Propiconazole/Pydiflumetofen

Propiconazole/Pydiflumetofen SE (A21573C) - Acute Oral Toxicity Study in Rats (Up and Down Procedure)

Final Report Amendment 1

DATA REQUIREMENT(S): OECD 425 (2008)
EPA 870.1100 (2002)

AUTHOR(S): Ádám Appl, M.Sc.

COMPLETION DATE: 04 May 2018

REPORT AMENDMENT 1 DATE: 19 June 2019

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

LABORATORY PROJECT ID: Report Number: 17/349-001P
Study Number: 17/349-001P
Task Number: TK0186809

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.

No chemical analysis of the dose formulation was performed as part of this study. Traceability (equipment used, quantities of test item weighed …) of dosing form preparations was checked and revealed no abnormalities of consequences. Furthermore, for this study, the formulations were prepared just before the treatment. Consequently, the absence of dose formulation analysis data was considered not to prejudice the overall GLP status of the study and the scientific reliability of the study conclusions.

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.

Ádám Appl, M.Sc.
Study Director

19 June 2019
Date

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

FLAGGING STATEMENT

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QUALITY ASSURANCE STATEMENT

Study Number: 17/349-001P

Study Title: Propiconazole/Pydiflumetofen SE (A21573C) - Acute Oral Toxicity Study in Rats (Up and Down Procedure)

Test Item: Propiconazole/Pydiflumetofen SE (A21573C)

This study has been inspected, and the report as well as the Final Report Amendment 1 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 and the Final Report Amendment 1 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
27 November 2017	Study Plan	27 November 2017	27 November 2017
28 November 2017	Observation	28 November 2017	28 November 2017
14 February 2018	Draft Report	14 February 2018	14 February 2018
04 May 2018	Final Report	04 May 2018	04 May 2018
19 June 2019	Final Report Amendment 1	19 June 2019	19 June 2019

Signature: Németh Nikolett Date: 19 June 2019

Nikolett Németh, B.Sc.
QA Inspector

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 "Propiconazole/Pydiflumetofen SE (A21573C) - Acute Oral Toxicity Study in Rats (Up and Down Procedure)" has been performed in compliance with the Principles of Good Laboratory Practice.

Signature: _____ Date: 19 June 2019

Christopher Banks, DABT
Site Director

GENERAL INFORMATION

Contributors

The following contributed to this report in the capacities indicated:

Name	Function
Ádám Appl, M.Sc.	Study Director
Zsolt Tarcai, M.Sc.	Assistant Scientist
Nikolett Németh, B.Sc.	Quality Assurance Unit
László Székelyhidi, D.V.M.	Veterinary Care
Gábor Boros, D.V.M.	Pathology
Tamás Mészáros, Ph.D.	Pharmacy
Monique Inforzato, B.Sc.	Syngenta Study Manager

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

Study dates

Study initiation date:	27 November 2017
Experimental starting date:	28 November 2017
Experimental completion date:	02 January 2018
Receipt of animals:	16 / 23 November and 08 December 2017
Acclimatization period:	At least 6 days
Treatment date:	28 November 2017 (female no. 1751) 29 November 2017 (female no. 1752) 30 November 2017 (female no. 1753) 03 December 2017 (female no. 1958) 05 December 2017 (female no. 1959) 07 December 2017 (female no. 1960) 13 December 2017 (female no. 1961) 14 December 2017 (female no. 2234) 19 December 2017 (female no. 2235)

Observation period:	28 November 2017 (female no. 1751) 29 November 2017 (female no. 1752) 30 November – 14 December 2017 (female no. 1753) 03 December – 17 December 2017 (female no. 1958) 05 December – 19 December 2017 (female no. 1959) 07 December – 21 December 2017 (female no. 1960) 13 December 2017 (female no. 1961) 14 December – 28 December 2017 (female no. 2234) 19 December 2017 – 02 January 2018 (female no. 2235)
Necropsy:	28 November 2017 (female no. 1751) 29 November 2017 (female no. 1752) 14 December 2017 (female no. 1753) 17 December 2017 (female no. 1958) 19 December 2017 (female no. 1959) 21 December 2017 (female no. 1960) 13 December 2017 (female no. 1961) 28 December 2017 (female no. 2234) 02 January 2018 (female no. 2235)

Deviations from the Study Plan

There was no deviation during the study.

Performing laboratory test substance reference number

170356

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

An acute oral toxicity study was conducted with 9 female Crl:WI Wistar rats. Animals were treated with a single oral (gavage) dose of propiconazole/pydiflumetofen SE (A21573C) at the dose levels of 5000, 2000, 550 and 175 mg/kg body weight (bw) followed by a 14-day observation period. The animals were fasted overnight prior to treatment and food was returned 3 hours after dosing.

Individual animals were dosed sequentially at no less than 48 hour intervals (except in cases where the animal was found dead, so it was not necessary to wait 48 hours to treat the next animal). The time intervals between doses were determined by the onset, duration and severity of clinical signs. The first animal was treated at a dose level of 5000 mg/kg bw. As this animal died on Day 0, the Limit Test at 5000 mg/kg bw was terminated and the next animal was treated at the dose level of 2000 mg/kg bw. As this animal died on Day 0, the Limit Test at 2000 mg/kg bw was terminated and a Main Test was conducted according to the OECD Guideline 425. In the Main Test, the first animal was treated at a dose level of 175 mg/kg bw. The dose selection for the next animals followed the recommendation of AOT425StatPgm software, based on available results.

Animals were observed individually after dosing at 30 minutes, then 1, 2, 3, 4 and 6 hours post treatment and once each day for 14 days thereafter or until death. Body weight was measured on Day -1, just before dosing (Day 0) and in case of the surviving animals weekly thereafter (Days 7 and 14 (before necropsy)). All animals were euthanised and examined macroscopically at necropsy at the end of the observation period.

1.2 Results

The animal treated at the dose level of 5000 mg/kg bw was found dead on Day 0.

2 out of the 5 animals treated at the dose level of 2000 mg/kg bw were found dead on Day 0.

No mortality occurred at the dose levels of 550 mg/kg bw (0 out of 2 animals was found dead) and 175 mg/kg bw (0 out of 1 animal was found dead).

At dose level of 5000 mg/kg bw the following test item related symptoms were observed until death: slightly to markedly decreased activity, hunched back, prone position, moderate to marked incoordination and piloerection.

At the dose level of 2000 mg/kg bw the following test item related symptoms were observed from Day 0 up to Day 2 or until death: slightly / slightly to moderately / markedly decreased activity (4 out of 5 animals), hunched back (4 out of 5 animals), slight / slight to moderate incoordination (4 out of 5 animals), piloerection (3 out of 5 animals) and prone position (1 out of 5 animals). The surviving animals were symptom free from Day 3.

At the dose level of 550 mg/kg bw the following test item related symptoms were observed up to Day 1: slightly /slightly to markedly decreased activity (2 out of 2 animals), hunched back (2 out of 2 animals), piloerection (2 out of 2 animals), prone position (1 out of 2 animals) and slight to moderate incoordination (1 out of 2 animals). All animals were symptom-free from Day 2.

The animal treated at the dose level of 175 mg/kg bw was symptom-free during the 14-day observation period.

There were no treatment related effects on body weight or body weight gain. Body weights were within the range commonly recorded for this strain and age.

In the animal treated at the dose level of 5000 mg/kg bw, found dead on Day 0, the digestive content was liquid and beige, the lungs were collapsed. In the animals treated at the dose level of 2000 mg/kg bw, found dead on Day 0, in one case the digestive content was liquid and white, in both cases the lungs were collapsed. There was no evidence of any gross findings in the surviving 3 rats treated at a dose level of 2000 mg/kg bw and in the animals treated at the dose levels of 550 and 175 mg/kg bw, necropsied at the end of the observation period on Day 14.

1.3 Conclusion

Under the conditions of this study, the acute oral median lethal dose (LD_{50}) of the test item, propiconazole/pydiflumetofen SE (A21573C) was equal to 2000 mg/kg bw in female Crl:WI Wistar rats (95% PL Confidence interval is 1009 to greater than 20000).

2.0 INTRODUCTION

2.1 Purpose

The purpose of the study was to assess the acute oral toxicity of the test item propiconazole/pydiflumetofen SE (A21573C) when administered as a single oral gavage dose to female rats at one or more defined dose levels.

This study was performed with vertebrate animals as no *in vitro* alternative is available. The study was designed such that the minimum numbers of animals were used.

The Final Report (dated 04 May 2018) was reissued on 19 June 2019 by the Final Report Amendment 1 to amend test item details (Section 3.1. Test substance) in agreement with the Sponsor, to add the new GLP Certificate of the Test Facility to Appendix 3 and to update the responsible Test Facility member in the Management Statement.

2.2 Guidelines

The study was performed according to the following guidelines:

- OECD Guideline Reference 425 (2008): Acute Oral Toxicity - Up-and-Down Procedure.

- United States Environmental Protection Agency, Health Effects Test Guidelines, OPPTS 870.1100 Acute Oral Toxicity EPA 712-C-02-190, December 2002.

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:	Propiconazole/Pydiflumetofen SE (A21573C)
Batch number:	1007839
Active ingredient content*:	Pydiflumetofen, 13.7 % w/w corresponding to 151 g/L Propiconazole, 11.6 % w/w corresponding to 128 g/L
Density:	1.100 g/cm^3
Appearance:	Beige liquid
Recertification date:	31 October 2020
Storage conditions:	Room temperature (<30°C)
Safety precautions:	Enhanced safety precautions were applied considering the supplied safety datasheet to assure personnel health and safety.

*No correction for purity of the test item was applied.

The Certificate of Analysis is presented in Appendix 2.

3.1.1 Identification and receipt

The test item of a suitable chemical purity together with all precautions required in the handling and disposal of the test item were supplied by the Sponsor. The identification of the test item was made in the Pharmacy of Citoxlab Hungary Ltd. on the basis of the information provided by Sponsor.

3.1.2 Formulation

The test item was freshly formulated on the day of dosing at the concentration of 350 mg/mL (dose level: 175 mg/kg bw) in the vehicle in the Pharmacy of Citoxlab Hungary Ltd. or was administered undiluted (dose levels: 550, 2000 and 5000 mg/kg bw). The formulation was stirred continuously until the completion of treatment.

Vehicle information:

Name:	Distilled water
Batch number:	71033Y25-2
Manufacturer:	B. Braun
Expiry Date:	29 February 2020
Storage condition:	Room temperature

3.2 Experimental Design

3.2.1 Animals

Species and strain:	Crl:WI Wistar rats
Source:	Charles River Laboratories, Research Models and Services, Germany GmbH, Sandhofer Weg 7, D-97633 Sulzfeld, Germany
Hygienic level:	SPF at arrival, standard housing conditions during study
Justification of strain:	Recognized by international guidelines as a recommended test system.
Number of animals:	9
Sex:	Female rats, nulliparous and non-pregnant.
Age when treated:	Young adult rats, 8-10 weeks old.
Body weight (at dosing):	179-236 g
Identification:	The animals were identified by numbers written on the tail with an indelible marker. The cages were marked with individual identity cards with information about study number, sex, cage number, dose group and individual animal number.
Randomization:	Selected by hand at time of delivery.
Acclimatisation time:	At least 6 days

3.2.2 Husbandry

Animal health:	Only healthy animals were used for the test. The health status was certified by the Veterinarian.
Room number:	522/9
Housing / Enrichment:	Animals were housed individually in Type II. polypropylene/polycarbonate cages. Rodents were housed with deep wood sawdust bedding to allow digging and other normal rodent activities.
Bedding and nesting:	"Lignocel 3/4-S" Hygienic Animal Bedding and "Arbocel crinklets natural" nesting material *(produced by J. Rettenmaier & Söhne GmbH + Co.KG, Germany)* were available to animals during the study.
Light:	12 hours daily, from 6.00 a.m. to 6.00 p.m.
Temperature:	20.6 – 24.1 °C
Relative humidity:	32 – 57%
Ventilation:	15-20 air exchanges/hour

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

3.2.3 Food and feeding

Animals received ssniff® SM R/M "Autoclavable complete diet for rats and mice – breeding and maintenance" produced by ssniff Spezialdiäten GmbH, D-59494, Soest, Germany (Lot number: 262 21592, Expiry date: 31 January 2018 and Lot number: 382 24962, Expiry date: 30 April 2018) *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. A detailed description of the contents of the lots used is archived with the raw data at Citoxlab Hungary Ltd.

3.2.4 Water supply and quality control

Animals received tap water from the municipal supply from 500 mL bottles *ad libitum*. The water was fit for human consumption and 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 Attila utca 36., Hungary). The quality control results are retained in the archive at Citoxlab Hungary Ltd.

3.3 Administration of the Test Item

3.3.1 Dosages

Justification of the dose:

A limit test with a starting dose of 5000 mg/kg bw was selected by the Study Director after discussion with the Sponsor. A total of 9 animals were tested at the dose levels of 5000, 2000, 550 and 175 mg/kg bw. The density of the test item was 1.100 g/cm^3 as provided by the Sponsor, therefore the dose volume for the first animal was 4.55 mL/kg bw. For the dose level of 175 mg/kg bw, a dose volume of 0.5 mL/kg bw in the selected vehicle was applied. The individual dose volumes used are shown below.

Animal Number	Dose [mg/kg body weight]	Volume Dosed [mL]	Bodyweight [g]	Mortality
1751	5000	0.97	214	Died
1752	2000	0.40	219	Died
1753	175	0.10	201	Survived
1958	550	0.11	210	Survived
1959	2000	0.38	210	Survived
1960	2000	0.40	220	Survived
1961	2000	0.43	236	Died
2234	550	0.09	179	Survived
2235	2000	0.35	193	Survived

The treatment was terminated as the stopping criteria (c) was met in accordance the OECD Guideline 425.

Rationale:

Oral administration was considered to be an appropriate dose route as it is a possible route of human exposure.

3.3.2 Procedure

A single oral (gavage) dose was followed by a 14-day observation period. The animals were fasted overnight prior to treatment. Water was still available, *ad libitum* overnight. Animals were weighed before dosing and the food was returned 3 hours after the treatment.

Individual animals were dosed sequentially following an interval of at least 48 hours (except in cases where the animal was found dead, so it was not necessary to wait 48 hours to treat the next animal). The time intervals between doses were determined by the onset, duration and severity of clinical signs.

3.4 Observations

3.4.1 Clinical observations

Animals were observed individually after dosing at 30 minutes, then at approximately 1, 2, 3, 4, and 6 hours after dosing and once each day for 14 days thereafter or until death. Individual observations were performed on the skin and fur, eyes and mucous membranes and also respiratory, circulatory, autonomic and central nervous system, somatomotor activity and behaviour pattern were assessed.

Particular attention was directed to observation of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma.

3.4.2 Body weight measurement

Body weight was measured on Day -1, just before dosing (Day 0) and in case of the surviving animals weekly thereafter (Days 7 and 14 (before necropsy)).

3.5 *Post Mortem* Investigations

All animals were subjected to gross macroscopic evaluation. All surviving animals were euthanised under pentobarbital anaesthesia (Euthanimal 40%, details in 3.5.1) at the end of the observation period. After examination of the external appearance, the cranial, thoracic and abdominal cavities were opened and the appearance of the tissues and organs were observed. All gross pathological changes were recorded for each animal on the *post mortem* record sheets and the animals were discarded.

3.5.1 Material used for euthanasia

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

3.6 Data Evaluation

The type, severity and duration of clinical observations are described in the tables and results of this document. Body weight and body weight changes are summarised in tabular form. Necropsy findings are described and summarised in tabular form.

The LD_{50} was calculated using the AOT425StatPgm program. This program was prepared for the US Environmental Protection Agency by Westat, May 2001 and updated by the US EPA June 2003. This program was constructed using the most appropriate method to estimate the LD_{50}.

4.0 RESULTS AND DISCUSSION

4.1 Mortality

The animal treated at the dose level of 5000 mg/kg bw was found dead on Day 0.

2 out of the 5 animals treated at the dose level of 2000 mg/kg bw were found dead on Day 0.

No mortality occurred at the dose levels of 550 mg/kg bw (0 out of 2 animals was found dead) and 175 mg/kg bw (0 out of 1 animal was found dead).

4.2 Clinical Signs

At dose level of 5000 mg/kg bw the following test item related symptoms were observed until death: slightly to markedly decreased activity, hunched back, prone position, moderate to marked incoordination and piloerection.

At the dose level of 2000 mg/kg bw the following test item related symptoms were observed from Day 0 up to Day 2 or until death: slightly / slightly to moderately / markedly decreased activity (4 out of 5 animals), hunched back (4 out of 5 animals), slight / slight to moderate incoordination (4 out of 5 animals), piloerection (3 out of 5 animals) and prone position (1 out of 5 animals). The surviving animals were symptom free from Day 3.

At the dose level of 550 mg/kg bw the following test item related symptoms were observed up to Day 1: slightly /slightly to markedly decreased activity (2 out of 2 animals), hunched back (2 out of 2 animals), piloerection (2 out of 2 animals), prone position (1 out of 2 animals) and slight to moderate incoordination (1 out of 2 animals). All animals were symptom-free from Day 2.

The animal treated at the dose level of 175 mg/kg bw was symptom-free during the 14-day observation period.

Individual clinical observations and mortality results are presented in Table 1.

4.3 Body Weights

There were no treatment related effects on body weight or body weight gain. Body weights were within the range commonly recorded for this strain and age.

Individual body weights are presented in Table 2.

4.4 Macroscopic Findings

In the animal treated at the dose level of 5000 mg/kg bw, found dead on Day 0, the digestive content was liquid and beige, the lungs were collapsed. In the animals treated at the dose level of 2000 mg/kg bw, found dead on Day 0, in one case the digestive content was liquid and white, in both cases the lungs were collapsed. There was no evidence of any gross findings in the surviving 3 rats treated at a dose level of 2000 mg/kg bw and in the animals treated at the dose levels of 550 and 175 mg/kg bw, necropsied at the end of the observation period on Day 14.

Macroscopic findings are presented in Table 3. The Pathology Report is presented in Appendix 1.

5.0 CONCLUSIONS

Under the conditions of this study, the acute oral median lethal dose (LD_{50}) of the test item, propiconazole/pydiflumetofen SE (A21573C) was equal to 2000 mg/kg bw in female Crl:WI Wistar rats (95% PL Confidence interval is 1009 to greater than 20000).

TABLES SECTION

TABLE 1 Individual Findings – Clinical Signs

DOSE LEVEL: 175 mg/kg bw, Treatment on Day 0 **SEX: FEMALE**

Cage No.	Animal Number	Observations	Observation days													Frequency
			0						1	2	3	4	5	6	7-14	
			30'	1h	2h	3h	4h	6h								
3	1753	Symptom-free	+	+	+	+	+	+	+	+	+	+	+	+	+	20/20

DOSE LEVEL: 550 mg/kg bw, Treatment on Day 0 **SEX: FEMALE**

Cage No.	Animal Number	Observations	Observation days													Frequency
			0						1	2	3	4	5	6	7-14	
			30'	1h	2h	3h	4h	6h								
4	1958	Activity decreased	1	1	2	3	2	2	-	-	-	-	-	-	-	6/20
		Hunched back	-	-	-	-	+	+	-	-	-	-	-	-	-	2/20
		Prone position	-	-	-	+	-	-	-	-	-	-	-	-	-	1/20
		Incoordination	1	2	2	-	2	2	-	-	-	-	-	-	-	5/20
		Piloerection	-	-	-	+	+	+	+	-	-	-	-	-	-	4/20
		Symptom-free	-	-	-	-	-	-	-	+	+	+	+	+	+	13/20
8	2234	Activity decreased	-	1	1	1	1	-	-	-	-	-	-	-	-	4/20
		Hunched back	-	+	+	+	+	+	-	-	-	-	-	-	-	5/20
		Piloerection	-	-	-	+	-	-	-	-	-	-	-	-	-	1/20
		Symptom-free	+	-	-	-	-	-	+	+	+	+	+	+	+	15/20

Remarks: + = present - = absent

h = hour (s) ' = minute

= Found dead

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

Severities: 1 = Slight/Small/Few; 2 = Moderate/Medium; 3 = Marked/Large/Many

TABLE 1 Individual Findings – Clinical Signs (Continued)

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

Cage No.	Animal Number	Observations	Observation days													Frequency
			0						1	2	3	4	5	6	7-14	
			30'	1h	2h	3h	4h	6h								
2	1752#	Activity decreased	-	3												1/2
		Hunched back	+	-												1/2
		Prone position	-	+												1/2
		Incoordination	1	-												1/2
		Found Dead	-	-	+											-
5	1959	Activity decreased	1	2	2	2	2	2	1	-	-	-	-	-	-	7/20
		Hunched back	+	+	+	+	+	+	+	-	-	-	-	-	-	7/20
		Incoordination	1	1	1	1	2	2	-	-	-	-	-	-	-	6/20
		Piloerection	-	-	+	+	+	+	+	+	-	-	-	-	-	6/20
		Symptom-free	-	-	-	-	-	-	-	-	+	+	+	+	+	12/20
6	1960	Activity decreased	1	1	1	1	1	1	-	-	-	-	-	-	-	6/20
		Hunched back	-	+	+	+	+	+	-	-	-	-	-	-	-	5/20
		Incoordination	1	2	2	2	2	1	-	-	-	-	-	-	-	6/20
		Piloerection	-	-	+	+	+	+	-	-	-	-	-	-	-	4/20
		Symptom-free	-	-	-	-	-	-	+	+	+	+	+	+	+	14/20
7	1961#	Found Dead	+													-

Remarks: + = present - = absent

h = hour (s) ' = minute

= Found dead

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

Severities: 1 = Slight/Small/Few; 2 = Moderate/Medium; 3 = Marked/Large/Many

TABLE 1 Individual Findings – Clinical Signs (Continued)

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

Cage No.	Animal Number	Observations	Observation days													Frequency
			0						1	2	3	4	5	6	7-14	
			30'	1h	2h	3h	4h	6h								
9	2235	Activity decreased	1	1	1	1	-	-	-	-	-	-	-	-	-	4/20
		Hunched back	+	+	+	+	+	+	-	-	-	-	-	-	-	6/20
		Incoordination	-	-	1	1	-	-	-	-	-	-	-	-	-	2/20
		Piloerection	-	+	+	+	+	+	-	-	-	-	-	-	-	5/20
		Symptom-free	-	-	-	-	-	-	+	+	+	+	+	+	+	14/20

DOSE LEVEL: 5000 mg/kg bw, Treatment on Day 0 **SEX: FEMALE**

Cage No.	Animal Number	Observations	Observation days													Frequency
			0						1	2	3	4	5	6	7-14	
			30'	1h	2h	3h	4h	6h								
1	1751#	Activity decreased	1	2	2	2	3									5/5
		Hunched back	-	-	-	+	-									1/5
		Prone position	-	-	-	-	+									1/5
		Incoordination	2	3	3	3	-									4/5
		Piloerection	-	-	-	+	-									1/5
		Found Dead	-	-	-	-	-	+								-

Remarks: + = present - = absent

h = hour (s) ' = minute

= Found dead

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

Severities: 1 = Slight/Small/Few; 2 = Moderate/Medium; 3 = Marked/Large/Many

TABLE 2 Body Weight and Body Weight Gain

DOSE LEVEL: 175 mg/kg bw, Treatment on Day 0 **SEX: FEMALE**

Cage No.	Animal Number	Body weight (g) Days				Day/Body Weight (g) Death	Body Weight Gain (g)			
		-1	0	7	14		-1-0	0-7	7- 14	-1 - 14
3	1753	213	201	232	240	-	-12	31	8	27
Mean:		213.0	201.0	232.0	240.0	-	-12.0	31.0	8.0	27.0
Standard deviation:		-	-	-	-	-	-	-	-	-

DOSE LEVEL: 550 mg/kg bw, Treatment on Day 0 **SEX: FEMALE**

Cage No.	Animal Number	Body weight (g) Days				Day/Body Weight (g) Death	Body Weight Gain (g)			
		-1	0	7	14		-1-0	0-7	7- 14	-1 - 14
4	1958	222	210	265	269	-	-12	55	4	47
8	2234	202	179	219	230	-	-23	40	11	28
Mean:		212.0	194.5	242.0	249.5	-	-17.5	47.5	7.5	37.5
Standard deviation:		14.1	21.9	32.5	27.6	-	7.8	10.6	4.9	13.4

TABLE 2 Body Weight and Body Weight Gain (Continued)

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

Cage No.	Animal Number	Body weight (g) Days				Day/Body Weight (g) Death	Body Weight Gain (g)			
		-1	0	7	14		-1-0	0-7	7- 14	-1 - 14
2	1752#	227	219	-	-	0/219	-8	-	-	-
5	1959	223	210	236	249	-	-13	26	13	26
6	1960	238	220	250	270	-	-18	30	20	32
7	1961#	256	236	-	-	0/236	-20	-	-	-
9	2235	207	193	222	227	-	-14	29	5	20
Mean:		230.2	215.6	236.0	248.7	-	-14.6	28.3	12.7	26.0
Standard deviation:		18.2	15.7	14.0	21.5	-	4.7	2.1	7.5	6.0

DOSE LEVEL: 5000 mg/kg bw, Treatment on Day 0 **SEX: FEMALE**

Cage No.	Animal Number	Body weight (g) Days				Day/Body Weight (g) Death	Body Weight Gain (g)			
		-1	0	7	14		-1-0	0-7	7- 14	-1 - 14
1	1751#	230	214	-	-	0/214	-16	-	-	-
Mean:		230.0	214.0	-	-	-	-16.0	-	-	-
Standard deviation:		-	-	-	-	-	-	-	-	-

= Found dead - = No data

TABLE 3 Macroscopic Findings

DOSE LEVEL: 175 mg/kg bw, Treatment on Day 0 **SEX: FEMALE**

Cage No.	Animal Number	Necropsy Date/ Necropsy Day	External Observations	Internal Observations	Organ/Tissue
3	1753	14 December 2017 Day 14	No external observations recorded	No internal observations recorded	Not applicable

DOSE LEVEL: 550 mg/kg bw, Treatment on Day 0 **SEX: FEMALE**

Cage No.	Animal Number	Necropsy Date/ Necropsy Day	External Observations	Internal Observations	Organ/Tissue
4	1958	17 December 2017 Day 14	No external observations recorded	No internal observations recorded	Not applicable
8	2234	28 December 2017 Day 14	No external observations recorded	No internal observations recorded	Not applicable

TABLE 3 Macroscopic Findings (Continued)

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

Cage No.	Animal Number	Necropsy Date/ Necropsy Day	External Observations	Internal Observations	Organ/Tissue
2	1752#	29 November 2017 Day 0	No external observations recorded	Collapsed	Lungs
				Digestive Content: Material, Liquid, White	Stomach
5	1959	19 December 2017 Day 14	No external observations recorded	No internal observations recorded	Not applicable
6	1960	21 December 2017 Day 14	No external observations recorded	No internal observations recorded	Not applicable
7	1961#	13 December 2017 Day 0	No external observations recorded	Collapsed	Lungs
9	2235	02 January 2017 Day 14	No external observations recorded	No internal observations recorded	Not applicable

DOSE LEVEL: 5000 mg/kg bw, Treatment on Day 0 **SEX: FEMALE**

Cage No.	Animal Number	Necropsy Date/ Necropsy Day	External Observations	Internal Observations	Organ/Tissue
1	1751#	28 November 2017 Day 0	No external observations recorded	Collapsed	Lungs
				Digestive Content: Material, Liquid, Beige	Stomach

= Found dead

APPENDICES SECTION

APPENDIX 1 Pathology Report

Citoxlab Hungary Ltd. Study code. 17/349-001P

PATHOLOGY REPORT

INTRODUCTION

The objective of the study was to assess the acute oral toxicity of the test item Propiconazole/Pydiflumetofen SE (A21573C) when administered in a single dose to rats.

METHODS

Surviving animals were euthanized upon completion of the observation period on Day 14. The rats were anaesthetized 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.

MORTALITY

The animal 1751 dosed at 5000 mg/kg bw died on Day 0 of the study. The digestive content was liquid and beige; the lungs were collapsed.
Two of the 5 animals, dosed at 2000 mg/kg bw died on Day 0 of the study. In one case the digestive content was liquid and white, in both cases the lungs were collapsed.

TERMINAL (DAY 14)

Macroscopic Findings

There was no evidence of any gross findings in the surviving 3/5 rats, at a dose level of 2000 mg/kg bw and in the animals dosed at 550 mg/kg bw and 175 mg/kg bw, necropsied at the end of the observation period on Day 14.

CONCLUSION

A single oral gavage of Propiconazole/Pydiflumetofen SE (A21573C) to Crl:WI female rats led to the death of the rat dosed at 5000 mg/kg bw and 2/5 rats at a dose level of 2000 mg/kg bw on the Day 0 of the study. The other experimental animals showed no macroscopic changes at necropsy on Day 14 of the study.

pp.

Gábor Boros, D.V.M.
Histopathologist

Date 02 May 2018

APPENDIX 2 Certificate of Analysis

syngenta

Syngenta Crop Protection, LLC
Analytical and Product Chemistry
Greensboro, NC 27409

Certificate of Analysis

A21573C

Batch ID 1007839 (GP170913)

Test Substance Name:	CGA64250/SYN545974 SE (125/150)
Common Name:	Propiconazole/Pydiflumetofen SE (125/150)
Design Code:	A21573C
Batch ID:	1007839
Other ID:	GP170913
Source:	Syngenta Crop Protection LLC.,US ,410 Swing Road,Greensboro, NC 27409,

Chemical Analysis

AI	% w/w	g/L
Pydiflumetofen	13.7	151
Propiconazole	11.6	128

Identity of the Active Ingredients: Confirmed

Methodology Used for Characterization: LC , mass spectrometry, oscillating density meter

The Active Ingredient(s) content is within the FAO limits.

Isomer Assay

Analyte	Isomer	% w/w	g/L
CGA93590	1H-1,2,4-triazole, 1-{[2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl]methyl}-, cis-	6.73	74
CGA93591	1H-1,2,4-triazole, 1-{[2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl]methyl}-, trans-	4.84	53.2

COA Number: USGR170462

Page 1 of 2

APPENDIX 2 Certificate of Analysis (Continued)

Physical Analysis

Property	Value	Units
Density	1.100	g/cm3

Appearance: Beige liquid

Storage Temperature: <30°C

Re-certification Date:End of Oct/2020

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

The stability of this test substance will be determined concurrently through reanalysis of material held in inventory under GLP conditions at Syngenta Crop Protection, LLC, Greensboro, NC.

This Certificate of Analysis is summarizing data from a study that has been performed in compliance with Good Laboratory Practices per 40 CFR Part 160. Raw data, documentation, protocols, any amendments to study protocols and reports pertaining to this study are maintained in the Syngenta Crop Protection Archives in Greensboro, NC.

Study Number: USGR170462

Authorization: Kirt Durand

Kirt Durand — Date: Oct. 13, 2017

Analytical and Product Chemistry Department

COA Number: USGR170462 Page 2 of 2

APPENDIX 3 GLP Certificates

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 3 GLP Certificates (Continued)

OGYÉI
National Institute of
Pharmacy and Nutrition

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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"