Chlorantraniliprole/Thiamethoxam

Chlorantraniliprole/Thiamethoxam DT (A19448E) - Acute Oral Toxicity Study in Rat

Final Report

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

AUTHOR(S): Éva Váliczkó, M.Sc.

COMPLETION DATE: 03 November 2015

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

LABORATORY PROJECT ID: Report Number: 15/288-001P
Study Number: 15/288-001P
Task Number: TK0156582

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.

The in life phase of the study was conducted by András Mátyás. Due to an unexpected leave of absence of the originally appointed Study Director, I took responsibility for the reporting phase of the study.

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 November 2015

Éva Váliczkó, 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: 15/288-001P

Study Title: Chlorantraniliprole/Thiamethoxam DT (A19448E) - Acute Oral Toxicity Study in Rat

Test Item: Chlorantraniliprole/Thiamethoxam DT (A19448E)

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
21 July 2015	Study Plan	21 July 2015	21 July 2015
23 July 2015	Treatment	23 July 2015	23 July 2015
21 September 2015	Amendment 1 to the Study Plan	21 September 2015	21 September 2015
22 September 2015	Draft Report	22 September 2015	22 September 2015
03 November 2015	Final Report	03 November 2015	03 November 2015

Signature: _____ Date: 03 November 2015

Agnes Rédl, M.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 "Chlorantraniliprole/Thiamethoxam DT (A19448E) - Acute Oral Toxicity Study in Rat" has been performed in compliance with the Principles of Good Laboratory Practice.

Signature: _____ Date: 03 Dec 2015

Alyson Leyshon, M.Sc.
Managing Director

GENERAL INFORMATION

Contributors

The following contributed to this report in the capacities indicated:

Name	Function
András Mátyás, M.Sc.	Study Director (in-life phase)
Éva Váliczkó, M.Sc.	Study Director (reporting phase)
Erika Matting, M.Sc.	Assistant Scientist
Ágnes Rédl, M.Sc.	Quality Assurance Unit
Katalin Böröczki, M.Sc.	Quality Assurance Unit
István Pásztor, DVM	Veterinary Control
Peter Maslej, DVM, PhD	Director of Pathology
Tamás Mészáros, PhD	Head of Pharmacy
Hannah Hobson, M.Sc.	Syngenta Study Manager
Harpreet Bhandal, B.Sc.	Syngenta Study Manager

Study dates

Study Initiation Date	22 July 2015
Experimental Starting Date	23 July 2015
Experimental Completion Date	13 August 2015
Receipt of Animals	09 July 2015
Acclimatisation	At least 14 days
Treatment	23 July 2015 (female no. 4252) 28 July 2015 (female no. 4253) 30 July 2015 (female no. 4254)
Observation	23 July – 06 August 2015 (female no. 4252) 28 July – 11 August 2015 (female no. 4253) 30 July – 13 August 2015 (female no. 4254)

Deviations from the Study Plan

The relative humidity was out of the target range of 30-70% during the study on three occasions (min. 32% and max 98%).

The temperature was out of the target range (22 ± 3 °C) during the study on three occasions (min. 20.0 and max. 27.6 °C).

Due to technical reason, the formulation was homogenized with a spatula (mixing applicator) prior to treatment instead of a magnetic stirrer as it was indicated in the study plan.

These deviations are considered to have no impact on the outcome of the study or the interpretation of the results.

Performing laboratory test substance reference number

150228

Other

The study documents and samples:

- study plan and its amendment,
- all raw data,
- sample of the test item,
- original study report and any amendments,
- correspondence

will be archived according to the Hungarian GLP regulations and to applicable SOP's in the archives of CiToxLAB Hungary Ltd. 8200 Veszprém, Szabadságpuszta, Hungary for a period of 15 years.

After the retention time of 15 years has elapsed, all the archived materials listed above will be returned to the Sponsor or retained for a further period if agreed by a contract. Otherwise the materials will be discarded.

TABLE OF CONTENTS

1.0 EXECUTIVE SUMMARY

1.1 Study Design

In this acute oral toxicity study, 3 female CRL:(WI) rats were given a single oral (gavage) dose of chlorantraniliprole/thiamethoxam DT (A19448E) formulated in 0.5% carboxymethyl cellulose at the limit dose of 5000 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. 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 no mortality was observed, 2 additional animals were sequentially dosed at 5000 mg/kg bw such that a total of 3 animals were tested.

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. Body weight was measured on Day -1, just before dosing and weekly thereafter. All animals were euthanised and examined macroscopically at the end of the observation period.

1.2 Results

There was no mortality during the study.

Treatment with chlorantraniliprole/thiamethoxam DT (A19448E) at a dose level of 5000 mg/kg bw did not cause any clinical signs.

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.

There was no evidence of macroscopic observations at a dose level of 5000 mg/kg bw.

1.3 Conclusion

Under the conditions of this study, the acute oral median lethal dose (LD_{50}) of the test item, chlorantraniliprole/thiamethoxam DT (A19448E), was greater than 5000 mg/kg bw (limit dose) in female CRL:(WI) rats.

2.0 INTRODUCTION

2.1 Purpose

The purpose of the study was to assess the oral toxicity of the test item chlorantraniliprole/thiamethoxam DT (A19448E) when administered as a single oral gavage dose to female rats at one or more defined dose levels. The results of the study allowed the test item to be ranked according to most classification systems currently in use.

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 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:	chlorantraniliprole/thiamethoxam DT (A19448E)
Batch number:	Virtako DT-15-001
Product code:	A19448E
Active ingredient content:	Content of thiamethoxam – 1.17 % w/w corresponding to 11.7 g/kg Content of chlorantraniliprole – 1.15 % w/w corresponding to 11.5 g/kg
Appearance:	Beige to brown tablets
Recertification date:	End of April 2019
Storage conditions:	Room temperature (< 30°C)
Safety precautions:	Routine safety precautions (lab coat, gloves, goggles, face mask) for unknown materials were applied to assure personnel health and safety.

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

Test item was powdered using a mortar and pestle and formulated with 0.5% carboxymethyl cellulose. Formulations were prepared just before the administration by Pharmacy of CiToxLAB Hungary Ltd. and the formulation was homogenized with a spatula (mixing applicator) prior to administration.

Components of vehicle:

Name:	Carboxymethyl cellulose
Batch number:	MKBQ8416V
Expiry date:	31 January 2019
Source:	SIGMA-ALDRICH

Name:	Distilled water
Lot number:	8110515
Expiry Date:	20 November 2015
Source:	Hungaropharma Zrt.

3.2 Experimental Design

3.2.1 Animals

Species and strain:	CRL:(WI) rats
Source:	Charles River Laboratories, Research Models and Services, Germany GmbH, Sandhofer Weg 7, D-97633 Sulzfeld
Hygienic level:	SPF at arrival, standard housing conditions during study
Justification of strain:	Recognized by international guidelines as a recommended test system.
Number of animals:	3
Sex:	Female rats, nulliparous and non-pregnant.
Age when treated:	Young adult rats, 9-10 weeks old.
Body weight (at dosing):	210-240 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.
Acclimatization time:	At least 14 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:	Lignocel ¾S Hygienic Animal Bedding *(produced by J. Rettenmaier & Söhne GmbH & Co.KG, Germany)* and Grade 5 *(produced by Johannes Brandenburg 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:	20.0 – 27.6 °C

Relative humidity: 32 – 98 %
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 batch number of the lots used in the study were: Lot number: 814 3108, Expiry date: August 2015; Lot number: 930 3907, Expiry date: December 2015. 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 A street 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:

A limit dose of 5000 mg/kg bw was selected by the Study Director after discussion with the Sponsor. A total of 3 animals were tested and the individual dose volumes used at this dose level are shown below.

Animal Number	Dose [mg/kg body weight]	Volume Dosed [mL]	Bodyweight [g]	Mortality
4252	5000	2.1	210	Survived
4253	5000	2.3	230	Survived
4254	5000	2.4	240	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.

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. 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

The body weights were recorded on Days -1, 0 (before treatment), 7 and 14.

3.5 *Post Mortem* Investigations

All animals were subjected to gross macroscopic evaluation. All 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)
Lot No.:	1409236-06
Expiry Date:	September 2017
Produced by:	AlfasanNederland BV, Kuipersweg 9, 3449 JA Woerden, The Netherlands

3.6 Data Evaluation

The 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 programme was constructed using the most appropriate method to estimate the LD_{50}.

4.0 RESULTS AND DISCUSSION

4.1 Mortality

No mortality occurred during the study.

4.2 Clinical Signs

Treatment with chlorantraniliprole/thiamethoxam DT (A19448E) at a dose level of 5000 mg/kg bw did not cause any clinical signs.

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. Individual body weights are presented in Table 2.

4.4 Macroscopic Findings

There was no evidence of abnormal macroscopic observations at a dose level of 5000 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, chlorantraniliprole/thiamethoxam DT (A19448E), was greater than 5000 mg/kg bw (limit dose) in female CRL:(WI) rats.

TABLES SECTION

TABLE 1 Individual Findings – Clinical Signs

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	6-14	
			30'	1h	2h	3h	4h	6h							
1	4252	Symptom Free	+	+	+	+	+	+	+	+	+	+	+	+	20/20
2	4253	Symptom Free	+	+	+	+	+	+	+	+	+	+	+	+	20/20
3	4254	Symptom Free	+	+	+	+	+	+	+	+	+	+	+	+	20/20

Remarks: + = present

h = hour (s) ' = minute

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

TABLE 2 Body Weight and Body Weight Gain

DOSE LEVEL: 5000 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	4252	235	210	245	258	-25	35	13	23
2	4253	251	230	260	262	-21	30	2	11
3	4254	248	240	269	274	-8	29	5	26
Mean:		244.7	226.7	258.0	264.7	-18.0	31.3	6.7	20.0
Standard deviation:		8.5	15.3	12.1	8.3	8.9	3.2	5.7	7.9

TABLE 3 Macroscopic Findings

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	4252	06 August 2015 Day 14	No external observations recorded	No internal observations recorded	Not applicable
2	4253	11 August 2015 Day 14	No external observations recorded	No internal observations recorded	Not applicable
3	4254	13 August 2015 Day 14	No external observations recorded	No internal observations recorded	Not applicable

APPENDICES SECTION

APPENDIX 1 Pathology Report

CiToxLAB Hungary Ltd. Study code 15/288-001P

PATHOLOGY REPORT

INTRODUCTION

The objective of the screening study was to assess the acute oral toxicity of the test items chlorantraniliprole/thiamethoxam DT (A19448E) when administered via a single oral gavage dose to female rats. Three rats were dosed at 5000 mg/kg bodyweight with the test item.

RESULTS AND DISCUSSION

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 5000 mg/kg bw.

CONCLUSION

A single oral gavage of chlorantraniliprole/thiamethoxam DT (A19448E) to the CRL:(WI) rats at a dose level of 5000 mg/kg bw with a 14 day observation period, was not associated with any macroscopic findings.

Gábor Boros, D.V.M.
Histopathologist

29. Oct. 2015.
Date

APPENDIX 2 Certificate of Analysis

syngenta

GLP Testing Facility WMU
Analytical Development &
Product Chemistry GS2131

Syngenta Crop Protection Münchwilen AG
Im Breitenloh 5
4333 Münchwilen, Switzerland

Certificate of Analysis

A19448E
thiamethoxam/chlorantraniliprole DT (1.2/1.2)
Virtako DT-15-001

Batch Identification	**Virtako DT-15-001**
Product Code	**A19448E**
Other Product Code(s)	thiamethoxam/chlorantraniliprole DT (1.2/1.2)

Chemical Analysis
(Active Ingredient Content)

– **Identity of the Active Ingredient(s)***	**confirmed**
– **Content of thiamethoxam***	**1.17 % w/w corresponding to 11.7 g/kg**
– **Content of chlorantraniliprole***	**1.15 % w/w corresponding to 11.5 g/kg**

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

Methodology used for Characterization / Recertification	HPLC

Physical Analysis

– **Appearance**	**beige to brown tablets**

Stability:

– **Storage Temperature**	**< 30°C**
– **Recertification Date**	**End of April 2019**

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 Münchwilen AG, Switzerland.

Study number of batch characterization:	CHMU150352
Study number(s) of batch recertification:	

Authorization: 28-APR-2015

Dr. Christopher Heppel
Analytical Development & Product Chemistry

APPENDIX 3 GLP Certificate

OGYÉI
National Institute of
Pharmacy and Nutrition

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

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

GOOD LABORATORY PRACTICE (GLP)
CERTIFICATE

It is hereby certified that the test facility

CiToxLAB Hungary Ltd.

H-8200 Veszprém, Szabadságpuszta

is able to carry out

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

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

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

Dr. József Reiter
Deputy Director-General