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Isocycloseram/Emamectin Benzoate

Isocycloseram/Emamectin Benzoate SC (A23220A) - Acute Oral Toxicity Study in Rats (Up and Down Procedure)

Final Report

TEST GUIDELINE(S): OECD 425 (2008)
EPA 870.1100 (2002)

AUTHOR(S): Nóra Krajcs, Ph.D.

COMPLETION DATE: 10 December 2020

PERFORMING LABORATORY: Charles River Laboratories Hungary Kft.
H-8200 Veszprém, Szabadságpuszta, hrsz. 028/1,
Hungary

LABORATORY PROJECT ID: Report Number: 20/080-001P
Study Number: 20/080-001P
Task Number: TK0416700

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

STATEMENT OF DATA CONFIDENTIALITY CLAIMS

The Following Statement Applies To The United States of America:

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS
UNDER SPECIFIED FIFRA PROVISIONS

No claim of confidentiality, on any basis whatsoever, is made for any information contained in this document. I acknowledge that information not designated as within the scope of FIFRA sec. 10(d)(1)(A), (B), or (C) and which pertains to a registered or previously registered pesticide is not entitled to confidential treatment and may be released to the public, subject to the provisions regarding disclosure to multinational entities under FIFRA 10(g).

Company: Syngenta Crop Protection, LLC
410 Swing Road
Post Office Box 18300
Greensboro, NC 27419-8300 USA

Submitter: _____ Date: _____

Syngenta is the owner of this information and data. Syngenta has submitted this material to the United States Environmental Protection Agency specifically under the provisions contained in FIFRA as amended and, hereby, consents to use and disclosure of this material by EPA according to FIFRA. In submitting this material to EPA according to method and format requirements contained in PR Notice 2011-3, we do not waive any protection or right involving this material that would have been claimed by the company if this material had not been submitted to the EPA, nor do we waive any protection or right provided under FIFRA Section 3 (concerning data exclusivity and data compensation) or FIFRA Section 10(g) (prohibiting disclosure to foreign and multinational pesticide companies or their agents).

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 Charles River Laboratories Hungary Kft. 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. By virtue of my dated signature I accept the responsibility for the validity of the data.

Signature: _____ Date: 10 December 2020

Nóra Krajcs, Ph.D.
Study Director

Performing Laboratory: Charles River Laboratories Hungary Kft.
H-8200 Veszprém, Szabadságpuszta, hrsz. 028/1,
Hungary

To be completed for USA EPA submission only:
Representative of Submitter/Sponsor:

_____ _____
Date

Submitter/Sponsor: Syngenta Crop Protection, LLC
410 Swing Road
Post Office Box 18300
Greensboro, NC 27419-8300 USA

FLAGGING STATEMENT

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QUALITY ASSURANCE STATEMENT

Study Number: 20/080-001P

Study Title: Isocycloseram/Emamectin Benzoate SC (A23220A) - Acute Oral Toxicity Study in Rats (Up and Down Procedure)

Test Item: Isocycloseram/Emamectin Benzoate SC (A23220A)

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
18 May 2020	Study Plan	18 May 2020	18 May 2020
21 May 2020	Treatment	21 May 2020	21 May 2020
06 October 2020	Draft Report	06 October 2020	06 October 2020
10 December 2020	Final Report	10 December 2020	10 December 2020

Signature: Sebestyén E.

Eszter Sebestyén, B.Sc.
On behalf of QA

Date: 10 December 2020

MANAGEMENT STATEMENT

According to the conditions of the research and development agreement between Syngenta Ltd. (as Sponsor) and Charles River Laboratories Hungary Kft. (as Test Facility) the study titled "Isocycloseram/Emamectin Benzoate SC (A23220A) - Acute Oral Toxicity Study in Rats (Up and Down Procedure)" has been performed in compliance with the Principles of Good Laboratory Practice.

Signature: _____ Date: 11 December 2020

Balázs Tóth, Ph.D.
General Manager

GENERAL INFORMATION

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

Name	**Function or Department**
Nóra Krajcs, Ph.D.	Study Director
Nikoletta Szalóki, Ph.D.	Assistant Scientist
Eszter Sebestyén, B.Sc	Quality Assurance
László Székelyhidi, D.V.M.	Veterinary Care
Peter Maslej, D.V.M., Ph.D.	Pathology
Tamás Mészáros, Ph.D.	Pharmacy
Ferenc Szűcs	Animal Service Laboratories
Carolina Vaccari	Syngenta Study Manager

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

Study dates

Study Initiation Date	19 May 2020
Experimental Starting Date	21 May 2020
Experimental Completion Date	30 June 2020
Draft Report (non QA audited)	20 August 2020
Draft Report (QA audited)	07 October 2020
Final Report:	10 December 2020
Receipt of Animals	14 May 2020
Acclimatisation	At least 7 days
Treatment	21 May 2020 (female no. 4554) 26 May 2020 (female no. 4555) 03 June 2020 (female no. 4556) 09 June 2020 (female no. 4557) 16 June 2020 (female no. 4558)
Observation	21 May – 04 June 2020 (female no. 4554) 26 May – 28 May 2020 (female no. 4555) 03 June – 17 June 2020 (female no. 4556) 09 June – 14 June 2020 (female no. 4557) 16 June – 30 June 2020 (female no. 4558)
Necropsy	04 June 2020 (female no. 4554) 28 May 2020 (female no. 4555) 17 June 2020 (female no. 4556) 14 June 2020 (female no. 4557)

30 June 2020 (female no. 4558)

Deviation from the guideline

Due to a technical reason, relative humidity values (maximum of 73%) outside the expected range of 30-70% were recorded during the study. However, this deviation has no effect on the outcome of the study.

Due to the rapid and lethal effects seen at 2000 mg/kg bw, it was agreed with the Sponsor that due to animal welfare reasons it was not justified to treat a third animal. Although this 3rd animal was not treated, it was counted as dead and a proposed LD50 was calculated on this basis, therefore this deviation has no effect on the outcome of the study.

Performing laboratory test substance reference number
200164

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 Charles River Laboratories Hungary Kft. H-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, 5 female Crl:WI rats were given a single oral (gavage) dose of isocycloseram/emamectin benzoate SC (A23220A) at a dose level of 2000 or 550 mg/kg body weight (bw).

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 550 mg/kg bw. The dose selection for the next animals followed the recommendation of AOT425StatPgm software, based on available results. Due to the rapid and lethal effects seen at 2000 mg/kg bw in 2 animals, for the third animal at 2000 mg/kg bw it was assumed to also die rapidly. This animal was counted as dead by AOT425StatPgm software and a proposed LD50 was calculated.

Animals were observed individually at 30 minutes, and 1, 2, 3, 4 and 6 hours post treatment and 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. All animals were euthanized and examined macroscopically at the end of the observation period or on the day of death.

1.2 Results

No mortality was observed at the dose level of 550 mg/kg bw during the study.
Two animals treated at 2000 mg/kg bw were pre-terminally euthanized due to their clinical conditions on Day 2 and Day 5.

At a dose level of 2000 mg/kg bw, decreased activity (slight to extreme), hunched back, coloured (red) discharge (on nose), liquid faeces, heightened startle response, increased salivation (moderate), recumbency, decreased respiratory rate (moderate), intermittent and continuous tremors (whole body), piloerection, prostration, splayed gait, red discharge (on both eyes, nose and snout) and vocalization were observed from Day 0 up to preterminal euthanasia.

At a dose level of 550 mg/kg bw, one animal was symptom-free from Day 0 up to the end of the 14-day observation period. Heightened startle response, hunched back, piloerection, splayed gait and intermittent tremors (whole body) were observed in the remaining two animals from Day 1 up to Day 12. These animals were symptom free on Day 0 and from Day 13 up to Day 14.

At a dose level of 550 mg, there was no test item related effect on body weight or body weight gain in any animals. Body weights were within the range commonly recorded for this strain and age.

At a dose level of 2000 mg/kg bw, the body weight of the animals decreased by approximately 15% and 28%, compared to their body weight measured on Day 0, before treatment.

A single oral gavage of isocycloseram/emamectin benzoate SC (A23220A) to Crl:WI female rats at dose level of 550 mg/kg bw was not associated with any gross observations at necropsy. At a dose level of 2000 mg/kg bw, test item-related gross findings were observed in the adrenals, stomach and/or spleen in these animals.

1.3 Conclusion

Under the conditions of this study, based on the outcome of AOT425StatPgm program, the estimated acute oral median lethal dose (LD_{50}) of the test item isocycloseram/emamectin benzoate SC (A23220A) was found to be 1049 mg/kg bw in female Crl:WI Wistar rats. The 95% confidence interval is 550 to 2000 mg/kg bw.

2.0 INTRODUCTION

2.1. Purpose

The purpose of the study was to assess the acute oral toxicity of the test item isocycloseram/emamectin benzoate SC (A23220A) when administered as a single oral gavage dose to female rats at two 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 Charles River Laboratories Hungary Kft. 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:	Isocycloseram/Emamectin Benzoate SC (A23220A)	
Batch number:	TSC002-041-001	
Product code:	A23220A	
Appearance:	Brown liquid	
Active ingredient content*:	Isocycloseram	17.5% w/w corresponding to 201 g/L
	emamectin benzoate	4.18% w/w corresponding to 48.1 g/L
Density:	1150 kg/m^3	
Recertification date:	31 Jan 2023	
Storage conditions:	Room temperature (<30°C)	
Safety precautions:	Enhanced safety precautions (half mask at least with P3 filter cartridge, nitrile gloves, lab coat, safety glasses) were applied considering the supplied safety datasheet to assure personnel health and safety.	

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

The Certificate of Analysis is presented in Appendix 3.

The integrity of supplied data relating to the identity, purity and stability of the test material is the responsibility of the Sponsor.

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 Charles River Laboratories Hungary Kft. on the basis of the information provided by the Sponsor.

3.1.2. Formulation

The test item was used undiluted, as supplied. 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:	5 (1 animal/step)
Sex:	Female rats, nulliparous and non-pregnant
Age when treated:	Young adult rats, 8-12 weeks old
Body weight (at dosing):	208 – 248 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 7 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 ¾-S certified wooden chips and SAFE 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 Charles River Laboratories Hungary Kft.
Light:	12 hours daily, from 6.00 a.m. to 6.00 p.m.
Temperature:	19.4-24.4°C
Relative humidity:	34-73%
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 *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 Charles River Laboratories Hungary Kft.

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 Charles River Laboratories Hungary Kft.

3.3. Administration of the Test Item

3.3.1. Dosages

Justification of the doses:

The starting dose of the main test was 550 mg/kg bw after a discussion with the Sponsor.

The density of the test item is 1150 kg/m^3 as provided by the Sponsor.

The animals were treated with a single oral (gavage) dose of isocycloseram/emamectin benzoate SC (A23220A) at the dose levels of 550 or 2000 mg/kg bw.

The individual dose volumes are shown below.

Animal Number	Dose [mg/kg body weight]	Volume Dosed [mL]	Bodyweight [g]	Mortality
4554	550	0.10	208	Survived
4555	2000	0.37	213	Pre-terminal euthanasia
4556	550	0.12	248	Survived
4557	2000	0.42	240	Pre-terminal euthanasia
4558	550	0.11	230	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 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. Body weights of pre-terminally euthanized animals were also recorded before 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 section 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. Gross macroscopic abnormalities were retained and preserved in buffered formaldehyde solution for possible histopathological examination. The preserved organs or tissues were discarded after the finalization of the Study Report.

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

No mortality was observed at the dose level of 550 mg/kg bw during the study.
Two animals treated at 2000 mg/kg bw were pre-terminally euthanized due to their clinical conditions on Day 2 and Day 5.

4.2. Clinical Signs

At a dose level of 2000 mg/kg bw, decreased activity (slight to extreme), hunched back, coloured (red) discharge (on nose), liquid faeces, heightened startle response, increased salivation (moderate), recumbency, decreased respiratory rate (moderate), intermittent and continuous tremors (whole body), piloerection, prostration, splayed gait, red discharge (on both eyes, nose and snout) and vocalization were observed from Day 0 up to preterminal euthanasia.

At a dose level of 550 mg/kg bw, one animal was symptom-free from Day 0 up to the end of the 14-day observation period. Heightened startle response, hunched back, piloerection, splayed gait and intermittent tremors (whole body) were observed in the remaining two animals from Day 1 up to Day 12. These animals were symptom free on Day 0 and from Day 13 up to Day 14.

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

4.3. Body Weights

At a dose level of 550 mg, there was no test item related effect on body weight or body weight gain in any animals. Body weights were within the range commonly recorded for this strain and age.

At a dose level of 2000 mg/kg bw, the body weight of the animals decreased by approximately 15% and 28%, compared to their body weight measured on Day 0, before treatment.

Individual body weights are presented in Table 2.

4.4. Macroscopic Findings

A single oral gavage of isocycloseram/emamectin benzoate SC (A23220A) to Crl:WI female rats at dose level of 550 mg/kg bw was not associated with any gross observations at necropsy. At a dose level of 2000 mg/kg bw, test item-related gross findings were observed in the adrenals, stomach and/or spleen in these animals.

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

5.0 CONCLUSIONS

Under the conditions of this study, based on the outcome of AOT425StatPgm program, the estimated acute oral median lethal dose (LD_{50}) of the test item isocycloseram/emamectin benzoate SC (A23220A) was found to be 1049 mg/kg bw in female Crl:WI Wistar rats. The 95% confidence interval is 550 to 2000 mg/kg bw.

TABLES SECTION

TABLE 1 Individual Findings – Clinical Signs

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	8	9	10	11	12	13	14	
			30'	1h	2h	3h	4h	6h															
1	4554	Symptom Free	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	20/20
3	4556	Symptom Free	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	+	16/20
		Heightened startle response	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	4/20
		Hunched back	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	4/20
5	4558	Symptom Free	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	+	8/20
		Heightened startle response	-	-	-	-	-	-	+	+	+	-	+	+	+	+	+	+	+	+	-	-	11/20
		Hunched back	-	-	-	-	-	-	+	+	+	+	+	+	+	-	-	-	-	-	-	-	7/20
		Piloerection	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	3/20
		Splayed gait	-	-	-	-	-	-	-	+	+	+	+	+	+	+	-	-	-	-	-	-	7/20
		Tremors Intermittent - Whole body	-	-	-	-	-	-	+	+	+	+	+	+	+	+	-	-	-	-	-	-	8/20

Standard footnotes:

+ = present
- = absent
h = hour (s)
' = minute
# = Found dead
M = Moribund
PE = Preterminal Euthanasia
'R = Red

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

Severities:

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

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	8	9	10	11	12	13	14	
			30'	1h	2h	3h	4h	6h															
2	4555PE	Symptom Free	+	-	-	-	-	-	-	-													1/8
		Activity decreased	-	-	-	-	-	-	-	Ex													1/8
		Discharge coloured - Nose	-	-	-	-	-	-	-	R													1/8
		Faeces liquid	-	+	-	-	-	-	-	-													1/8
		Heightened startle response	-	-	+	+	+	+	+	+													6/8
		Hunched back	-	-	-	+	+	+	+	+													5/8
		Increased Salivation	-	-	-	-	-	-	Mo	Mo													2/8
		Recumbency	-	-	-	-	-	-	+	+													2/8
		Respiratory rate decreased	-	-	-	-	-	-	-	Mo													1/8
		Tremors Intermittent - Whole Body	-	-	-	-	-	-	+	-													1/8
		Preterminal Euthanasia	-	-	-	-	-	-	-	+													-

Standard footnotes: + = present
- = absent
h = hour (s)
' = minute
# = Found dead
M = Moribund
PE = Preterminal Euthanasia
'R = Red

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

Severities:

Sl = Slight/Small/Few/Small amount

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Ex = Severe/Large/Many/Large/Extreme amount

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	8	9	10	11	12	13	14	
			30'	1h	2h	3h	4h	6h															
4	4557PE	Symptom Free	+	-	-	-	-	-	-	-													1/11
		Activity decreased	-	-	-	-	-	-	Sl	Sl	Mo	Ex	Ex										5/11
		Heightened startle response	-	+	+	+	+	+	+	+	+	+	+										10/11
		Hunched back	-	-	-	-	-	+	+	+	+	+	+										6/11
		Piloerection	-	-	-	-	-	-	-	+	+	+	+										4/11
		Prostration	-	-	-	-	-	-	-	-	-	-	+										1/11
		Red discharge - Both Eyes	-	-	-	-	-	-	-	-	-	-	+										1/11
		Red discharge - Nose	-	-	-	-	-	-	-	+	+	+	+										4/11
		Red discharge - Snout	-	-	-	-	-	-	-	+	-	+	+										3/11
		Splayed gait	-	-	-	-	-	-	-	-	+	+	-										2/11
		Tremors Continous - Whole Body	-	-	-	-	-	-	+	+	+	+	+										5/11
		Tremors Intermittent - Whole Body	-	-	-	-	-	+	-	-	-	-	-										1/11
		Vocalisation	-	-	-	-	-	-	+	+	+	+	+										5/11
		Preterminal Euthanasia	-	-	-	-	-	-	-	-	-	-	+										-

Standard footnotes: + = present - = absent

h = hour (s) ' = minute

= Found dead M = Moribund

PE = Preterminal Euthanasia 'R = Red

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

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

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		0-7	7- 14	0 - 14
1	4554	228	208	243	263	-	35	20	55
3	4556	252	248	256	263	-	8	7	15
5	4558	248	230	257	273	-	27	16	43
Mean:		242.7	228.7	252.0	266.3	-	23.3	14.3	37.7
Standard deviation:		12.9	20.0	7.8	5.8	-	13.9	6.7	20.5

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		0-7	7- 14	0 - 14
2	4555PE	238	213	-	-	2/180	-	-	-
4	4557PE	255	240	-	-	5/173	-	-	-
Mean:		246.5	226.5	-	-	-	-	-	-
Standard deviation:		12.0	19.1	-	-	-	-	-	-

Standard footnotes: **# = Found dead** **M = Moribund**
- = No data
PE = Preterminal Euthanasia

Note: Day -1 prior to fasting, Day 0 prior to administration.

TABLE 3 Macroscopic Findings

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

Cage No.	Animal Number	Necropsy Day	External Observations	Internal Observations	Organ/Tissue
1	4554	Day 14	No external observations recorded	No internal observations recorded	Not applicable
3	4556	Day 14	No external observations recorded	No internal observations recorded	Not applicable
5	4558	Day 14	No external observations recorded	No internal observations recorded	Not applicable

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

Cage No.	Animal Number	Necropsy Day	External Observations	Internal Observations	Organ/Tissue
2	4555PE	Day 2	Fur: Material, red, perinasal, perioral:dry	Enlargement, bilateral	Adrenal gland
				Discoloration, dark red, diffuse, all lobes	Lungs
				Focus, black, many, glandular, mucosa	Stomach
				Focus, white, many, glandular, mucosa	
4	4557PE	Day 5	Fur: Material, red, perinasal: dry area	Enlargement, bilateral	Adrenal gland
			Fur: Material, red, periorbital: dry area	Small	Spleen

Standard footnotes: **# = Found dead** **M = Moribund** **- = No data** **PE = Preterminal Euthanasia**

APPENDICES SECTION

APPENDIX 1 Pathology Report

PATHOLOGY REPORT

INTRODUCTION

The objective of the study was to assess the acute oral toxicity of isocycloseram/emamectin benzoate SC (A23220A) when administered in a single dose to rats at dose levels of 550 and 2000 mg/kg bw.

METHODS

Surviving animals were euthanized upon completion of the observation period on Day 14. These 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.

MORTALITY

Two animals dosed at 2000 mg/kg bw were preterminally euthanized on Day 2 and 5. In the female 4555, increased salivation, decreased activity, decreased respiratory rate and whole body tremor were major clinical observations observed prior preterminal euthanasia. Decreased activity, vocalisation, heightened startle response, whole body tremor and red discharge of nose/eye/snout were major clinical observations seen in female 4557 prior preterminal euthanasia.

PRETERMINAL EUTHANASIA

Macroscopic Findings

At necropsy, the enlargement of adrenals in 2/2 females, black/white foci of glandular stomach in 1/2 females and small spleen in 1/2 females, were attributed to the administration of the test item.

Other changes were agonal or post mortem.

TERMINAL (DAY 14)

Macroscopic Findings

There was no evidence of the test item-related macroscopic findings in females dosed at 550 mg/kg bw and terminated on Day 14.

CONCLUSION

A single oral gavage of isocycloseram/emamectin benzoate SC (A23220A) to Crl:(WI) female rats led to the preterminal euthanasia two females dosed at 2000 mg/kg bw. The test item-related gross findings were observed in the adrenals, stomach and/or spleen in these animals.

There were no test item-related macroscopic findings noted at a dose level of 550 mg/kg bw on necropsy Day 14.

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

09 Dec 2020
Date

APPENDIX 2 AOT 425 Report (Main Test)

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

Date/Time: 2020. augusztus 19., szerda, 16:11:18
Data file name: AOT.dat
Last modified: 2020. 08. 19. 16:11:14

Test/Substance: Enter test description.
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	1	550	O	O
2	2	2000	X	X
3	3	550	O	O
4	4	2000	X	X
5	5	550	O	O
6	6	2000	X	X

(X = Died, O = Survived)

Dose Recommendation: The main test is complete.

Stopping criteria met: 5 reversals in 6 tests. LR criterion.

SUMMARY OF LONG-TERM RESULTS:

Dose	O	X	Total
550	3	0	3
2000	0	3	3
All Doses	3	3	6

Statistical Estimate based on long term outcomes:

Estimated LD50 = 1049 (Based on an assumed sigma of 0.5).
Approximate 95% confidence interval is 550 to 2000.

APPENDIX 3 Certificate of Analysis

syngenta

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

Certificate of Analysis

A23220A

isocycloseram/emamectin benzoate SC (200/050)

TSC002-041-001

Batch Identification	**TSC002-041-001**
Other Batch ID	1122866
Product Code	**A23220A**
Other Product Code(s)	isocycloseram/emamectin benzoate SC (200/050)
Chemical Analysis (Active Ingredient content)	
- **Identity of the Active Ingredient(s)***	**confirmed**
- **Content of isocycloseram***	**17.5 % w/w corresponding to 201 g/l**
- **Content of emamectin benzoate***	**4.18 % w/w corresponding to 48.1 g/l**
	The Active Ingredient(s) content is within the FAO limits.
Methodology used for Characterization / Recertification	LC, chiral LC, oscillating density meter
Physical Analysis	
- **Appearance**	**brown liquid**
- **Density***	**1150 kg/m^3**
Stability:	
- **Storage Temperature**	**< 30°C**
- **Recertification Date**	**End of January 2023**

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: CHMU200180
Study number(s) of batch recertification:

Authorization: 19-Feb-2020

Dr. Karine Heintz
Analytical Development & Product Chemistry

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APPENDIX 4 Structured Study Summary

Structured Study Summary Table

Test substance design codes	Isocycloseram/Emamectin Benzoate SC (A23220A)
Test substance batch code	TSC002-041-001
Test substance purity (% w/w)	Active ingredient content of Isocycloseram/Emamectin Benzoate SC (A23220A), 100% w/w isocycloseram 17.5% w/w corresponding to 201 g/L emamectin benzoate 4.18% w/w corresponding to 48.1 g/L
Study number	20/080-001P
Study type	MAMMALIAN ACUTE ORAL
Lab Reference	Charles River Laboratories Hungary Kft.
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
Dosing approach	undiluted
LD_{50} - Male	
LD_{50} - Female	Estimated LD_{50} is 1049 mg/kg bw, 95% confidence interval between 550 and 2000 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	2	0	decreased activity (slight to extreme), hunched back, coloured (red) discharge (on nose), liquid faeces, heightened startle response, increased salivation (moderate), recumbency, decreased respiratory rate (moderate), intermittent and continuous tremors (whole body), piloerection, prostration, splayed gait, red discharge (on both eyes, nose and snout),vocalization
Female	550	3	3	heightened startle response, hunched back, piloerection, splayed gait and intermittent tremors (whole body)

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

Remark: Translation of the Stamp on the official document ("Országos Gyógyszerészeti és Élelmezésegészségügyi Intézet"): ("National Institute of Pharmacy and Nutrition").
The legal name of Citoxlab Hungary Ltd. (formerly shown as CiToxLAB Hungary Ltd.) was changed on 28 December 2019 to Charles River Laboratories Hungary Kft."