citoxlab
Safety and Health Research

Pydiflumetofen

Pydiflumetofen FS (A22657B) - 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): Zsolt Tarcai, M.Sc.

COMPLETION DATE: 30 October 2018

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

LABORATORY PROJECT ID: Report Number: 18/132-005N
Study Number: 18/132-005N
Task Number: TK0337350

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

STATEMENT OF DATA CONFIDENTIALITY CLAIMS

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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 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: 30 October 2018

Zsolt Tarcai, 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: 18/132-005N

Study Title: Pydiflumetofen FS (A22657B) - Acute Eye Irritation Study in Rabbits

Test Item: Pydiflumetofen FS (A22657B)

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
19 July 2018	Study Plan	19 July 2018	19 July 2018
25 July 2018	Treatment	25 July 2018	25 July 2018
16 October 2018	Draft Report	16 October 2018	16 October 2018
30 October 2018	Final Report	30 October 2018	30 October 2018

Signature: Merazga Leila Date: 30 October 18

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 "Pydiflumetofen FS (A22657B) - Acute Eye Irritation Study in Rabbits" was performed, in compliance with the Principles of Good Laboratory Practice.

Signature: _____ Date: 30 Oct 2018

Alyson Leyshon, M.Sc.
Managing Director

GENERAL INFORMATION

Contributors

The following contributed to this report in the capacities indicated:

Name	Function
Zsolt Tarcai, M.Sc.	Study Director
Viktória Zelenák, M.Sc.	Assistant Scientist
Leila Merazga, M.Sc.	Quality Assurance Unit
Tamás Mészáros, Ph.D.	Pharmacy
László Székelyhidi, D.V.M.	Veterinary Control
Sophie van der Kamp, B.Sc.	Syngenta Study Manager

(*Other trained/ competent personnel may worked on the study as required)*

Study dates

Study Initiation Date:	24 July 2018
Experimental Starting Date:	25 July 2018
Experimental Completion Date:	30 July 2018
Acclimation:	20 June – 24/26 July 2018
Treatment:	25/27 July 2018

Deviations from the guidelines

There were no deviations during the study from the guideline.

Performing laboratory test substance reference number

180162

Other

The study documents and samples:
- Study Plan,
- all raw data,
- sample of the test item,
- Final Report and any amendments,
- correspondence

will be archived according to the Hungarian GLP and 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.

TABLE OF CONTENTS

1.0 EXECUTIVE SUMMARY

1.1 Study Design

The primary eye irritation effect of the test item pydiflumetofen FS (A22657B) 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 eye serving as 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, 72 hours in all animals after the 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 pydiflumetofen FS (A22657B):

Animal No.		Scores after treatment *				Mean scores (24-72 h)	Reversible (day)
		1 h	24 h	48 h	72 h		
958	Corneal opacity	0	0	0	0	0	-
	Iritis	0	0	0	0	0	-
	Redness conjunctivae	1	0	0	0	0	1
	Chemosis conjunctivae	1	0	0	0	0	1
	Discharge	1	0	0	0	0	1
956	Corneal opacity	0	0	0	0	0	-
	Iritis	0	0	0	0	0	-
	Redness conjunctivae	1	0	0	0	0	1
	Chemosis conjunctivae	1	0	0	0	0	1
	Discharge	1	0	0	0	0	1
569	Corneal opacity	0	0	0	0	0	-
	Iritis	0	0	0	0	0	-
	Redness conjunctivae	1	0	0	0	0	1
	Chemosis conjunctivae	1	0	0	0	0	1
	Discharge	1	0	0	0	0	1

* scores in the range of 0 to 4 for cornea opacity and chemosis, 0 to 3 for redness of conjunctivae and 0 to 2 for iritis

All animals became symptom free after 24 hours.

Fluorescein staining was negative in all rabbit test item treated eyes.

The control eye of all animals was symptom-free during the 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 pydiflumetofen FS (A22657B) was graded as a minimal irritant (Class 3 on a 1 to 8 scale) to the rabbit eye according to the modified Kay and Calandra classification system.

2.0 INTRODUCTION

2.1 Purpose

The purpose of this eye irritation study was to assess the irritancy potential of pydiflumetofen FS (A22657B), 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 assay in the Isolated Chicken Eyes (Citoxlab study code: 18/132-038CS) with the test item pydiflumetofen FS (A22657B) concluded that the test item is non-irritant. This *in vivo* study was conducted for classification of eye irritation, for regional 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 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 (EC) 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 authorized the conduct of the study.

3.0 MATERIALS AND METHODS

3.1 Test Substance

The following information was provided by the Sponsor.

Name:	Pydiflumetofen FS (A22657B)
Batch number:	SGBW180118-GLP1
Active Ingredient Content*:	17.7% w/w corresponding to 199 g/L
Density:	1125 kg/m^3
Appearance:	Red liquid
Recertification date:	31 January 2021
Storage conditions:	Room temperature (<30°C)
Safety precautions:	Routine safety precautions (lab coat, gloves, safety glasses and face mask) for unknown materials were applied to assure personnel health and safety.

**No adjustment for the 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 treatment, washing and fluorescein control:

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

Name:	Saline (0.9% NaCl)
Lot number:	72522Y05-2 / 80353Y05-1
Expiry date:	31 May 2020 / 31 December 2020
Produced by:	B. Braun Pharmaceuticals SA, Germany

Name:	Insulin disposable syringe, 1 mL
Lot number:	2017-04-26
Expiry date:	26 April 2022
Produced by:	Jiangxi Hongda Medical Equipment Group Ltd.

Name:	Fluorescein 100 mg/mL
Batch number:	271594F
Expiry date:	31 October 2018
Produced by:	Alcon Pharma GmbH

Systemic opiate analgesic:

Name:	Bupredine Multidose (0.3 mg/mL buprenorphine)
Batch number:	16D188
Expiry date:	31 September 2018
Produced by:	Le Vet. Beheer B.V.

Topical ocular anaesthetic:

Name:	Benoxi (4 mg/mL oxybuprocaine)
Batch number:	050416
Expiry date:	31 August 2018
Produced by:	Unimed Pharma, spol. s r.o., Slovakia

Nonsteroidal anti-inflammatory drug:

Name:	Loxicom (5 mg/mL meloxicam)
Batch number:	7041-94C
Expiry date:	31 July 2018
Manufacturer:	Norbrook Laboratories Limited, UK

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:	~14 weeks
Body weight range at dosing:	3377 – 3800 g
Body weight range at termination:	3433 – 3865 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.
Acclimation time:	at least 35 days

3.3.2 Husbandry

Animal health:	Only healthy animals were used for the study, as certified by the veterinarian.
Room:	034
Housing/Enrichment:	Rabbits were individually housed in AAALAC approved metal wire rabbit cages. Cages were of an open wire structure and cages were placed together to allow some social interaction with rabbit(s) in adjoining cages.
Light:	12 hours daily from 6.00 a.m. to 6.00 p.m. (and during the analgesic/anaesthetic treatment)
Temperature:	18.9–22.6°C
Relative humidity:	44–68%
Ventilation:	15-20 air exchanges/hour.

The temperature and relative humidity values were measured continuously. The measured range was checked regularly during the acclimation 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:

- 0005017453, expiry date: 24 August 2018,
- 0005052863, expiry date: 08 September 2018.

The food was not considered 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 once every 3 months and microbiological assessment is performed monthly, by Veszprém County Institute of State Public Health and Medical Officer Service (ÁNTSZ, H-8201 Veszprém, József A.u.36., Hungary). The quality control results are retained in the archive at Citoxlab Hungary Ltd.

3.4 Pre-Study and Analgesic and Anaesthetic Treatment Procedures

3.4.1 *In vitro* study results

An *in vitro* eye irritation study was performed prior to the treatment of any animal. The results from the *in vitro* eye irritation study (Citoxlab code: 18/132-038CS) in the Isolated Chicken Eye model with pydiflumetofen FS (A22657B), in accordance with the guidance from the OECD 438 for this method, indicated that the test item was non-irritant.

3.4.2 Identification of pH

The pH of the test item was measured as pH 6.31, 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. As the local effect in the first rabbit was non-irritant (all scores were zero) at 24 hours, then two further rabbits were treated with test item after the 48 hour observation of the first rabbit.

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 nonsteroidal anti-inflammatory drug (NSAID) were administered by subcutaneous (SC) injection under direct Veterinary supervision. The systemic opiate analgesic was again injected ~12 hours and ~12 hours after the post-treatment analgesic.

Systemic opiate analgesic: Bupredine Multidose (buprenorphine) 0.01 mg/kg.
Topical ocular anaesthetic: Benoxi (oxybuprocaine) one-two drops per eye.
Nonsteroidal anti-inflammatory drug: Loxicom (meloxicam) 0.5 mg/kg.

3.5 Administration of the Test Item

3.5.1 Dosage

A single volume of 0.1 mL of pydiflumetofen FS (A22657B) was administered to the left eye of each animal.

3.5.2 Application of the test item

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

The untreated contralateral eye served as the control.

3.5.3 Duration of exposure

Both eyes of the test animal were rinsed with physiological saline solution following fluorescein control: 24, 48, 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, 72 hours in all animals after test material installation. Observations with fluorescein staining were made approximately 24 hours before treatment and then 24, 48, 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 (1956) 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 4).

3.7 *Post Mortem* Investigations

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

3.7.1 Materials used for euthanasia

Name:	Ketanest (100 mg/mL ketamine)
Batch number:	J4213-06
Expiry date:	31 October 2020
Produced by:	Bela-pharm GmbH & Co. KG, Germany

Name:	Nerfasin (20 mg/mL xylazine)
Batch number:	16A134
Expiry date:	31 December 2018
Produced by:	Le Vet B.V., The Netherlands

Name:	Euthanimal 40% (Pentobarbital sodium, 400 mg/mL)
Lot No.:	1609291-03
Expiry Date:	31 October 2019
Produced by:	Alfasan Nederland BV, Kuipersweg 9, Woerden, The Netherlands

4.0 RESULTS AND DISCUSSION

4.1 Ocular Reactions

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

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

First animal (No: 958) clinical observation
At one hour after application, conjunctival redness (score 1), chemosis (score 1) and discharge (score 1) were noted in the rabbit.
At 24, 48 and 72 hours after the application, no clinical signs and no conjunctival or corneal effects were observed. Fluorescein staining was negative during the observations.

Second animal (No: 956) clinical observation
At one hour after application, conjunctival redness (score 1), chemosis (score 1) and discharge (score 1) were noted in the rabbit.
At 24, 48 and 72 hours after the application, no clinical signs and no conjunctival or corneal effects were observed. Fluorescein staining was negative during the observations.

Third animal (No: 569) clinical observation
At one hour after application, conjunctival redness (score 1), chemosis (score 1) and discharge (score 1) were noted in the rabbit.
At 24, 48 and 72 hours after the application, no clinical signs and no conjunctival or corneal effects were observed. Fluorescein staining was negative during the observations.

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

4.2 Body Weight

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

4.3 Clinical Signs

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

4.4 Mortality

No mortality occurred during this study.

5.0 CONCLUSIONS

The test item pydiflumetofen FS (A22657B) was graded as a minimal irritant (Class 3 on a 1 to 8 scale) to the rabbit eye according to the modified Kay and Calandra classification system.

6.0 REFERENCES

Kay JH, Calandra JC. 1962. Interpretation of eye irritation tests. J Soc Cosmet Chem 13:281–289.

Internal references

Orovecz B, 2018. Pydiflumetofen FS (A22657B) - *In Vitro* Eye Irritation Test in Isolated Chicken Eyes

TABLES SECTION

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

Rabbit number and sex	**958 Male**				**956 Male**				**569 Male**			
IPR	0				0				0			
PR	0	0	0	0	0	0	0	0	0	0	0	0
Time after treatment	1 Hr	24 Hr	48 Hr	72 Hr	1 Hr	24 Hr	48 Hr	72 Hr	1 Hr	24 Hr	48 Hr	72 Hr
CORNEA												
E = Degree of Opacity	0	0	0	0	0	0	0	0	0	0	0	0
F = Area of Cornea involved	0	0	0	0	0	0	0	0	0	0	0	0
* Score (E x F) x 5	0	0	0	0	0	0	0	0	0	0	0	0
IRIS												
D	0	0	0	0	0	0	0	0	0	0	0	0
* Score (D x 5)	0	0	0	0	0	0	0	0	0	0	0	0
CONJUNCTIVAE												
A = Redness	1	0	0	0	1	0	0	0	1	0	0	0
B = Chemosis	1	0	0	0	1	0	0	0	1	0	0	0
C = Discharge	1	0	0	0	1	0	0	0	1	0	0	0
* Score (A+B+C) x 2	6	0	0	0	6	0	0	0	6	0	0	0
* Total Score	6	0	0	0	6	0	0	0	6	0	0	0

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

TABLE 2 Individual Total Scores and Group Mean Scores for Ocular Irritation Calculated from the Draize Scores

Rabbit Number and Sex	* Individual Total Scores At:			
	1 Hour	24 Hours	48 Hours	72 Hours
958 Male	6	0	0	0
956 Male	6	0	0	0
569 Male	6	0	0	0
* Group Total	18	0	0	0
* Group Mean Score	6.00	0.00	0.00	0.00

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

TABLE 3 Individual Fluorescein Staining

Rabbit Number and sex	Fluorescein Staining (treated eye)			
	24 Hours Prior to Instillation	24 Hours After Instillation	48 Hours After Instillation	72 Hours After Instillation
958 Male	-	-	-	-
956 Male	-	-	-	-
569 Male	-	-	-	-

- : Absence of Fluorescein Stain
+ : Presence of Fluorescein Stain

TABLE 4 Individual Bodyweights and Bodyweight Change

Rabbit Number and Sex	Individual Bodyweight (g)		Bodyweight Change (g)
	Before treatment	At termination	
958 Male	3725	3792	67
956 Male	3800	3865	65
569 Male	3377	3433	56

TABLE 5 Individual Clinical Signs

Rabbit Number and Sex	Day 0	Day 1	Day 2	Day 3
958 Male	N	N	N	N
956 Male	N	N	N	N
569 Male	N	N	N	N

N: Symptom-free

APPENDICES SECTION

APPENDIX 1 Certificate of Analysis

syngenta

GLP Testing Facility GOA
Analytical & Product Chemistry

Syngenta Biosciences Pvt. Ltd.
Santa Monica Works,
Corlim, Ilhas Goa 403 110
India

Certificate of Analysis

A22657B
Pydiflumetofen FS (200)
SGBW180118-GLP1

Batch Identification	**SGBW180118-GLP1**
Other Batch ID	1026746
Product Code	**A22657B**
Other Product Code(s)	SYN545974 FS (200)
Chemical Analysis (Active Ingredient content)	
– **Identity of the Active Ingredient***	**Confirmed**
– **Content of pydiflumetofen (SYN545974)***	**17.7 % w/w corresponding to 199 g/l**

The Active Ingredient content is within the FAO limits.

Methodology used for Characterization	HPLC, oscillating density meter
Physical Analysis	
– **Appearance**	**Red liquid**
– **Density***	**1125 kg/m^3**
Stability:	
– **Storage Temperature**	**< 30 °C**
– **Recertification Date**	**End of January 2021**

If stored under the conditions given above, this test substance can be considered stable until the recertification date is reached.

This Certificate of Analysis is summarizing data which originate 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 study(ies) are stored under the study number(s) referenced below within the archives of the GLP Testing Facility Goa at Syngenta Biosciences Pvt. Ltd., Santa Monica Works, Corlim, Ilhas, Goa 403110.

Study number of batch characterization:	SMG14605
Study number(s) of batch recertification:	---

Authorization: 15-Mar-2018

Sunil B. Khot
Analytical and Product Chemistry, Goa

Page 1 of 1

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

Study number	18/132-005N
Study type	EYE IRRITATION (DRAIZE)
Lab Reference	Citoxlab Hungary Ltd.
Study guidelines	OECD 405 (2017), OPPTS 870.2400 (1998), EC 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
958	Symptom-free	N	-	-
956	Symptom-free	N	-	-
569	Symptom-free	N	-	-

APPENDIX 6 GLP Certificate

OGYÉI
National Institute of
Pharmacy and Nutrition

H-1051 Budapest, Zrínyi u. 3.
1372 P.O. Box:450.
Tel: +36 1 88 69-300, Fax: +36 1 88 69 460
E-mail: ogyei@ogyei.gov.hu, Web: www.ogyei.gov.hu

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

GOOD LABORATORY PRACTICE (GLP) CERTIFICATE

It is hereby certified that the test facility

CiToxLAB Hungary Ltd.

H-8200 Veszprém, Szabadságpuszta

is able to carry out

physico-chemical testing, toxicity studies, in vitro studies and mutagenicity studies, environmental toxicity studies on aquatic or terrestrial organisms, studies on behaviour in water, soil and air; bio-accumulation, reproduction toxicology, inhalation toxicology, analytical chemistry and contract archiving

in compliance with the Principles of GLP (Good Laboratory Practice) and also complies with the corresponding OECD/European Community requirements.

Date of the inspection: **02-04. June 2015.**

Dr. József Reiter
Deputy Director-General

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

APPENDIX 6 GLP Certificate (continued)

OGYÉI
National Institute of
Pharmacy and Nutrition

H-1051 Budapest, Zrínyi u. 3.
1372 P.O. Box:450.
Tel: +36 1 88 69-300, Fax: +36 1 88 69 460
E-mail: ogyei@ogyei.gov.hu, Web: www.ogyei.gov.hu

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")