Benzovindiflupyr/Cyproconazole

Benzovindiflupyr/Cyproconazole WG (A22801A) - Acute Oral Toxicity Study in Rats (Up and Down Procedure)

Final Report

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

AUTHOR(S): Zsolt Tarcai, M.Sc.

COMPLETION DATE: 10 August 2019

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

LABORATORY PROJECT ID: Report Number: 19/024-001P
Study Number: 19/024-001P
Task Number: TK0298264

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 consequence. 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.

Signature: _____ Date: 10 August 2019

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: 19/024-001P

Study Title: Benzovindiflupyr/Cyproconazole WG (A22801A) - Acute Oral Toxicity Study in Rats (Up and Down Procedure)

Test Item: Benzovindiflupyr/cyproconazole WG (A22801A)

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
15 April 2019	Study Plan	15 April 2019	15 April 2019
17 April 2019	Treatment	17 April 2019	17 April 2019
01 August 2019	Draft Report	01 August 2019	01 August 2019
09 August 2019	Final Report	09 August 2019	09 August 2019

Signature: _____ Date: 10 August 2019

Ágnes Rédl, 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 "Benzovindiflupyr/Cyproconazole WG (A22801A) - Acute Oral Toxicity Study in Rats (Up and Down Procedure)" has been performed in compliance with the Principles of Good Laboratory Practice.

Signature: _____ Date: 10 August 2019

David J. Esdaile, M.Sc.
Director of Science and Regulatory Affairs

GENERAL INFORMATION

Contributors

The following contributed to this report in the capacities indicated:

Name	Function
Zsolt Tarcai, M.Sc.	Study Director
Máté Weisz, M.Sc.	Assistant Scientist
Ágnes Rédl, M.Sc.	Senior QA Inspector
László Székelyhidi, D.V.M.	Veterinary Care
Peter Maslej, D.V.M., Ph.D.	Pathology
Babu Gangadharan, BVSc&AH., MVSc., PhD., DipRCPath.	Pathology
Tamás Mészáros, Ph.D.	Pharmacy
Pontes Merielen Garcia, Ph.D.	Syngenta Study Manager

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

Study dates

Study Initiation Date	16 April 2019
Experimental Starting Date	17 April 2019
Experimental Completion Date	16 May 2019
Draft Report (non-audited)	24 July 2019*
Draft Report (audited)	05 August 2019
Receipt of Animals	11 and 18 April 2019
Acclimatisation	At least 5 days

*Note: Deviation to the Study Plan, the Draft Report was issued later than proposed, however this has no impact on the study.

Treatment	17 April 2019 (female no. 7640) 18 April 2019 (female no. 7641) 24 April 2019 (female no. 7642) 26 April 2019 (female no. 7643) 30 April 2019 (female no. 7644) 01 May 2019 (female no. 7654) 02 May 2019 (female no. 7717) 09 May 2019 (female no. 7729)
Observation	17 April 2019 (female no. 7640) 18 April – 02 May 2019 (female no. 7641) 24 April – 08 May 2019 (female no. 7642) 26 April – 10 May 2019 (female no. 7643) 30 April 2019 (female no. 7644)

	01 May 2019 (female no. 7654) 02-16 May 2019 (female no. 7717) 09-10 May 2019 (female no. 7729)
Necropsy	17 April 2019 (female no. 7640) 02 May 2019 (female no. 7641) 08 May 2019 (female no. 7642) 10 May 2019 (female no. 7643) 30 April 2019 (female no. 7644) 01 May 2019 (female no. 7654) 16 May 2019 (female no. 7717) 10 May 2019 (female no. 7729)

Deviation from the guideline

Due to technical reason relative humidity values (maximum of 80%) and temperature values (maximum of 25.7°C) outside the expected range of 30-70% and 19-25°C were recorded occasionally during the study, however these deviations have no effect on the outcome of the study.

Performing laboratory test substance reference number
190104

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

In this acute oral toxicity (up and down procedure) study, 8 female Crl:WI rats were given a single oral (gavage) dose of benzovindiflupyr/cyproconazole WG (A22801A) at a dose level of 55, 175, 550 or 2000 mg/kg body weight (bw). The animals were fasted overnight prior to treatment and food was returned 3 hours after dosing. The test item was formulated in distilled water at concentration of 5.5, 17.5, 55, 200 mg/mL. The animals were dosed with the formulated test item at dose volume of 10 mL/kg bw.

Individual animals were dosed sequentially at no less than 48-hour intervals, if no mortality occurred. The time intervals between doses were determined by the onset, duration and severity of clinical signs. Based on the available information about the test item a main test was started from a dose level of 175 mg/kg bw. The dose selection for each step followed the recommendation of AOT425StatPgm software, based on available results.

Animals were observed individually at least at 30 minutes, and 1, 2, 3, 4 and 6 hours post treatment and once each day for 14 days thereafter or until the day of death. Body weight was measured on Day -1 (prior to removal of food), before dosing (on Day 0), on Day 7 and on Day 14 or on the day of death. All survived animals were euthanized and examined macroscopically at the end of the observation period. The animals found dead were examined macroscopically after death was noted.

1.2 Results

At dose level of 2000 mg/kg bw 1 out of 1 animal, at 550 mg/kg bw 2 out of 3 animals and at 175 mg/kg bw 1 out of 3 animals died on Day 0 or Day 1. The single animal treated at dose level of 55 mg/kg bw survived.

At a dose level of 2000 mg/kg bw, decreased activity (score 2), prone position and clonic convulsion were observed prior to death.

At a dose level of 550 mg/kg bw, decreased activity (score 1, 2 or 3, in 3/3 animals), hunched back (in 1/3 animals), incoordination (score 1, 2 or 3, in 3/3 animals), piloerection (in 2/3 animals) and prone position (in 2/3 animals) were observed up to Day 3 or until death was noted. From Day 4 the one animal that survived was symptom-free.

At a dose level of 175 mg/kg bw, decreased activity (score 1 or 2, in 2/3 animals), stereotypy (in 1/3 animals), hunched back (in 2/3 animals), prone position (in 1/3 animals), incoordination (score 1, in 3/3 animals), clonic convulsion (in 1/3 animals) and piloerection (in 2/3 animals) were observed on the day of treatment. From Day 1 the two surviving animals were symptom-free.

The single animal treated at a dose level of 55 mg/kg bw, was symptom-free throughout the study.

There was no treatment related effect on body weight or body weight gain in the surviving animals. Body weights were within the range commonly recorded for this strain and age.

In the female given 2000 mg/kg/bw (No.7644), diffuse red discolouration in all lobes of non-collapsed lungs, creamy beige material in the esophagus and stomach, and diffuse red discolouration of the glandular mucosa of the stomach were observed at necropsy.

In the female given 550 mg/kg/bw (No.7654), diffuse red discolouration in all lobes of lungs and creamy beige digestive material in the stomach were observed at necropsy.

In the female given 550 mg/kg/bw (No.7729), diffuse dark red discolouration in all lobes of non-collapsed lungs and foamy white material in the trachea were observed at necropsy.

In the female given 175 mg/kg/bw (No.7640), a few red foci in all lobes of non-collapsed lungs was observed at necropsy.

There was no evidence of any macroscopic observations in the terminal animals at dose levels of 55, 175 or in one animal at 550 mg/kg bw.

1.3 Conclusion

Under the conditions of this study, the acute oral median lethal dose (LD_{50}) of the test item benzovindiflupyr/cyproconazole WG (A22801A) was found to be 311.9 mg/kg bw (approximate 95% confidence interval 0 to 20,000) in female Crl:WI Wistar rats.

2.0 INTRODUCTION

2.1 Purpose

The purpose of the study was to assess the acute oral toxicity of the test item benzovindiflupyr/cyproconazole WG (A22801A) 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.

2.2 Guidelines

The study was performed according to the following guidelines:

- OECD Guidelines 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:	Benzovindiflupyr/cyproconazole WG (A22801A)
Batch number:	SMU8IP003
Design code:	A22801A
Appearance:	Brown solid
Active ingredient content*:	Content of benzovindiflupyr, 14.8 % w/w corresponding to 148 g/kg Content of cyproconazole, 37.5 % w/w corresponding to 375 g/kg Content of diastereomer A of cyproconazole, 19.9 % w/w corresponding to 199 g/kg Content of diastereomer B of cyproconazole, 17.6 % w/w corresponding to 176 g/kg Content of water 1.79 % w/w
Recertification date:	31 October 2021
Storage conditions:	Room temperature (<30°C)
Safety precautions:	Enhanced safety precautions above the routine (lab coat, gloves, safety glasses, face mask) were applied considering the supplied safety data sheet to assure personnel health and safety.

**No adjustment for the active ingredient content was applied.*

The Certificate of Analysis is presented in Appendix 2.

3.1.1 Identification and receipt

The test item of a suitable active ingredient content 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 the Sponsor.

3.1.2 Formulation

The test item was freshly formulated at concentrations of 200, 55, 17.5 and 5.5 mg/mL in the vehicle (distilled water) in the Pharmacy of Citoxlab Hungary Ltd. on the day of administration. The formulation was stirred with a magnetic stirrer until completion of treatment.

Vehicle information:

Name:	Distilled water
Batch number:	8000119
Manufacturer:	Hungaro-Gal Kft.
Expiry Date:	02 July 2019
Storage condition:	Room temperature

There was no chemical analysis of the dose formulation.

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:	8
Sex:	Female rats, nulliparous and non-pregnant
Age when treated:	Young adult rats, 8-10 weeks old
Body weight (at dosing):	198 – 242 g (the weight variation in animals in the study did not exceed ± 20 % of the mean weight)
Identification:	The animals were identified by numbers written on the tail with an indelible pen. 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 5 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:	522/3
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 / Nesting:	SAFE 3/4 S certified wooden chips and Arbocel crinklets natural nest building material produced by

	J. Rettenmaier & Söhne GmbH + Co.KG (D-73494 Rosenberg, Germany) were available to animals during the study. Copies of the Certificate of Analysis are retained in the archive at Citoxlab Hungary Ltd.
Light:	12 hours daily, from 6.00 a.m. to 6.00 p.m.
Temperature:	19.1 – 25.7°C
Relative humidity:	33 – 80%
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 *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. Details of the diets are 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-8200 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 doses:

Based on the available information by the Sponsor, the calculated LD_{50} value of the test item was approximately 263 mg/kg bw, therefore a main test was started from a dose level of 175 mg/kg bw.

The animals were treated with a single oral (gavage) dose of benzovindiflupyr/cyproconazole WG (A22801A) at the dose levels of 55, 175, 550 and 2000 mg/kg bw according to the recommendation of the AOT 425 StatPgm program. The dose volume was 10 mL/kg bw of the formulated test item in the appropriate vehicle. The individual dose volumes used are shown below.

Animal Number	Dose [mg/kg body weight]	Volume Dosed [mL]	Bodyweight [g]	Mortality
7640	175	2.0	199	Died
7641	55	2.0	198	Survived
7642	175	2.1	213	Survived
7643	550	2.1	205	Survived
7644	2000	2.2	219	Died
7654	550	2.1	214	Died
7717	175	2.2	220	Survived
7729	550	2.4	242	Died

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 (surviving animals) or until the day of death. 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 (if no death occurred). The time intervals between doses were determined by the onset, duration and severity of toxic signs.

3.4 Observations

3.4.1 Clinical observations

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

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

3.4.2 Body weight measurement

The body weights were recorded on Day -1 (prior to removal of food), on Day 0 (before dosing), on Day 7 and on Day 14 (before necropsy) in all animals until termination or death.

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 then 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% (sodium pentobarbital)
Lot No.:	1811347-03
Expiry Date:	31 December 2021
Produced by:	Alfasan Nederland BV, Kuipersweg 9, Woerden, The Netherlands

3.6 Data Evaluation

Type, severity and duration of clinical observations are described. 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

At dose level of 2000 mg/kg bw, 1 out of 1 animal; at 550 mg/kg bw 2 out of 3 animals; and at 175 mg/kg bw 1 out of 3 animal died on Day 0 or Day1. The single animal treated at dose level of 55 mg/kg bw survived.

4.2 Clinical Signs

At a dose level of 2000 mg/kg bw, decreased activity (score 2), prone position and clonic convulsion were observed prior to death.

At a dose level of 550 mg/kg bw, decreased activity (score 1, 2 or 3, in 3/3 animals), hunched back (in 1/3 animals), incoordination (score 1, 2 or 3, in 3/3 animals), piloerection (in 2/3 animals) and prone position (in 2/3 animals) were observed up to Day 3 or until death was noted. From Day 4 the one animal that survived was symptom-free.

At a dose level of 175 mg/kg bw, decreased activity (score 1 or 2, in 2/3 animals), stereotypy (in 1/3 animals), hunched back (in 2/3 animals), prone position (in 1/3 animals), incoordination (score 1, in 3/3 animals), clonic convulsion (in 1/3 animals) and piloerection (in 2/3 animals) were observed on the day of treatment. From Day 1, the two surviving animals were symptom-free.

The single animal treated at a dose level of 55 mg/kg bw, was symptom-free during the study.

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

4.3 Body Weights

There was no test item related effect on body weight or body weight gain in the surviving animals. 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 female given 2000 mg/kg/bw (No.7644), diffuse red discolouration in all lobes of non-collapsed lungs, creamy beige material in the esophagus and stomach, and diffuse red discolouration of the glandular mucosa of the stomach were observed at necropsy.

In the female given 550 mg/kg/bw (No.7654), diffuse red discolouration in all lobes of lungs and creamy beige digestive material in the stomach were observed at necropsy.

In the female given 550 mg/kg/bw (No.7729), diffuse dark red discolouration in all lobes of non-collapsed lungs and foamy white material in the trachea were observed at necropsy.

In the female given 175 mg/kg/bw (No.7640), a few red foci in all lobes of non-collapsed lungs were observed at necropsy.

There was no evidence of any macroscopic observations in the terminal animals at dose level 55, 175 or in one animal at 550 mg/kg bw.

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 benzovindiflupyr/cyproconazole WG (A22801A) was found to be 311.9 mg/kg bw (approximate 95% confidence interval 0 to 20,000) in female Crl:WI Wistar rats.

TABLES SECTION

TABLE 1 Individual Findings – Clinical Signs

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	
			20'	30'	1h	2h	3h	4h	6h								
5	7644#	Activity decreased	Mo														1/1
		Prone position	+														1/1
		Clonic convulsion - Whole body	+														1/1
		Found dead	-	+													-

Remarks:
+ = present
- = absent
h = hour (s)
' = minute
= Found dead
Frequency of observation = number of occurrence of observation / total number of observations

Occurrent severities:
Sl = Slight/Small/Few/Small amount
Mo = Moderate/Several/Moderate amount
Ex = Severe/Large/Many/Large/Extreme amount

TABLE 1 Individual Findings – Clinical Signs (continued)

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	
			20'	30'	1h	2h	3h	4h	6h								
4	7643	Symptom Free	/	-	-	-	-	-	-	-	-	-	+	+	+	+	11/20
		Activity decreased	/	-	Sl	Sl	Sl	Mo	Mo	Sl	Sl	Sl	-	-	-	-	8/20
		Hunched back	/	+	+	+	+	+	+	+	+	+	-	-	-	-	9/20
		Incoordination	/	-	-	Sl	Mo	Mo	Ex	-	-	-	-	-	-	-	4/20
		Piloerection	/	-	-	-	+	+	+	+	+	+	-	-	-	-	6/20
6	7654#	Activity decreased	Mo	Mo													2/2
		Prone position	+	+													2/2
		Incoordination	Ex	Ex													2/2
		Found dead	-	-	+												-
8	7729#	Activity decreased	Sl	Mo	Ex	Ex	Ex	Ex	Ex								7/7
		Prone position	-	+	+	+	+	+	+								6/7
		Incoordination	Sl	Mo	-	-	-	-	-								2/7
		Piloerection	-	-	+	+	+	+	+								5/7
		Found dead	-	-	-	-	-	-	-	+							-

Remarks:
+ = present - = absent
h = hour (s) ' = minute
# = Found dead
/ = No clinical observation (was) done at 20'
Frequency of observation = number of occurrence of observation / total number of observations

Occurrent severities:
Sl = Slight/Small/Few/Small amount
Mo = Moderate/Several/Moderate amount
Ex = Severe/Large/Many/Large/Extreme amount

TABLE 1 Individual Findings – Clinical Signs (continued)

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	
			20'	30'	1h	2h	3h	4h	6h								
1	7640#	Activity decreased	-	-	Sl	Mo	Mo	Mo									4/6
		Stereotypy	-	-	+	+	-	-									2/6
		Hunched back	-	+	-	-	-	-									1/6
		Prone position	-	-	+	+	+	+									4/6
		Incoordination	Sl	Sl	-	-	-	-									2/6
		Clonic convulsion - Whole body	-	-	-	-	+	+									2/6
		Piloerection	-	-	-	+	+	+									3/6
		Found dead	-	-	-	-	-	-	+								-
3	7642	Symptom Free	/	-	-	-	-	-	-	+	+	+	+	+	+	+	14/20
		Activity decreased	/	-	Sl	Sl	Sl	Sl	Sl	-	-	-	-	-	-	-	5/20
		Hunched back	/	-	-	+	+	+	+	-	-	-	-	-	-	-	4/20
		Incoordination	/	Sl	Sl	Sl	Sl	Sl	-	-	-	-	-	-	-	-	5/20
		Piloerection	/	-	-	-	+	+	+	-	-	-	-	-	-	-	3/20
7	7717	Symptom Free	/	+	+	+	-	-	+	+	+	+	+	+	+	+	18/20
		Incoordination	/	-	-	-	Sl	Sl	-	-	-	-	-	-	-	-	2/20

Remarks:
+ = present
- = absent
h = hour (s)
' = minute
= Found dead
/ = No clinical observation (was) done at 20'
Frequency of observation = number of occurrence of observation / total number of observations

Occurrent severities:
Sl = Slight/Small/Few/Small amount
Mo = Moderate/Several/Moderate amount
Ex = Severe/Large/Many/Large/Extreme amount

TABLE 1 Individual Findings – Clinical Signs (continued)

DOSE LEVEL: 55 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	
			20'	30'	1h	2h	3h	4h	6h								
2	7641	Symptom Free	/	+	+	+	+	+	+	+	+	+	+	+	+	+	20/20

Remarks: + = present - = absent
h = hour (s) ' = minute
/ = No clinical observation (was) done at 20'
Frequency of observation = number of occurrence of observation / total number of observations

TABLE 2 Body Weight and Body Weight Gain

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	7643	219	205	229	248	-	-14	24	19	29
6	7654#	229	214	-	-	0/214	-15	-	-	-
8	7729#	256	242	-	-	1/227	-14	-	-	-
Mean:		234.7	220.3	229.0	248.0	-	-14.3	24.0	19.0	29.0
Standard deviation:		19.1	19.3	-	-	-	0.6	-	-	-

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
5	7644#	233	219	-	-	0/219	-14	-	-	-

- = No data
= Found dead

TABLE 2 Body Weight and Body Weight Gain (continued)

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
1	7640#	217	199	-	-	0/199	-18	-	-	-
3	7642	229	213	234	256	-	-16	21	22	27
7	7717	233	220	241	249	-	-13	21	8	16
Mean:		226.3	210.7	237.5	252.5	-	-15.7	21.0	15.0	21.5
Standard deviation:		8.3	10.7	4.9	4.9	-	2.5	0.0	9.9	7.8

DOSE LEVEL: 55 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	7641	211	198	234	265	-	-13	36	31	54

- = No data
= Found dead

TABLE 3 Macroscopic Findings

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	7643	10 May 2019 Day 14	No external observations recorded	No internal observations recorded	Not applicable
6	7654#	01 May 2019 Day 0	No external observations recorded	Discoloration, red, diffuse, all lobes	Lungs
				Digestive content: Material, creamy, beige	Stomach
8	7729#	10 May 2019 Day 1	No external observations recorded	Non-collapsed	Lungs
				Discoloration, dark red, diffuse, all lobes	
				Material, foamy, white	Trachea

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
5	7644#	30 April 2019 Day 0	No external observations recorded	Non-collapsed	Lungs
				Discoloration, red, diffuse, all lobes	
				Material, creamy, beige	Esophagus
				Discoloration, red, diffuse, glandular mucosa	Stomach
				Digestive content: Material, creamy, beige	

= Found dead

TABLE 3 Macroscopic Findings (continued)

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
1	7640#	17 April 2019 Day 0	No external observations recorded	Non-collapsed	Lungs
				Focus red few all lobes	
3	7642	08 May 2019 Day 14	No external observations recorded	No internal observations recorded	Not applicable
7	7717	16 May 2019 Day 14	No external observations recorded	No internal observations recorded	Not applicable

DOSE LEVEL: 55 mg/kg bw, Treatment on Day 0 **SEX: FEMALE**

Cage No.	Animal Number	Necropsy Date/ Necropsy Day	External Observations	Internal Observations	Organ/Tissue
2	7641	02 May 2019 Day 14	No external observations recorded	No internal observations recorded	Not applicable

= Found dead

APPENDICES SECTION

APPENDIX 1 Pathology Report

Citoxlab Hungary Ltd. Study code 19/024-001P

PATHOLOGY REPORT

INTRODUCTION

The objective of the study was to assess the acute oral toxicity of benzovindiflupyr/cyproconazole WG (A22801A) when administered in a single dose to rats at dose levels of 55, 175, 550 or 2000 mg/kg bw.

METHODS

All surviving animals were euthanized upon completion of the observation period on Day 14. Rats were anesthetized with pentobarbital, followed by exsanguination and subjected to necropsy. Macroscopic examination of found dead and euthanized animals consisted of external examination, as well as a detailed internal examination of cranial, thoracic and abdominal cavities. Histopathological examination was not performed.

FOUND DEAD

One female given 175 mg/kg bw (No. 7640), one female given 550 mg/kg bw (No. 7654) and one female given 2000 mg/kg bw (No. 7644) were found dead on Day 0 of treatment. In addition, one female given 550 mg/kg bw (No. 7729) was found dead on Day 1 of treatment.

The female given 175 mg/kg bw (No.7640) showed slight to moderate decreased activity, stereotypy, hunched back, prone position, incoordination, whole body clonic convulsion and piloerection prior to death. Few red focus in all lobes of non-collapsed lungs was observed at necropsy.

The female given 550 mg/kg bw (No.7654) showed moderate decreased activity, prone position, and incoordination prior to death. Diffuse red discolouration in all lobes of lungs and creamy beige digestive material in the stomach were observed at necropsy.

The female given 550 mg/kg bw (No.7729) showed slight to extreme decreased activity, prone position, incoordination and piloerection prior to death. Diffuse dark red discolouration in all lobes of non-collapsed lungs and foamy white material in the trachea were observed at necropsy.

The female given 2000 mg/kg bw (No.7644) showed moderate decreased activity, prone position and whole body clonic convulsions prior to death. Diffuse red discolouration in all lobes of non-collapsed lungs, creamy beige material in the esophagus and stomach, and diffuse red discolouration of the glandular mucosa of the stomach were observed at necropsy.

TERMINAL (DAY 14)

One female given 55 mg/kg bw, two females given 175 mg/kg bw and one female given 550 mg/kg bw survived until the 14-day observation period.

APPENDIX 1 Pathology Report (Continued)

Macroscopic Findings

There was no evidence of any macroscopic observations in the terminal animals at dose level 55, 175 or in one animal at 550 mg/kg bw.

CONCLUSION

A single oral gavage administration of benzovindiflupyr/cyproconazole WG (A22801A) to Crl:WI rats at dose levels of 175 mg/kg bw (one female), 550 mg/kg bw (two females) and 2000 mg/kg bw (one female) resulted in mortality on Day 0 or Day 1 of treatment.

One female given 55 mg/kg bw, two females given 175 mg/kg bw and one female given 550 mg/kg bw survived the observation period and subjected to necropsy on Day 14, was not associated with any macroscopic findings.

Babu Gangadharan, BVSc&AH., MVSc., PhD., DipRCPath.
Pathologist

09 August 2019
Date

APPENDIX 2 Certificate of Analysis

syngenta

Syngenta Crop Protection AG
GLP Testing Facility WMU
Analytical Development & Product Chemistry
Breitenloh 5
4333 Münchwilen, Switzerland

Certificate of Analysis

A22801A

benzovindiflupyr/cyproconazole WG (15/37.5)

SMU8IP003

Batch Identification	**SMU8IP003**
Other Batch ID	1067924
Product Code	**A22801A**
Other Product Code(s)	benzovindiflupyr/cyproconazole WG (15/37.5)
Chemical Analysis (Active Ingredient content)	
– **Identity of the Active Ingredient(s)***	**confirmed**
– **Content of benzovindiflupyr***	**14.8 % w/w corresponding to 148 g/kg**
– **Content of cyproconazole***	**37.5 % w/w corresponding to 375 g/kg**
– **Content of diastereomer A of cyproconazole***	**19.9 % w/w corresponding to 199 g/kg**
– **Content of diastereomer B of cyproconazole***	**17.6 % w/w corresponding to 176 g/kg**
– **Content of water***	**1.79 % w/w** The Active Ingredient(s) content is within the FAO limits.
Methodology used for Characterization / Recertification	HPLC, Karl Fischer Titration,
Physical Analysis	
– **Appearance**	**brown solid**
Stability:	
– **Storage Temperature**	**< 30 °C**
– **Recertification Date**	**End of October 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 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:	CHMU180809
Study number(s) of batch recertification:	---

Authorization: January 08, 2019

Elke Ebi
Analytical Development & Product Chemistry

Page 1 of 1

APPENDIX 3 Structured Study Summary

Structured Study Summary Table

Test substance design codes	A22801A
Test substance batch code	SMU8IP003
Test substance purity (% w/w)	Content of benzovindiflupyr, 14.8 % w/w corresponding to 148 g/kg Content of cyproconazole, 37.5 % w/w corresponding to 375 g/kg Content of diastereomer A of cyproconazole, 19.9 % w/w corresponding to 199 g/kg Content of diastereomer B of cyproconazole, 17.6 % w/w corresponding to 176 g/kg Content of water 1.79 % w/w
Study number	19/024-001P
Study type	MAMMALIAN ACUTE ORAL
Lab Reference	Citoxlab Hungary Ltd.
Study guidelines	OECD 425 (2008), OPPTS 870.1100 (2002)
Nonstandard elements	
Species	Rat
Strain	Crl:WI Wistar
TK data collected?	No
Dose units	mg/kg bw
Substance vehicle	Distilled water
Dosing approach	Constant Concentration
LD_{50} - Male	
LD_{50} - Female	311.9 mg/kg bw

Structured Study Results Table

Gender	Dose (mg/kg bw)	Number of animals dosed	Number of animals survived	Adverse Clinical Observations
Female	2000	1	0	decreased activity, prone position and clonic convulsion
Female	550	3	1	decreased activity, hunched back, incoordination, piloerection and prone position
Female	175	3	2	decreased activity, stereotypy, hunched back, prone position, incoordination, clonic convulsion and piloerection
Female	55	1	1	symptom-free

APPENDIX 4 AOT 425 Report (Main Test)

AOT425statpgm (Version: 1.0) Test Results and Recommendations
Acute Oral Toxicity (OECD Test Guideline 425) Statistical Program

Date/Time: 2019. július 23., kedd, 15:40:10
Data file name: 19_024-001P AOT 425 Dosage.dat
Last modified: 2019. 05. 10. 12:08:28

Test/Substance: 19/024-001P Dosage
Test type: Main Test
Limit dose (mg/kg): 2000
Assumed LD50 (mg/kg): Default
Assumed sigma (mg/kg): 0.5

Recommended dose progression: 2000, 550, 175, 55, 17.5, 5.5, 1.75

DATA:

Test Seq.	Animal ID	Dose (mg/kg)	Short-term Result	Long-term Result
1	001	175	X	X
2	002	55	O	O
3	003	175	O	O
4	004	550	O	O
5	005	2000	X	X
6	006	550	X	X
7	007	175	O	O
8	008	550	X	X

(X = Died, O = Survived)

Dose Recommendation: The main test is complete.

Stopping criteria met: LR criterion.

SUMMARY OF LONG-TERM RESULTS:

Dose	O	X	Total
55	1	0	1
175	2	1	3
550	1	2	3
2000	0	1	1
All Doses	4	4	8

Statistical Estimate based on long term outcomes:

Estimated LD50 = 311.9 (Based on maximum likelihood).
95% PL Confidence interval is 0 to Greater than 20,000.

APPENDIX 5 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: 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")