citoxlab
A Charles River Company

A22988A

A22988A - Acute Oral Toxicity Study in Rats (Up and Down Procedure)

Final Report

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

AUTHOR(S): Balázs Oroszlány, DVM

COMPLETION DATE: 26 July 2019

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

LABORATORY PROJECT ID: Report Number: 19/053-001P
Study Number: 19/053-001P
Task Number: TK0345607

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

STATEMENT OF DATA CONFIDENTIALITY CLAIMS

This page is intentionally left blank.

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: 26 July 2019

Balázs Oroszlány, DVM
Study Director

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

FLAGGING STATEMENT

This page is intentionally left blank.

QUALITY ASSURANCE STATEMENT

Study Number: 19/053-001P

Study Title: A22988A -
Acute Oral Toxicity Study in Rats (Up and Down Procedure)

Test Item: A22988A

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
04 June 2019	Study Plan	04 June 2019	04 June 2019
06 June 2019	Treatment	06 June 2019	06 June 2019
22 July 2019	Draft Report	22 July 2019	22 July 2019
26 July 2019	Final Report	26 July 2019	26 July 2019

Signature: Varga Viktória Date: 26 July 2019

Viktória Varga, B.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 "A22988A - Acute Oral Toxicity Study in Rats (Up and Down Procedure)" has been performed in compliance with the Principles of Good Laboratory Practice.

Signature: _____ Date: 26 July 2019

Christopher Banks, DABT
Site Director

GENERAL INFORMATION

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

Name	Function
Balázs Oroszlány, DVM	Study Director
Ádám Appl, M.Sc.	Assistant Scientist
Viktória Varga, B.Sc.	QA Inspector
László Székelyhidi, DVM	Veterinary Care
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	05 June 2019
Receipt of Animals	23 May 2019
Experimental Starting Date	06 June 2019
Experimental Completion Date	04 July 2019
Draft Report Date	22 July 2019
Treatment	06 June 2019 (female no. 8020) 12 June 2019 (female no. 8021) 14 June 2019 (female no. 8022) 18 June 2019 (female no. 8023) 20 June 2019 (female no. 8024)
Observation	06 June – 20 June 2019 (female no. 8020) 12 June – 26 June 2019 (female no. 8021) 14 June – 28 June 2019 (female no. 8022) 18 June – 02 July 2019 (female no. 8023) 20 June – 04 July 2019 (female no. 8024)
Necropsy	20 June 2019 (female no. 8020) 26 June 2019 (female no. 8021) 28 June 2019 (female no. 8022) 02 July 2019 (female no. 8023) 04 July 2019 (female no. 8024)

Deviation from the Guideline
There were no deviations from the Guideline.

Performing laboratory test substance reference number
190147

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 (up and down procedure) study, 5 female Crl:WI Wistar rats were given a single oral (gavage) dose of A22988A 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.

There were no test item related effects on body weight or body weight gain.

There was no evidence of macroscopic findings 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, A22988A, 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 A22988A 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:	A22988A
Batch number:	001/19
Appearance:	Brown color, homogeneous suspension
Purity*:	15% Total organic carbon 0.7% Nitrogen 3.12% Linoleic Acid 1.65% Palmitic Acid 1.25% Oleic Acid
Recertification date:	31 January 2021
Storage conditions:	Room temperature (<30^{o}C), protected from light
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 purity 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):	223 – 238 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 14 days

3.2.2 Husbandry

Animal health:	Only healthy animals were used for the test. The health status was certified by the Veterinarian.
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 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:	20.6 – 24.8 °C
Relative humidity:	37 – 70%
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: 797 46230, Expiry date: 31 August 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 A22988A at a dose level of 2000 mg/kg bw. Based on the Sponsor's information, the density of the Test Item is between 1.15-1.20 g/mL. To ensure that the animals were treated with an adequate amount of Test Item, 1.15 g/mL was used for volume calculations; therefore the dose volume was 1.74 mL/kg bw. The volume of individual doses used are shown below:

Animal Number	Dose [mg/kg body weight]	Volume Dosed [mL]	Bodyweight [g]	Mortality
8020	2000	0.40	228	Survived
8021	2000	0.41	238	Survived
8022	2000	0.40	232	Survived
8023	2000	0.40	231	Survived
8024	2000	0.39	223	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) in all animals until termination or death.

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.:	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

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

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 changes 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, A22988A, 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	8020	Symptom Free	+	+	+	+	+	+	+	+	+	+	+	+	+	20/20
2	8021	Symptom Free	+	+	+	+	+	+	+	+	+	+	+	+	+	20/20
3	8022	Symptom Free	+	+	+	+	+	+	+	+	+	+	+	+	+	20/20
4	8023	Symptom Free	+	+	+	+	+	+	+	+	+	+	+	+	+	20/20
5	8024	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	8020	241	228	243	261	-13	15	18	20
2	8021	251	238	259	284	-13	21	25	33
3	8022	244	232	268	279	-12	36	11	35
4	8023	246	231	254	265	-15	23	11	19
5	8024	230	223	236	255	-7	13	19	25
Mean:		242.4	230.4	252.0	268.8	-12.0	21.6	16.8	26.4
Standard deviation:		7.8	5.5	12.7	12.3	3.0	9.0	5.9	7.3

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	8020	20 June 2019 Day 14	No external observations recorded	No internal observations recorded	Not applicable
2	8021	26 June 2019 Day 14	No external observations recorded	No internal observations recorded	Not applicable
3	8022	28 June 2019 Day 14	No external observations recorded	No internal observations recorded	Not applicable
4	8023	02 July 2019 Day 14	No external observations recorded	No internal observations recorded	Not applicable
5	8024	04 July 2019 Day 14	No external observations recorded	No internal observations recorded	Not applicable

APPENDICES SECTION

APPENDIX 1 Pathology Report

Citoxlab Hungary Ltd. Study code 19/053-001P

PATHOLOGY REPORT

INTRODUCTION

The objective of the study was to assess the acute oral toxicity of A22988A when administered in a single dose to rats at 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 and subjected to necropsy. Macroscopic examination consisted of external examination, as well as a detailed internal examination of cranial, thoracic and abdominal cavities. Histopathological examination was not performed.

TERMINAL (DAY 14)

All animals survived until the 14-day observation period.

Macroscopic Findings

There was no evidence of any macroscopic observations in the terminal animals at dose level 2000 mg/kg bw (five females).

CONCLUSION

A single oral gavage administration of A22988A to Crl:WI rats at dose level of 2000 mg/kg bw and subjected to necropsy on Day 14, was not associated with any macroscopic findings.

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

25 July 2019

APPENDIX 2 Certificate of Analysis

SinergiaAgro

Certificate of Analysis

Comercial name: Epivio Sky
Classification: Class A Organomineral Fertilizer
Application Method: Via Seed
MAPA Record: SP 81007 10104-6
Batch Number: 001
Manufacture Date: 15/10/2018
Expiration Date: 15/10/2019
Formulation Base: 1L
Batch: 001/19
Physical Properties:

Physical State:	Liquid – Homogeneous Suspension
Density:	1.15 – 1.20 g/ml
pH:	6.0-7.0
Solubility:	Water Soluble
Color:	Brown
Smell:	Characteristics

COMPONENTS	COMPONENTS' CONCENTRATION W/V in formulation	NUTRIENT'S GUARANTEES W/W in formulation	NUTRIENT'S GUARANTEES W/V in formulation
Vegetal Extract	380 g/L	15% Total Organic Carbon 0.7% Nitrogen 3.12% Linoleic Acid 1.65% Palmitic Acid 1.25% Oleic Acid	180 g/L Total Organic Carbon 8.4 g/L Nitrogen 37.4 g/L Linoleic Acid 19.8 g/L Palmitic Acid 15.0 g/L Oleic Acid
Epivio Vigor (MAPA Record: EI SP 81057 10016-7) Guarantees: 1%N; 0.4%Fe;1%Zn.	330 g/L	0.3% Nitrogen 0.1% Iron 0.3% Zinc	3.6 g/L Nitrogen 1.2 g/L Iron 3.6 g/L Zinc
Sodium Molybdate 39% (CAS 7631-95-0)	60 g/L	2.0% Molybdenum	24.0 g/L Molybdenum
Tensoactive/ Surfactant (Cumene Sulfonate os Ammonia – 58%) (CAS 37475-88-0)	275 g/L	Not Applicable For Additive	Not Applicable For Additive
Tensoactive/ Surfactant (Silicone Emulsion – 60%) (CAS 63148-62-9)	100 g/L	Not Applicable For Additive	Not Applicable For Additive
Acidifying (Phosphoric Acid – 85%) (CAS 7664-38-2)	50 g/L	Not Applicable For Additive	Not Applicable For Additive

Adriano da Cruz Silva
Chemist

APPENDIX 3 Structured Study Summary

Structured Study Summary Table

Test substance design code	A22988A
Test substance batch code	001/19
Test substance purity (% w/w)	15% Total organic carbon 0.7% Nitrogen 3.12% Linoleic Acid 1.65% Palmitic Acid 1.25% Oleic Acid
Study number	19/053-001P
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")