Name: Benoxi (4 mg/mL oxybuprocaine-hydrochloride)
Batch No.: 050817
Expiry Date: 31 December 2019
Produced by: Unimed Pharma

Non-steroidal anti-inflammatory drug:

Name: Melovem® (5 mg/mL meloxicam)
Batch No.: 17I22-04C3
Expiry Date: 30 September 2019
Produced by: Dopharma Resarch B.V.

3.3 Experimental Design

3.3.1 Animals

Species and strain:	New Zealand white rabbit
Source:	S&K-LAP Kft. 2173 Kartal, Császár út 135, Hungary
Justification of strain:	The New Zealand White rabbit is one of the standard strains used for acute irritation toxicity studies.
Number of animals:	3 animals
Sex:	Male
Age of animals at dosing:	~11-13 weeks
Body weight range at dosing:	3446 – 3759 g
Body weight range at termination:	3608 – 3817 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 / 21 / 26 days

3.3.2 Husbandry

Animal health:	Only healthy animals were used for the study, as certified by the veterinarian.
Room:	033
Housing/Enrichment:	Rabbits were individually housed in AAALAC approved metal wire rabbit cages. Cages were of an open wire structure and cages were placed together to allow some social interaction with rabbit(s) in adjoining cages.
Light:	12 hours daily from 6.00 a.m. to 6.00 p.m. (and during the analgesic/anaesthetic treatment)
Temperature:	19.9 – 22.8 °C
Relative humidity:	36 – 70%
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 batch numbers of the lots used in the study were as follows:

- Batch number: 0005195239, expiry date: 10 November 2018,
- Batch number: 0005279068, expiry date: 21 December 2018,
- Batch number: 0005356688, expiry date: 26 January 2019.

The food was considered not to contain any contaminants that could reasonably be expected to affect the purpose or integrity of the study. A detailed description of the contents of the lot used is archived with the raw data at Citoxlab Hungary Ltd.

3.3.4 Water supply and quality control

The animals received tap water, fit for human consumption, *ad libitum*, from the automatic system supplied by the communal water network. The water was considered not to contain any contaminants that could reasonably be expected to affect the purpose or integrity of the study.

The quality control analysis is performed 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-8201 Veszprém, József Attila u. 36., Hungary). The quality control results are retained in the archive at Citoxlab Hungary Ltd.

3.4 Pre-Study and Analgesic and Anaesthetic Treatment Procedures

3.4.1 *In vitro* study results

An *in vitro* eye irritation study was performed prior to treatment on any animal. The results from the *in vitro* eye irritation study (Citoxlab code: 18/212-038CS) in the Isolated Chicken Eye model with SYN549522 SC (A22011B) (Batch number: SMU7JP001), in accordance with the guidance from the OECD 438 for this method, indicated that the test item is not classified as a severe irritant and not classified as non-irritant, therefore further information is required for classification.

3.4.2 Identification of pH

The pH of the test item was measured as pH 6.0, permitting the test item to be used in the animal studies.

3.4.3 Pre-study examination

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

3.4.4 Chronology of animal use

The first animal (ID 2427) showed scores of above zero at 1, 24 and 48-hour observation points after treatment, and the fluorescein staining was positive at 24 and 48-hour observation points after treatment. This animal became symptom-free after 72-hour, thus a second animal (ID 2839) was treated after the 72-hour observation of the first animal. The second animal showed scores of above zero at 1, 24 and 48-hour observation points after treatment, the fluorescein staining was positive at the 24-hour observation point after treatment, and became symptom-free at 72-hour observation point, thus a third animal (ID 2838) was treated after the 72-hour observation of the second animal. The third animal showed scores of above zero at 1, 24 and 48-hour observation points after treatment, the fluorescein staining was negative during the 72-hour observation period. This animal became symptom-free at 72-hour observation point.

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 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 eye scores were zero.

Systemic opiate analgesic: Bupredine Multidose (0.3 mg/mL buprenorphine) 0.01 mg/kg.
Topical ocular anaesthetic: Benoxi (4 mg/mL oxybuprocaine-hydrochloride) one-two drops/eye.
Non-steroidal anti-inflammatory drug: Melovem® (5 mg/mL meloxicam) 0.5 mg/kg.

3.5 Administration of the Test Item

3.5.1 Dosage

A single amount of 0.1 mL of SYN549522 SC (A22011B) was administered to the left eye of each animal.

3.5.2 Application of the test item

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

The untreated contralateral eye served as the control.

3.5.3 Duration of exposure

Both eyes of the test animals were rinsed with physiological saline solution following fluorescein control: 24, 48, 72 hours after test item application as part of the fluorescein observation process.

3.6 Observations and Scoring

3.6.1 Clinical observations and evaluation of ocular irritation

Scoring of irritation effects was performed approximately 1, 24, 48 and 72 hours 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 euthanised by intramuscular injections of ketamine 10% (Ketanest) and xylazine 2% (Nerfasin) followed by intravenous pentobarbital sodium (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 Materials used for euthanasia

Name:	Ketanest (100 mg/mL ketamine)
Batch No.:	J4213-06
Expiry Date:	31 October 2020
Produced by:	bela-pharm GmbH & Co. KG, Germany

Name:	Nerfasin (20 mg/mL xylazine)
Batch No.:	16A134
Expiry Date:	31 December 2018
Produced by:	Le Vet B.V., The Netherlands

Name:	Euthanimal 40% (400 mg/ mL pentobarbital sodium)
Lot No.:	1609291-03
Expiry Date:	31 October 2019
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 Tables 1, 2, and 3.

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

Animal (no. 2427) clinical observation

One hour after the application, redness (score 2), chemosis (score 2) and discharge (score 3) were noted in the animal. Test item remnant was noted in the treated eye of the animal.

At 24 hours after the treatment, redness (score 2) and chemosis (score 1) were noted in the animal. The rabbit showed a positive reaction to fluorescein staining.

At 48 hours after the treatment, redness (score 2) and chemosis (score 1) were noted in the animal. The rabbit showed a positive reaction to fluorescein staining.

At 72 hours after the treatment, no clinical signs and no conjunctival or corneal effects were observed in this animal. Fluorescein staining was negative.

Animal (no. 2839) clinical observation

One hour after the application, redness (score 2), chemosis (score 2), discharge (score 3) and corneal opacity (score 1, area 4) were noted in the animal. No remaining test item was noted in the treated eye of the animal.

At 24 hours after the treatment, redness (score 2) and chemosis (score 1) were noted in the animal. The rabbit showed a positive reaction to fluorescein staining.

At 48 hours after the treatment, redness (score 2) and chemosis (score 1) were noted in the animal. Fluorescein staining was negative.

At 72 hours after the treatment, no clinical signs and no conjunctival or corneal effects were observed in this animal. Fluorescein staining was negative.

Animal (no. 2838) clinical observation

One hour after the application, redness (score 2), chemosis (score 1) and discharge (score 1) were noted in the animal. No remaining test item was noted in the treated eye of the animal.

At 24 hours after the treatment, redness (score 1) was noted in the animal. Fluorescein staining was negative.

At 48 hours after the treatment, redness (score 1) was noted in the animal. Fluorescein staining was negative.

At 72 hours after the treatment, no clinical signs and no conjunctival or corneal effects were observed in this animal. Fluorescein staining was negative.

Fluorescein staining is presented in Table 4.

The study was terminated 72 hours 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 considered to be within the normal range of variability.

Individual bodyweights and bodyweight changes are given in Table 5.

4.3 Clinical Signs

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

4.4 Mortality

No mortality occurred during the study.

5.0 CONCLUSIONS

The test item SYN549522 SC (A22011B) was graded as a mild irritant (Class 4 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.

Orosz, I. (2018): SYN549522 SC (A22011B) - *In Vitro* Eye Irritation Test in Isolated Chicken Eyes. Citoxlab Hungary Ltd. H-8200 Veszprém, Szabadságpuszta Hungary. 18/212-038CS.

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	**2427, male**							**2839, male**							**2838, 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	1	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	4	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	20	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	2	2	2	0	NA	NA	NA	2	2	2	0	NA	NA	NA	2	1	1	0	NA	NA	NA
B = Chemosis	2	1	1	0	NA	NA	NA	2	1	1	0	NA	NA	NA	1	0	0	0	NA	NA	NA
C = Discharge	3	0	0	0	NA	NA	NA	3	0	0	0	NA	NA	NA	1	0	0	0	NA	NA	NA
* Score (A+B+C) x 2	14	6	6	0	NA	NA	NA	14	6	6	0	NA	NA	NA	8	2	2	0	NA	NA	NA
* Total Score	14	6	6	0	NA	NA	NA	34	6	6	0	NA	NA	NA	8	2	2	0	NA	NA	NA

IPR: Initial pain reaction
PR: Pain reaction
Hr: Hour(s)
W: Weeks(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)
2427	male	0	0	1.5	1
2839	male	0.25	0	1.5	1
2838	male	0	0	1	0.25

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
2427, Male	14	6	6	0	NA	NA	NA
2839, Male	34	6	6	0	NA	NA	NA
2838, Male	8	2	2	0	NA	NA	NA
* Group Total	56	14	14	0	NA	NA	NA
* Group Mean Score	18.67	4.67	4.67	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
2427 Male	N	Y	Y	N	NA	NA	NA
2839 Male	N	Y	N	N	NA	NA	NA
2838 Male	N	N	N	N	NA	NA	NA

Remarks:
N : Absence of Fluorescein Stain
Y : 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	
2427, Male	3446	3608	162
2839, Male	3525	3689	164
2838, Male	3759	3817	58

TABLE 6 Individual Clinical Signs

Rabbit Number and Sex	Observations	Day 0	Day 1	Day 2	Day 3	Day 4-7	Day 8-14	Day 15-21
2427, Male	Symptom-free	Y	Y	Y	Y	NA	NA	NA
2839, Male	Symptom-free	Y	Y	Y	Y	NA	NA	NA
2838, Male	Symptom-free	Y	Y	Y	Y	NA	NA	NA

Y: Present
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

A22011B
SYN549522 SC (450)
SMU7JP001

Batch Identification	**SMU7JP001**
Other Batch ID	1013163
Product Code	**A22011B**
Other Product Code(s)	SYN549522 SC (450)
Chemical Analysis (Active Ingredient content)	
– **Identity of the Active Ingredient(s)***	**confirmed**
– **Content of SYN549522***	**38.1 % w/w corresponding to 448 g/l**
– **Content of SYN547386***	**34.3 % w/w corresponding to 403 g/l**
– **Content of SYN548941***	**3.83 % w/w corresponding to 45.0 g/l**
	The Active Ingredient(s) content is within the FAO limits.
Methodology used for Characterization / Recertification	HPLC, chiral HPLC, oscillating density meter
Physical Analysis	
– **Appearance**	**beige liquid**
– **Density***	**1175 kg/m³**
Stability:	
– **Storage Temperature**	**< 30 °C**
– **Recertification Date**	**End of November 2020**

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: CHMU170748
Study number(s) of batch recertification: ---

Authorization: 30-November-2017

Dr. Christian Mink
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	A22011B
Test substance batch code	SMU7JP001
Test substance purity (% w/w)	SYN549522 (a mixture of SYN547386 and SYN548941), 38.1% w/w corresponding to 448 g/L SYN547386, 34.3 % w/w corresponding to 403 g/L SYN548941, 3.83 % w/w corresponding to 45.0 g/L
Study number	18/212-005N
Study type	EYE IRRITATION (DRAIZE)
Lab Reference	Citoxlab Hungary Ltd.
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
2427	No clinical signs were observed	No	-	hour
2839	No clinical signs were observed	No	-	hour
2838	No clinical signs were observed	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"