citoxlab
Safety and Health Research

Thiamethoxam/Chlorantraniliprole

Thiamethoxam/Chlorantraniliprole OD (A22629A) - Acute Oral Toxicity Study in Rats (Up and Down Procedure)

Final Report

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

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

COMPLETION DATE: 03 April 2019

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

LABORATORY PROJECT ID: Report Number: 18/249-001P
Study Number: 18/249-001P
Task Number: TK0300513

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

STATEMENT OF DATA CONFIDENTIALITY CLAIMS

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GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

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

This study was conducted in accordance with a written Study Plan, authorized by the Sponsor and Citoxlab Hungary Ltd. Management, and followed applicable Standard Operating Procedures.

I, the undersigned, declare that this report constitutes a true record of the actions undertaken and the results obtained in the course of this study.

Signature: _____ Date: 03 April 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: 18/249-001P

Study Title: Thiamethoxam/Chlorantraniliprole OD (A22629A) - Acute Oral Toxicity Study in Rats (Up and Down Procedure)

Test Item: Thiamethoxam/chlorantraniliprole OD (A22629A)

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
17 October 2018	Study Plan	17 October 2018	17 October 2018
25 October 2018	Treatment	25 October 2018	25 October 2018
06 March 2019	Draft Report	06 March 2019	06 March 2019
03 April 2019	Final Report	03 April 2019	03 April 2019

Signature: Merazga Leila Date: 03 April 2019

Leila Merazga, M.Sc.
Deputy Head 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 "Thiamethoxam/Chlorantraniliprole OD (A22629A) - Acute Oral Toxicity Study in Rats (Up and Down Procedure)" has been performed in compliance with the Principles of Good Laboratory Practice.

Signature: _____ Date: 03 April 2019

Alyson Leyshon, M.Sc.
Managing Director

GENERAL INFORMATION

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

Name	Function
Zsolt Tarcai, M.Sc.	Study Director
Ádám Appl, M.Sc.	Assistant Scientist
Leila Merazga, M.Sc.	Deputy Head of QA
László Székelyhidi, D.V.M.	Veterinary Care
Peter Maslej, D.V.M., Ph.D.	Pathology
Tamás Mészáros, Ph.D.	Pharmacy
Sophie van der Kamp, B.Sc.	Syngenta Study Manager

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

Study dates

Study Initiation Date	24 October 2018
Receipt of Animals	27 September and 25 October 2018
Experimental Starting Date	25 October 2018
Experimental Completion Date	22 November 2018
Draft Report Date	08 March 2019*

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

Treatment	25 October 2018 (female no. 5485) 29 October 2018 (female no. 5486) 31 October 2018 (female no. 5487) 06 November 2018 (female no. 5858) 08 November 2018 (female no. 5859)
Observation	25 October – 08 November 2018 (female no. 5485) 29 October – 12 November 2018 (female no. 5486) 31 October – 14 November 2018 (female no. 5487) 06 – 20 November 2018 (female no. 5858) 08 – 22 November 2018 (female no. 5859)
Necropsy	08 November 2018 (female no. 5485) 12 November 2018 (female no. 5486) 14 November 2018 (female no. 5487) 20 November 2018 (female no. 5858) 22 November 2018 (female no. 5859)

Deviation from the Guideline
Due to a technical reason, relative humidity values (minimum of 29% and maximum of 73 %) outside the expected range of 30-70 % were recorded during the study. However, these minor differences of the environmental parameters were considered not to adversely affect the results or integrity of the study.

Performing laboratory test substance reference number
180335

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.

TABLE OF CONTENTS

1.0 EXECUTIVE SUMMARY

1.1 Study Design

In this acute oral toxicity study, 5 female Crl:WI Wistar rats were given a single oral (gavage) dose of thiamethoxam/chlorantraniliprole OD (A22629A) at a dose level of 2000 mg/kg body weight (bw). 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, if no mortality occurred. 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 2000 mg/kg bw. The dose selection for the next animal followed the recommendation of AOT425StatPgm software, based on available results.

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

1.2 Results

There was no mortality during the study.

All animals were symptom-free during the 14-day observation period.

Slight body weight loss between Day 7-14 was observed in one female animal, however this proved to be incidental as no effects were observed on body weight or body weight gain in any other animals during the study. There were no test item related effects on body weight or body weight gain.

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

1.3 Conclusion

Under the conditions of this study, the acute oral median lethal dose (LD_{50}) of the test item, thiamethoxam/chlorantraniliprole OD (A22629A), was greater than 2000 mg/kg bw 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 thiamethoxam/chlorantraniliprole OD (A22629A) 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:	Thiamethoxam/chlorantraniliprole OD (A22629A)
Batch number:	TAD001-020-001
Active Ingredient Content*:	Thiamethoxam 7.32% w/w corresponding to 75.7 g/L Chlorantraniliprole 7.31% w/w corresponding to 75.6 g/L
Density:	1034 kg/m^3
Appearance:	Beige liquid
Recertification date:	30 June 2021
Storage conditions:	Room temperature (<30°C)
Safety precautions:	Routine safety precautions (gloves, goggles, face mask, lab coat) for unknown materials were applied 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 Sponsor.

3.1.2 Formulation

The test item was administered undiluted as supplied.

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:	5
Sex:	Female rats, nulliparous and non-pregnant
Age when treated:	Young adult rats, 9-12 weeks old
Body weight (at dosing):	222 – 237 g
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 12 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:	“Lignocel 3/4-S Hygienic Animal Bedding” 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:	20.4 – 24.8 °C
Relative humidity:	29 – 73 %
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: 840 33675, Expiry date: 31 January 2019 and Lot number: 639 38520, Expiry date: 30 April 2019) *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 diet 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-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 doses:

The starting dose of the limit test was 2000 mg/kg bw. Animals were treated with a single oral (gavage) dose of thiamethoxam/chlorantraniliprole OD (A22629A) at a dose level of 2000 mg/kg bw. The density of the test item was 1034 kg/m^3 as provided by the Sponsor, therefore the dose volume for the first animal was 1.93 mL/kg bw. The individual dose volumes used are shown below:

Animal Number	Dose [mg/kg body weight]	Volume Dosed [mL]	Bodyweight [g]	Mortality
5485	2000	0.44	228	Survived
5486	2000	0.43	222	Survived
5487	2000	0.46	237	Survived
5858	2000	0.43	223	Survived
5859	2000	0.45	232	Survived

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. 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 30 minutes, 1, 2, 3, 4 and 6 hours after dosing then once each day for 14 days thereafter. 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 Days -1 (prior to removal of food), 0 (before treatment), 7 and 14 (before necropsy).

3.5 *Post Mortem* Investigations

All animals were subjected to gross macroscopic evaluation. All animals were euthanized 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.:	1609291-03
Expiry Date:	31 October 2019
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

There was no mortality during the study.

4.2 Clinical Signs

All animals were symptom-free during the 14-day observation period.

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

4.3 Body Weights

Slight body weight loss between Day 7-14 was observed in one female animal, however this proved to be incidental as no effects were observed on body weight or body weight gain in any other animals during the study. There were no test item related effects on body weight or body weight gain.

Individual body weights are presented in Table 2.

4.4 Macroscopic Findings

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

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, thiamethoxam/chlorantraniliprole OD (A22629A), was greater than 2000 mg/kg bw 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	
			30'	1h	2h	3h	4h	6h								
1	5485	Symptom Free	+	+	+	+	+	+	+	+	+	+	+	+	+	20/20
2	5486	Symptom Free	+	+	+	+	+	+	+	+	+	+	+	+	+	20/20
3	5487	Symptom Free	+	+	+	+	+	+	+	+	+	+	+	+	+	20/20
4	5858	Symptom Free	+	+	+	+	+	+	+	+	+	+	+	+	+	20/20
5	5859	Symptom Free	+	+	+	+	+	+	+	+	+	+	+	+	+	20/20

Remarks: + = present ' = minute

h = hour (s)

Frequency of observation = number of occurrence 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: FEMALE**

Cage No.	Animal Number	Body weight (g) Days				Body Weight Gain (g)			
		-1	0	7	14	-1-0	0-7	7- 14	-1 - 14
1	5485	248	228	258	259	-20	30	1	11
2	5486	229	222	241	248	-7	19	7	19
3	5487	255	237	248	265	-18	11	17	10
4	5858	242	223	261	257	-19	38	-4	15
5	5859	236	232	250	274	-4	18	24	38
Mean:		242.0	228.4	251.6	260.6	-13.6	23.2	9.0	18.6
Standard deviation:		10.1	6.3	8.0	9.7	7.5	10.7	11.5	11.4

TABLE 3 Macroscopic Findings

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
1	5485	08 November 2018 Day 14	No external observations recorded	No internal observations recorded	Not applicable
2	5486	12 November 2018 Day 14	No external observations recorded	No internal observations recorded	Not applicable
3	5487	14 November 2018 Day 14	No external observations recorded	No internal observations recorded	Not applicable
4	5858	20 November 2018 Day 14	No external observations recorded	No internal observations recorded	Not applicable
5	5859	22 November 2018 Day 14	No external observations recorded	No internal observations recorded	Not applicable

APPENDICES SECTION

APPENDIX 1 Pathology Report

Study code. 18/249-001P

PATHOLOGY REPORT

INTRODUCTION

The objective of the study was to assess the acute oral toxicity of thiamethoxam/chlorantraniliprole OD (A22629A) when administered in a single dose to rats at a dose level of 2000 mg/kg bw.

METHODS

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 macroscopic observations at a dose level of 2000 mg/kg bw.

CONCLUSION

A single oral gavage of thiamethoxam/chlorantraniliprole OD (A22629A) to Crl: WI female rats dosed at 2000 mg/kg bw followed by 14-day observation, did not produce any gross observations.

Peter Maslej, D.V.M., Ph.D.
Director of Pathology

29 March 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

A22629A
Thiamethoxam/Chlorantraniliprole OD (077.43/077.43)
TAD001-020-001

Batch Identification	**TAD001-020-001**
Other Batch ID	1042405
Product Code	**A22629A**
Other Product Code(s)	Thiamethoxam/Chlorantraniliprole OD (077.43/077.43)
Chemical Analysis (Active Ingredient content)	
– **Identity of the Active Ingredient(s)***	**confirmed**
– **Content of Thiamethoxam***	**7.32 % w/w corresponding to 75.7 g/l**
– **Content of Chlorantraniliprole***	**7.31 % w/w corresponding to 75.6 g/l**
	The Active Ingredient(s) content is within the FAO limits.
Methodology used for Characterization / Recertification	HPLC, oscillating density meter
Physical Analysis	
– **Appearance**	**beige liquid**
– **Density***	**1034 kg/m³**
Stability:	
– **Storage Temperature**	**< 30 °C**
– **Recertification Date**	**End of June 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: CHMU180412
Study number(s) of batch recertification:

Authorization: July 17, 2018

C. Simonin
Dr. Céline Simonin
Analytical Development & Product Chemistry

Page 1 of 1

APPENDIX 3 Structured Study Summary

Structured Study Summary Table

Test substance design code	A22629A
Test substance batch code	TAD001-020-001
Test substance purity (% w/w)	Thiamethoxam 7.32% w/w corresponding to 75.7 g/L Chlorantraniliprole 7.31% w/w corresponding to 75.6 g/L
Study number	18/249-001P
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	none
Dosing approach	Constant Concentration
LD50 - Male	
LD50 - Female	>2000

Structured Study Results Table

Gender	Dose (mg/kg bw)	Number of animals dosed	Number of animals survived	Adverse Clinical Observations
Female	2000	5	5	Symptom-free

APPENDIX 4 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")