Cyproconazole/Isopyrazam

Cyproconazole/Isopyrazam SC (A19022A) - Acute Dermal Toxicity Study in Rats

Final Report

DATA REQUIREMENT(S): OECD 402 (1987)
EPA OPPTS 870.1200 (1998)
EC 440/2008 (2008)

AUTHOR(S): Istvánné Kiss, M.Sc.

STUDY COMPLETION DATE: 10 July 2012

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

LABORATORY PROJECT ID: Report Number: 12/055-002P
Study Number: 12/055-002P
Task Number**:** TK0006575

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: 9/2001. (III. 30.) EüM-FVM joint decree of the Minister of Health and the Minister of Agriculture and Regional Development which corresponds to the OECD GLP, ENV/MC/CHEM (98) 17.).

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.

K. Istvánné	10 July 2012
Istvánné Kiss, M.Sc. Study Director	Date

Performing Laboratory: CiToxLAB Hungary Ltd.
H-8200 Veszprém, Szabadságpuszta
Hungary

FLAGGING STATEMENT

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QUALITY ASSURANCE STATEMENT

Study Code: 12/055-002P

Study Title: Cyproconazole/Isopyrazam SC (A19022A) - Acute Dermal Toxicity Study in Rats

Test Item: Cyproconazole/Isopyrazam SC (A19022A)

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
08 March 2012	Study Plan	08 March 2012	08 March 2012
21 March 2012	Treatment	21 March 2012	21 March 2012
23 April 2012	Draft Report	23 April 2012	23 April 2012
10 July 2012	Final Report	10 July 2012	10 July 2012

Signature: _____ Date: 10 JUL 2012

Katalin Böröcki, M.Sc.
QA Inspector

STATEMENT OF THE MANAGEMENT

According to the conditions of the research and development agreement between Syngenta Limited (as Sponsor) and CiToxLAB Hungary Ltd. (as Test Facility) the study titled "Cyproconazole/Isopyrazam SC (A19022A) - Acute Dermal Toxicity Study in Rats" was performed, in compliance with the Principles of Good Laboratory Practice.

Signature: _____ Date: 10 July 2012

Christopher Banks, DABT
Managing Director

GENERAL INFORMATION

Contributors

The following contributed to this report in the capacities indicated:

Name	Function
Istvánné Kiss, M.Sc.	Study Director
Viktória Zelenák, M.Sc.	Assistant Scientist
István Pásztor, DVM	Veterinary Control
Peter Maslej, DVM, PhD	Head of Pathology
Tamás Mészáros, PhD	Technical Team Leader of Central Dispensary Unit
Claire Elliott	Syngenta Study Manager

Study dates

Study initiation date: 09 March 2012
Experimental start date: 21 March 2012
Experimental termination date: 04 April 2012

Retention of samples

See in other below

Performing laboratory test substance reference number

12003B

Other

The study documents:
- 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 SOP's in the archives of CiToxLAB Hungary Ltd. 8200 Veszprém, Szabadságpuszta, Hungary. This is for a period of 15 years.

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

A single administration of cyproconazole/isopyrazam SC (A19022A) at a dose of 2000 mg/kg body weight was applied dermally to 5 male and 5 female CRL:(WI) rats, followed by a 14-day observation period. The test item was applied as supplied by the Sponsor. The application period was 24 hours.

Clinical observations were assessed in all animals at 1 and 5 hours after dosing and daily for 14 days thereafter. Body weight was measured prior to dosing on Day 0 and on Days 7 and 14. All animals were euthanized and subjected to a gross macroscopic examination at the end of the 14-day observation period (Day 14).

1.2 Results

No mortality occurred during the study.

No adverse clinical signs were observed after treatment with the test item or during the 14 day observation period and no effects were observed at the site of application.

Slight body weight loss was observed in one female animal on the second week. In the absence of a consistent response, this variation was ascribed to individual variability, unrelated to treatment.

There was no evidence of the test item-related observations at a dose level of 2000 mg/kg bw at necropsy.

1.3 Conclusion

The median lethal dose of cyproconazole/isopyrazam SC (A19022A) after a single dermal administration was found to be greater than 2000 mg/kg bw in male and female CRL:(WI) rats.

2.0 INTRODUCTION

2.1 Purpose

The purpose of the study was to assess the acute dermal toxicity of cyproconazole/isopyrazam SC (A19022A) when administered to rats by a single semi-occlusive dermal application, followed by an observation period of 14 days.

This study was designed to provide a rational basis for hazard classification.

2.2 Guidelines

The study was performed according to the following guidelines:

OECD Guidelines for Testing of Chemicals, Section 4, Number 402 "Acute Dermal Toxicity", adopted February 24, 1987.

United States Environmental Protection Agency Health Effects Division Test Guidelines, OPPTS 870.1200 Acute Dermal Toxicity EPA 712-C-98-192, August 1998

Commission Regulation (EC) No 440/2008, B.3 (L 142, 30 May 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

Data as supplied by the Sponsor.

The certificate of analysis is attached in Appendix 1.

Name:	Cyproconazole/isopyrazam SC (A19022A)
Batch number:	J8657/147
Product code:	A19022A
Purity:	Content of cyproconazole – 79.9 g/l corresponding to 7.45 % w/w Content of isopyrazam (sum of epimers) – 129 g/l corresponding to 12.0 % w/w Content of SYN534969 (syn-epimer of isopyrazam) – 112 g/l corresponding to 10.4 % w/w Content of SYN534968 (anti-epimer of isopyrazam) – 17.0 g/l corresponding to 1.58 % w/w
Density:	1073 kg/m^3
Appearance:	beige liquid
Recertification date:	End of October 2013
Storage conditions:	Room temperature (<30 ^oC)
Safety Precautions:	Routine safety precautions (lab coat, gloves, goggles, face mask) for unknown materials were applied to assure personnel health and safety

3.1.1 Identification and receipt of the test substance

The test item of a suitable chemical purity was provided by the Sponsor. All precautions required in the handling and disposal of the test item were outlined by the Sponsor. The test item was identified by the Central Dispensary of CiToxLAB Hungary Ltd. on the basis of the information supplied by the Sponsor.

3.1.2 Formulation

The test item was administered as a single dose, as supplied by the Sponsor.

3.1.3 Other materials

For euthanasia:

Name:	Euthasol® 40%
Lot No.:	11H15 8
Expiry Date:	July 2014
Produced by:	Produlab Pharma B.V.

3.2 Experimental Design

3.2.1 Doses

A limit dose of 2000 mg/kg bw was chosen by the Sponsor.

3.2.2 Experimental design

Dose Group	Number of cages	Number of animals
Male group 2000 mg/kg bw	Cages 1-5	5
Female group 2000 mg/kg bw	Cages 6-10	5

A single administration was performed by the dermal route and was followed by a 14-day observation period.

The application volume was 1.86 mL/kg bw (x 1073 mg/mL = 2000 mg/kg).

3.2.3 Procedure

The backs of the animals were shaven (approximately 10% area of the total body surface) approximately 24 hours prior to treatment. Only those animals without injury or irritation on the skin were used in the test.

On test day 0, the test item was applied at a single dose of 2000 mg/kg body weight applied uniformly over the skin and remained on the skin throughout a 24- hour exposure period. Sterile gauze pads were placed on the skin of rats at the site of application. These gauze pads were kept in contact with the skin by a patch with adhesive hypoallergenic plaster. The entire trunk of the animal was then wrapped with semi occlusive plastic wrap for 24 hours. At the end of the exposure period, residual test item was removed, using body temperature water.

3.2.4 Clinical observations

A clinical examination was performed on the day of treatment, at 1 and 5 hours after the application of the test item, and once each day for 14 days thereafter.

Observations included assessments of the skin and fur, eyes and mucous membranes, the respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and behavior patterns. Particular attention was directed to the observation of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma.

3.2.5 Measurement of body weight

The body weight of all animals was recorded on Day 0 (beginning of the experiment) and on Days 7 and 14.

3.2.6 Animals

Species and strain:	CRL:(WI) rats
Source:	Charles River Laboratories, Research Models and Services, Germany GmbH, Sandhofer Weg 7, D-97633 Sulzfeld
Hygienic level at arrival:	SPF
Hygienic level during the study:	Standard housing conditions
Justification of strain:	Recognized by international guidelines as a recommended test system
Number of animals:	5 animals/sex
Sex:	Male and female, female rats were nulliparous and non-pregnant
Age of animals:	Young adult rats
Body weight range at dosing:	Between 207 g and 276 g
Acclimatization time:	8 days

3.2.7 Husbandry

Animal health:	Only healthy animals were used for the study. The veterinarian certified the health status.
Room-Box:	242/4
Housing:	Individual caging
Cage type:	Type II. polypropylene/polycarbonate
Bedding:	Laboratory bedding was available to animals during the study (Lignocel® Hygienic Animal Bedding produced by J. Rettenmaier & Söhne GmbH+Co.KG).
Light:	12 hours daily, from 6.00 a.m. to 6.00 p.m.
Temperature:	22 ± 3°C

Relative humidity:	30 - 70 %
Ventilation:	15 - 20 air exchanges per hour
Enrichment:	Rodents were housed with deep wood sawdust bedding to allow digging and other normal rodent activities.

The temperature and relative humidity were recorded twice daily during the study.

3.2.8 Food and water supply

Animals received ssniff® SM R/M-Z+H "Autoclavable complete diet for rats and mice – breeding and maintenance" produced by ssniff Spezialdiäten GmbH, D-59494, Soest, Germany *ad libitum* (Lot number: 719 6627). Tap water from the municipal supply (fit for human consumption) was provided *ad libitum* from 500 mL bottles. The food and water was considered not to contain any contaminants that could reasonably be expected to affect the purpose or integrity of the study.

Water quality control analysis is performed once every three 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 archives at CiToxLAB Hungary Ltd.

3.2.9 Identification

Animals were identified by numbers written on the tail with an indelible marker. The numbers were allocated on the basis of the CiToxLAB Hungary Ltd. master file.

The boxes were identified by cards holding information on the study code, the sex of animals, the dose group, the cage number and the individual animal number.

3.3 *Post Mortem* Investigations

All animals were subjected to gross macroscopic examination. All animals were anesthetized with Euthasol® 40% (details in 3.1.3.) and exsanguinated. After examination of the external appearance, the cranial, thoracic and abdominal cavities were opened and the appearance of the tissues and organs were observed. Any gross macroscopic findings were recorded.

3.4 Data Evaluation

The type, severity and duration of clinical observations were described. Body weight and body weight changes were summarized in tabular form. Necropsy findings were described and summarized in tabular form.

4.0 RESULTS AND DISCUSSION

4.1 Mortality

No mortality occurred on the study.

4.2 Clinical Signs

There were no adverse clinical signs noted in any animals throughout the study.

4.3 Local Dermal Signs

No findings were observed in any animals throughout the study.

4.4 Body Weight

Slight body weight loss was observed in one female animal on the second week. In the absence of a consistent response, this variation was ascribed to individual variability, unrelated to treatment.

4.5 Necropsy

No treatment related macroscopic findings were observed.

There was no evidence of the test item-related observations at a dose level of 2000 mg/kg bw at necropsy.

5.0 CONCLUSIONS

The median lethal dose of cyproconazole/isopyrazam SC (A19022A) after single dermal administration was found to be greater than 2000 mg/kg bw in male and female CRL:(WI) rats.

TABLES SECTION

GLOSSARY FOR TABLE 1

Grading of Skin Reactions

ERYTHEMA AND ESCHAR FORMATION

No erythema	0
Very slight erythema	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beef redness) or eschar formation (injuries in depth preventing erythema) reading	4

OEDEMA FORMATION

No oedema	0
Very slight oedema (barely perceptible)	1
Slight oedema (edges of area well-defined by definite raising)	2
Moderate oedema (edges raised approximately 1 mm)	3
Severe oedema (raised more than 1 mm and extending beyond the area of exposure)	4

TABLE 1 Clinical Observations

DOSE LEVEL: 2000 mg/kg bw, Treatment on Day 0 **SEX: MALE**

Cage No.	Animal No.	Observations	Observation days																Frequency
			0		1	2	3	4	5	6	7	8	9	10	11	12	13	14	
			1h	5h															
1	1861	Symptom Free	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	16/16
2	1862	Symptom Free	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	16/16
3	1863	Symptom Free	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	16/16
4	1864	Symptom Free	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	16/16
5	1865	Symptom Free	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	16/16

DOSE LEVEL: 2000 mg/kg bw, Treatment on Day 0 **SEX: FEMALE**

Cage No.	Animal No.	Observations	Observation days																Frequency
			0		1	2	3	4	5	6	7	8	9	10	11	12	13	14	
			1h	5h															
6	1871	Symptom Free	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	16/16
7	1872	Symptom Free	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	16/16
8	1873	Symptom Free	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	16/16
9	1874	Symptom Free	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	16/16
10	1875	Symptom Free	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	16/16

Remarks: + = present

h = hour (s)

Frequency of observation = number of occurence of observation / total number of observations

TABLE 2 Body Weight and Body Weight Gain

DOSE LEVEL: 2000 mg/kg bw, Treatment on Day 0 **SEX: MALE**

Cage No.	Animal No.	Body weight (g) Days			Body Weight Gain (g)		
		0	7	14	0-7	7-14	0-14
1	1861	274	328	366	54	38	92
2	1862	272	303	355	31	52	83
3	1863	274	317	360	43	43	86
4	1864	265	302	346	37	44	81
5	1865	276	331	380	55	49	104
Mean:		272.2	316.2	361.4	44.0	45.2	89.2
Standard deviation:		4.3	13.6	12.7	10.5	5.4	9.3

DOSE LEVEL: 2000 mg/kg bw, Treatment on Day 0 **SEX: FEMALE**

Cage No.	Animal No.	Body weight (g) Days			Body Weight Gain (g)		
		0	7	14	0-7	7-14	0-14
6	1871	207	211	229	4	18	22
7	1872	216	224	240	8	16	24
8	1873	234	252	248	18	-4	14
9	1874	229	241	258	12	17	29
10	1875	226	253	260	27	7	34
Mean:		222.4	236.2	247.0	13.8	10.8	24.6
Standard deviation:		10.8	18.3	12.9	9.0	9.4	7.5

TABLE 3 Necropsy Findings

DOSE LEVEL: 2000 mg/kg bw, Treatment on Day 0 **SEX: MALE**

Cage No.	Animal No.	Necropsy Day	External Observations	Internal Observations	Organ/ Tissue
1	1861	Day 14	No external observations	No internal observations	Not applicable
2	1862	Day 14	No external observations	No internal observations	Not applicable
3	1863	Day 14	No external observations	No internal observations	Not applicable
4	1864	Day 14	No external observations	No internal observations	Not applicable
5	1865	Day 14	No external observations	No internal observations	Not applicable

DOSE LEVEL: 2000 mg/kg bw, Treatment on Day 0 **SEX: FEMALE**

Cage No.	Animal No.	Necropsy Day	External Observations	Internal Observations	Organ/ Tissue
6	1871	Day 14	No external observations	No internal observations	Not applicable
7	1872	Day 14	No external observations	No internal observations	Not applicable
8	1873	Day 14	No external observations	No internal observations	Not applicable
9	1874	Day 14	No external observations	No internal observations	Not applicable
10	1875	Day 14	No external observations	No internal observations	Not applicable

APPENDICES SECTION

APPENDIX 1 Certificate of Analysis

syngenta

GLP Testing Facility WMU
Analytical Development &
Product Chemistry

Syngenta Crop Protection
Münchwilen AG
Breitenloh 5
CH-4333 Münchwilen

Certificate of Analysis

A19022A
cyproconazole/isopyrazam SC (080/125)
J8657/147

Batch Identification	**J8657/147**
Product Code	**A19022A**
Other Product Code(s)	SAN619/SYN520453 SC (080/125)

Chemical Analysis
(Active Ingredient Content)

– **Identity of the Active Ingredients***	**confirmed**
– **Content of Cyproconazole***	**79.9 g/l corresponding to 7.45 % w/w**
– **Content of Isopyrazam (sum of epimers)***	**129 g/l corresponding to 12.0 % w/w**
– **Content of SYN534969 (syn-epimer of isopyrazam)***	**112 g/l corresponding to 10.4 % w/w**
– **Content of SYN534968 (anti-epimer of isopyrazam)***	**17.0 g/l corresponding to 1.58 % w/w**

The Active Ingredients content is within the FAO limits.

Methodology used for Characterization: HPLC

Physical Analysis

– **Appearance**	**beige liquid**
– **Density***	**1073 kg/m^3**

Stability:

– **Storage Temperature**	**< 30°C**
– **Recertification Date**	**End of October 2013**

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 Muenchwilen AG.

Study number of batch characterization: 123779

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

Authorisation:

14 November 2011

Dr. R. Kettner
Analytical Development & Product Chemistry

APPENDIX 2 Pathology Report

PATHOLOGY REPORT

Introduction

The objective of the study was to assess the acute dermal toxicity of cyproconazole/isopyrazam SC (A19022A) when administered in a single 24 hour dermal application to rats at one or more defined dose levels followed by 14 days observation.

Results and Discussion

All rats survived until the scheduled termination of the study.

All animals were euthanized upon completion of the observation period on Day 14. Rats were anesthetized with pentobarbital, followed by exsanguination. Gross pathology consisted of an external examination, including identification of all clinically-recorded lesions, as well as a detailed internal examination. Histopathological examination was not performed.

TERMINAL (DAY 14)

MACROSCOPIC FINDINGS

There was no evidence of the any observations at a dose level of 2000 mg/kg bw at necropsy.

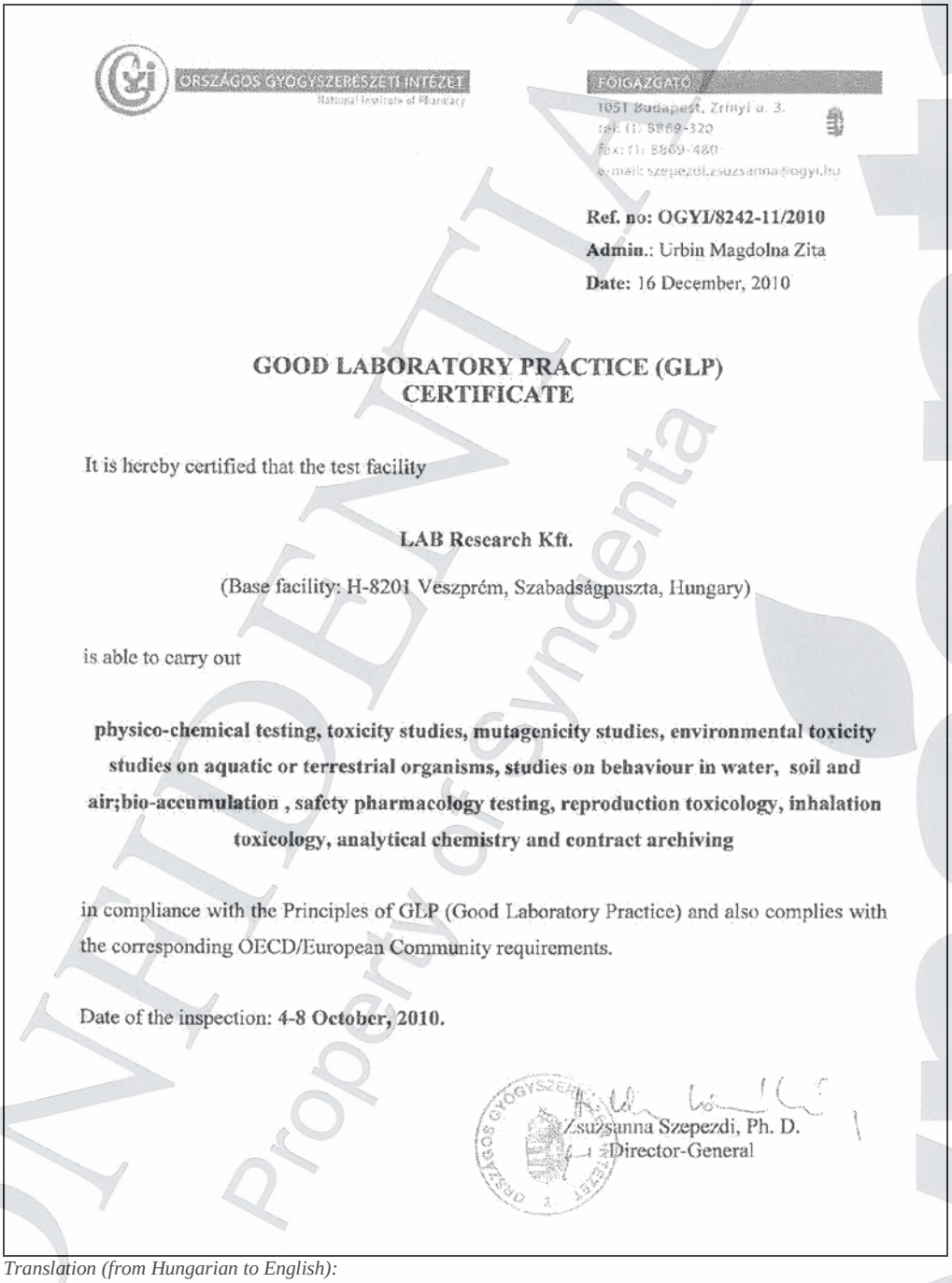
Conclusion

A single 24 hour dermal application of cyproconazole/isopyrazam SC (A19022A) to CRL: (WI) rats at a dose level of 2000 mg/kg bw followed by 14 days observation, was not associated with any macroscopic findings.

09/Aug 2012

Peter Maslej, D.V.M., Ph.D. Date
Head, Pathology Department

APPENDIX 3 GLP Certificate



ORSZÁGOS GYÓGYSZERÉSZETI INTÉZET
National Institute of Pharmacy

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e-mail: szepezdi.zsuzsanna@ogyi.hu

Ref. no: OGYI/8242-11/2010
Admin.: Urbin Magdolna Zita
Date: 16 December, 2010

GOOD LABORATORY PRACTICE (GLP) CERTIFICATE

It is hereby certified that the test facility

LAB Research Kft.

(Base facility: H-8201 Veszprém, Szabadságpuszta, Hungary)

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 , safety pharmacology testing, 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: **4-8 October, 2010.**

Zsuzsanna Szepezdi, Ph. D.
Director-General

Translation (from Hungarian to English):
Stamp Translation = Országos Gyógyszerészeti Intézet (OGYI) = National Institute of Pharmacy
Főigazgató = Director-General

The facility name was LAB Research Ltd until 1st September 2011. From this date, the registered name is now CiToxLAB Hungary Ltd.,this information has been transmitted to the GLP competent authority. The above GLP certificate is valid for this facility (now known as CiToxLAB Hungary Ltd.) until the certificate expires (16 December 2012).