Sedaxane/Fludioxonil/Imidacloprid

Sedaxane/Fludioxonil/Imidacloprid FS (A20183B) - Acute Oral Toxicity Study in Rat (Up and Down Procedure)

Final Report

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

AUTHOR(S): András Mátyás, M.Sc.

STUDY COMPLETION DATE: 01 April 2015

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

LABORATORY PROJECT ID: Report Number: 14/414-001P
Study Number: 14/414-001P
Task Number: TK0146594

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: 01 April 2015

András Mátyás, M.Sc.
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: 14/414-001P

Study Title: Sedaxane/Fludioxonil/Imidacloprid FS (A20183B) - Acute Oral Toxicity Study in Rat

Test Item: sedaxane/fludioxonil/imidacloprid FS (A20183B)

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
20 November 2014	Study Plan	20 November 2014	20 November 2014
25 November 2014	Treatment	25 November 2014	25 November 2014
05 March 2015	Draft Report	05 March 2015	05 March 2015
01 April 2015	Final Report	01 April 2015	01 April 2015

Signature: _____ Date: 01 April 2015

Ágnes Rédl, M.Sc.
On behalf of QA

MANAGEMENT STATEMENT

According to the conditions of the research and development agreement between Syngenta Ltd (as Sponsor) and CiToxLAB Hungary Ltd. (Test Facility) the study titled "Sedaxane/Fludioxonil/Imidacloprid FS (A20183B) - Acute Oral Toxicity Study in Rat" has been performed in compliance with the Principles of Good Laboratory Practice.

Signature: _____ Date: 01 April 2015

Szabolcs Gáty, M.Sc.
Senior Director of Operations

GENERAL INFORMATION

Contributors

The following contributed to this report in the capacities indicated:

Name	Function
András Mátyás, M.Sc.	Study Director
Éva Váliczkó, M.Sc.	Assistant scientist
Katalin Böröczki, M.Sc.	Quality Assurance
Ágnes Rédl, M.Sc.	Quality Assurance
István Pásztor, DVM	Veterinary control
Peter Maslej, DVM, PhD	Director of Pathology Unit
Tamás Mészáros, PhD	Head of Pharmacy
Hannah Hobson, M.Sc.	Syngenta Study Manager

Study dates

Study Initiation Date	20 November 2014
Experimental Starting Date	25 November 2014
Experimental Completion Date	26 December 2014
Reception of Animals	06 November 2014 / 27 November 2014
Acclimatization	At least 5 days
Treatment	25 November 2014 (female no. 964) 27 November 2014 (female no. 965) 01 December 2014 (female no. 966) 03 December 2014 (female no. 967) 05 December 2014 (female no. 968) 08 December 2014 (female no. 980) 10 December 2014 (female no. 1173) 12 December 2014 (female no. 1174) 16 December 2014 (female no. 1175)
Observation	25 November – 09 December 2014 (female no. 964) 27 November 2014 (female no. 965) 01 December - 15 December 2014 (female no. 966) 03 December 2014 (female no. 967) 05 December – 19 December 2014 (female no. 968) 08 December – 22 December 2014 (female no. 980) 10 December 2014 (female no. 1173) 12 December – 26 December 2014 (female no. 1174) 16 December 2014 (female no. 1175)

Performing laboratory test substance reference number

1402A3

Deviations from the Study Plan

In addition to the items listed in the Study Plan, certified wood chips „GRADE 5" produced by Johannes Brandenburg GmbH & Co. KG, Arkeburger Str. 31, D-49424 Goldenstedt was used as bedding, and RELEASE® 300 mg/mL sodium-pentobarbital solution was used for the purpose of euthanasia, since the items listed in the Study Plan ran out during the study.

Due to the miscalculation of the dose volume for the first animal, the female rat was dosed (648 mg/kg bw) slightly above the target dose level (550 mg/kg bw). Since there were no differences in clinical signs, body weights or necropsy findings comparing to the other animals at the dose level of 550 mg/kg bw, this rat was also regarded as dosed at 550 mg/kg bw, especially for LD_{50} calculation.

The experiment was completed later than indicated in the Study Plan.

The LD_{50} calculation was performed by SPSS/PC+ Statistical Program instead of AOT425StatPgm program since the AOT425StatPgm program was over-ruled by the Study Director in agreement with the Sponsor due animal ethical reason.

The Draft Report was issued later than stated in the Study Plan.

These deviations are presumed to have no effect on the outcome or integrity of the study.

Other

The study documents and sample(s):
- 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 SOP's in the archives of CiToxLAB Hungary Ltd. 8200 Veszprém, Szabadságpuszta, Hungary. This is 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

An acute oral toxicity study was conducted with 9 animals (female Crl:WI rats). Four animals were treated at the dose level of 550 mg/kg bw and five animals were dosed at the dose level of 2000 mg/kg bw. Animals were treated with a single oral (gavage) dose of sedaxane/fludioxonil/imidacloprid FS (A20183B), followed by a 14-day observation period. The animals were fasted overnight prior to treatment and food was returned 3 hours after dosing.

All animals were observed individually after dosing at 30 minutes, 1, 2, 3, 4 and 6 hours post treatment and once each day for 14 days thereafter (or at the time points listed above, until the time point closest to the death of the animal). Body weight was measured on Day -1 (prior to removal of food), Day 0 (prior to administration), and weekly thereafter. All animals were examined macroscopically at the end of the observation period.

1.2 Results

Administration of sedaxane/fludioxonil/imidacloprid FS (A20183B) at the dose level of 2000 mg/kg bw caused death in 4/5 rats on Day 0 or Day 1. No mortality was noted at the dose level of 550 mg/kg bw.

Administration of the test item at the dose level of 550 mg/kg bw did not cause any test item related effect.

Administration of the test item at the dose level of 2000 mg/kg bw caused decreased activity (slight to severe) in 5/5 animals, intermittent tremors in 4/5 animals, hunched back position in 2/5 animals, prone position in 3/5 rats, and piloerection in 1/5 rat. One animal was cold when touched before its death. The single surviving animal at 2000 mg/kg bw was symptom free from Day 2.

There were no treatment-related changes in the body weights in the surviving animals. The body weights of these animals were within the range commonly recorded for this strain and age.

At necropsy, diffuse, dark red discoloration in all lobes of the collapsed lungs and red liquid material mixed with diet was noted in the gastrointestinal tract were noted in the found dead animals at the dose level of 2000 mg/kg bw. No observations were recorded in all surviving animals at 550 mg/kg bw or 2000 mg/kg bw.

1.3 Conclusion

Under the conditions of this study, the acute oral median lethal dose (LD_{50}) of the test item, sedaxane/fludioxonil/imidacloprid FS (A20183B) in Crl: WI female rats was 1596 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 sedaxane/fludioxonil/imidacloprid FS (A20183B) according to OECD 425 guideline.

This study was being 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 for the Testing of Chemicals, Section 4, *Test No.* 425 Acute Oral Toxicity: Up-and-Down Procedure. OECD Publishing, 2008.

- 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:	sedaxane/fludioxonil/imidacloprid FS (A20183B)
Synonyms:	SYN524464/CGA173506/imidacloprid FS (050/025/350) sedaxane/fludioxonil/imidacloprid FS (050/025/350)
Product code:	A20183B
Batch number:	SMU4EP001
Appearance:	Red liquid
Density:	1205 kg/m^3
Purity[1]:	sedaxane – 4.17 % (w/w) corresponding to 50.2 g/L fludioxonil – 2.05 % (w/w) corresponding to 24.7 g/L imidacloprid – 28.9 % (w/w) corresponding to 348 g/L
Recertification date:	End of July 2017
Storage conditions:	Room temperature (<30^{o}C)
Safety precautions:	Routine safety precautions (gloves, goggles, face mask, lab coat) for unknown materials were applied to assure personnel health and safety.

The Certificate of Analysis is shown in Appendix 2.

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 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 Department of CiToxLAB Hungary Ltd. on the basis of the information provided by Sponsor.

3.1.2 Formulation

The test item was administered undiluted.

[1] = *No correction for purity of the test item was applied.*

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, 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:	9 animals
Sex:	Female rats, nulliparous and non-pregnant.
Age when treated:	Young adult rats, between 9 and 11 weeks old.
Body weight at dosing:	205 – 244 g The weight variation in animals involved in the study did not exceed ± 20 % of the mean weight.
Identification:	Animals were individually identified by numbers written on the tail with an indelible pen. The numbers were given on the basis of CiToxLAB Hungary Ltd.'s master file, for each animal allocated to the study. The housing boxes were identified by cards holding information on the study code, the sex of animals, the dose group, the cage number and the individual animal number.
Randomisation:	Animals were selected by hand at time of delivery. No computer generated randomization program.
Acclimatisation time:	At least 5 days under laboratory conditions.

3.2.2 Husbandry

Animal health:	Only healthy animals were used for the test. The health status was certified by the veterinarian
Room number:	245/9
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 or Grade 5 bedding for laboratory animals was available to animals during the study
Light:	12 hours daily, from 6:00 a.m. to 6:00 p.m.
Temperature:	19.0 – 25.0 °C
Relative humidity:	31 – 60 %
Ventilation:	15-20 air exchanges/hour

The temperature and relative humidity was recorded twice daily during the study and the acclimation period.

3.2.3 Food and water supply

The 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 numbers of the lots used in the study were: 190 1786, expiry date: January 2015, and 680 2237, expiry date: March 2015. The food is considered not to contain any contaminants that could reasonably be expected to affect the purpose or integrity of the study. A detailed description of the contents of the lot used is archived with the raw data at CiToxLAB Hungary Ltd.

3.2.4 Water supply and quality control

The animals received tap water, fit for human consumption, *ad libitum*, from the automatic system supplied by the communal water network. The water was considered not to contain any contaminants that could reasonably be expected to affect the purpose or integrity of the study.

The 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.u.36., Hungary). Copies of the relevant Certificates of Analysis are retained in the archives at CiToxLAB Hungary Ltd.

3.3 Administration of the Test Item

3.3.1 Doses

Justification of the doses:

An acute oral toxicity (up and down procedure) study was conducted with 9 animals (female Crl:WI rats). The starting dose was 550 mg/kg bw. Animals were treated with a single oral (gavage) dose of sedaxane/fludioxonil/imidacloprid FS (A20183B) at dose levels of 550 or 2000 mg/kg bw. The density of the test item was 1205 kg/m^3 and the applied dose volume was calculated based on the actual body weights.

The individual dose volumes used are shown in the following table.

Animal Number	Dosage [mg/kg body weight]	Dose volume [mL/animals]	Body weight at dosing [g]	Viability/Mortality
964	550 (648*)	0.12	223	survived
965	2000	0.37	223	died
966	550	0.10	215	survived
967	2000	0.37	221	died
968	550	0.09	205	survived
980	2000	0.37	225	survived
1173	2000	0.36	216	died
1174	550	0.10	212	survived
1175	2000	0.41	244	died

*: for details see Deviations from the Study Plan

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) administration 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 food was returned 3 hours after treatment.

Initially one animal was dosed at the dose level of 550 mg/kg bw according to the Sponsor's request. The further dose level selection was made by use of the AOT425StatPgm program (as per the OECD guideline) Animals were dosed sequentially following an interval of at least 48 hours. The time intervals between dosing were determined by the onset, duration and severity of toxic signs.

3.4 Observations

3.4.1 Clinical observations

All animals were observed individually after dosing at 30 minutes, 1, 2, 3, 4 and 6 hours post treatment and once each day for 14 days thereafter (or at the time points listed above, until the time point closest to the death of the animal). 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 behavior pattern.

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

3.4.2 Body weight measurement

The body weights of the animals were recorded on Day -1 and Days 0 (prior to administration), 7, and 14. Found dead animal was measured on Day -1 and Day 0.

3.5 *Post Mortem* Investigations

All animals were subjected to macroscopic examination, following exsanguination under pentobarbital anaesthesia (where applicable, details in 3.5.1). 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.

3.5.1 Materials used for euthanasia

Name:	Euthasol® 40 %
Lot No.:	13E22 9
Expiry Date:	April 2016
Produced by:	Produlab Pharma B.V., The Netherlands

Name:	RELEASE® 300 mg/mL sodium-pentobarbital solution
Batch No.:	085093
Expiry Date:	September 2016
Produced by:	Wirtschaftsgenossenschaft deutscher Tierarzte eG Siemensstr. 14 30827 Garbsen, Germany

3.6 Data Evaluation

Type, severity and duration of clinical observations are described. Body weight and body weight changes are summarized in tabular form. Necropsy findings are described and summarized in tabular form.

As the AOT425StatPgm program could not estimate an exact LD_{50} in this case, due to the pattern of mortality. In line with the OECD guideline, an alternative calculation method was used. Probit analysis was performed, with the SPSS/PC+ 4.0. This is the standard validated statistical software package used at this facility for Probit analysis on acute studies for LD_{50}.

4.0 RESULTS AND DISCUSSION

4.1 Mortality

Administration of sedaxane/fludioxonil/imidacloprid FS (A20183B) at the dose level of 2000 mg/kg bw caused death in 4/5 rats on Day 0 or Day 1. No mortality was noted at the dose level of 550 mg/kg bw.

4.2 Clinical Signs

Administration of the test item at the dose level of 550 mg/kg bw did not cause any test item related effect.

Administration of the test item at the dose level of 2000 mg/kg bw caused decreased activity (slight to severe) in 5/5 animals, intermittent tremors in 4/5 animals, hunched back position in 2/5 animals, prone position in 3/5 rats, and piloerection in 1/5 rat. One animal was cold when touched before its death. The single surviving animal at 2000 mg/kg bw was symptom free from Day 2.

4.3 Body Weights

There were no treatment-related changes in the body weights in the surviving animals. The body weights of these animals were within the range commonly recorded for this strain and age.

4.4 Macroscopic Findings

At necropsy, diffuse, dark red discoloration in all lobes of the collapsed lungs and red liquid material mixed with diet was noted in the gastrointestinal tract were noted in the found dead animals at the dose level of 2000 mg/kg bw. No observations were recorded in all surviving animals at 550 mg/kg bw or 2000 mg/kg bw.

5.0 CONCLUSIONS

Under the conditions of this study, the acute oral median lethal dose (LD_{50}) of the test item, sedaxane/fludioxonil/imidacloprid FS (A20183B) in Crl: WI female rats was 1596 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**	**7-14**	
			30'	**1h**	**2h**	**3h**	**4h**	**6h**								
1	964	Symptom free	+	+	+	+	+	+	+	+	+	+	+	+	+	20/20
3	966	Symptom free	+	+	+	+	+	+	+	+	+	+	+	+	+	20/20
5	968	Symptom free	+	+	+	+	+	+	+	+	+	+	+	+	+	20/20
8	1174	Symptom free	+	+	+	+	+	+	+	+	+	+	+	+	+	20/20

Remarks: + = present

h = hour (s) ' = minute

Frequency of observation = number of occurrence of observation / total number of observations
Severities: 1 = Slight/Small/Few; 2 = Moderate/Medium; 3 = Marked/Large/Many

TABLE 1 Individual Findings – Clinical Signs (continued)

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**								
2	965#	Symptom free	+	+	+	-	-	-								3/6
		Activity decreased	-	-	-	1	2	2								3/6
		Hunched back	-	-	-	+	+	+								3/6
		Tremors (intermittent)	-	-	-	-	-	+								1/6
		Found dead	-	-	-	-	-	-	+							-
4	967#	Symptom free	+	-	-	-	-									1/5
		Activity decreased	-	1	2	2	3									4/5
		Prone position	-	-	+	+	+									3/5
		Tremors (intermittent)	-	-	-	+	+									2/5
		Cold to touch	-	-	-	-	+									1/5
		Found dead	-	-	-	-	-	+								-
6	980	Symptom free	+	+	+	+	-	-	-	+	+	+	+	+	+	17/20
		Activity decreased	-	-	-	-	1	2	1	-	-	-	-	-	-	3/20
		Hunched back	-	-	-	-	+	+	+	-	-	-	-	-	-	3/20
		Piloerection	-	-	-	-	-	+	-	-	-	-	-	-	-	1/20

Remarks: + = present - = absent

h = hour (s) ' = minute

= Found dead

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

Severities: 1 = Slight/Small/Few; 2 = Moderate/Medium; 3 = Marked/Large/Many

TABLE 1 Individual Findings – Clinical Signs (continued)

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**								
7	1173#	Symptom free	+	-	-	-	-									1/5
		Activity decreased	-	1	3	3	3									4/5
		Prone position	-	+	+	+	+									4/5
		Tremors (intermittent)	-	-	-	+	+									2/5
		Found dead	-	-	-	-	-	+								-
9	1175#	Symptom free	+	+	-	-	-									2/5
		Activity decreased	-	-	1	2	2									3/5
		Prone position	-	-	+	+	+									3/5
		Tremors (intermittent)	-	-	-	+	+									2/5
		Found dead	-	-	-	-	-	+								-

Remarks: + = present - = absent

h = hour (s) ' = minute

= Found dead

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

Severities: 1 = Slight/Small/Few; 2 = Moderate/Medium; 3 = Marked/Large/Many

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)**				**Day/Body Weight (g) at Death**	**Body Weight Gain (g)**			
		Days								
		-1	**0**	**7**	**14**		**-1-0**	**0-7**	**7- 14**	**-1 - 14**
1	964	240	223	255	258	-	-17	32	3	18
3	966	235	215	232	249	-	-20	17	17	14
5	968	215	205	210	224	-	-10	5	14	9
8	1174	236	212	251	268	-	-24	39	17	32
Mean:		231.5	213.8	237.0	249.8	-	-17.8	23.3	12.8	18.3
Standard deviation:		11.2	7.5	20.6	18.8	-	5.9	15.2	6.7	9.9

- = No data

TABLE 2 Body Weight and Body Weight Gain (continued)

DOSE LEVEL: 2000 mg/kg bw, Treatment on Day 0 SEX: FEMALE										
Cage No.	**Animal Number**	**Body weight (g)**				**Day/Body Weight (g) at Death**	**Body Weight Gain (g)**			
		Days								
		-1	**0**	**7**	**14**		**-1-0**	**0-7**	**7- 14**	**-1 - 14**
2	965#	238	223	-	-	1/221	-15	-	-	-
4	967#	241	221	-	-	0/221	-20	-	-	-
6	980	236	225	229	245	-	-11	4	16	9
7	1173#	239	216	-	-	0/216	-23	-	-	-
9	1175#	264	244	-	-	0/244	-20	-	-	-
Mean:		243.6	225.8	229.0	245.0	-	-17.8	4.0	16.0	9.0
Standard deviation:		11.5	10.7	-	-	-	4.8	-	-	-

- = No data
= Found dead

TABLE 3 Macroscopic Findings

DOSE LEVEL: 550 mg/kg bw, Treatment on Day 0 SEX: FEMALE					
Cage No.	**Animal Number**	**Necropsy Date/ Necropsy Day**	**External Observations**	**Internal Observations**	**Organ/Tissue**
1	964	09 December 2014 Day 14	No external observations recorded	No internal observations recorded	Not applicable
3	966	15 December 2014 Day 14	No external observations recorded	No internal observations recorded	Not applicable
5	968	19 December 2014 Day 14	No external observations recorded	No internal observations recorded	Not applicable
8	1174	26 December 2014 Day 14	No external observations recorded	No internal observations recorded	Not applicable

TABLE 3 Macroscopic Findings (continued)

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**
2	965#	28 November 2014 Day 1	No external observations recorded	Collapsed	Lungs
				Dark discoloration, red, diffuse, all lobes	
				Digestive Content: Liquid, material, red	Stomach
4	967#	03 December 2014 Day 0	No external observations recorded	Collapsed	Lungs
				Dark discoloration, red, diffuse, all lobes	
				Digestive Content: Liquid, material, red	Stomach and Duodenum and Jejunum
6	980	22 December 2014 Day 14	No external observations recorded	No internal observations recorded	Not applicable
7	1173#	10 December 2014 Day 0	No external observations recorded	Collapsed	Lungs
				Dark discoloration, red, diffuse, all lobes	
				Digestive Content: Liquid, material, red	Stomach and Duodenum and Jejunum
9	1175#	16 December 2014 Day 0	No external observations recorded	Collapsed	Lungs
				Dark discoloration, red, diffuse, all lobes	
				Digestive Content: Liquid, material, red	Stomach and Duodenum and Jejunum

= Found dead

APPENDICES SECTION

APPENDIX 1 Pathology Report

INTRODUCTION

The objective of the study was to assess the acute oral toxicity of the test item sedaxane/fludioxonil/imidacloprid FS (A20183B) when administered in a single dose to rats. Five female rats were dosed at 2000 mg/kg bw and 4/5 animals died at this dose level. Four rats were dosed at dose level of 550 mg/kg bw. All of these animals survived.

RESULTS AND DISCUSSION

Surviving animal was euthanized upon completion of the observation period on Day 14. The rat was 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

Four of the five rats dosed at 2000 mg/kg bw were found dead on Day 0 or Day 1.

FOUND DEAD

Macroscopic Findings

In all of the found dead rats, red liquid material was observed in the digestive content and was considered to be associated with administration of the test item. In the collapsed lungs, diffuse, dark red discoloration was recorded at the necropsy and was regarded as agonal.

TERMINAL (DAY 14)

Macroscopic Findings

There was no evidence of any gross findings in the 1/5 surviving rat, dosed at 2000 mg/kg bw and in the 4/4 surviving rats at a dose level of 550 mg/kg bw on Day 14.

CONCLUSION

A single oral gavage of sedaxane/fludioxonil/imidacloprid FS (A20183B) to Crl:WI female rats at a dose level of 2000 mg/kg bw led to the death in 4/5 of the experimental rats. The red, liquid material observed in the digestive content were considered to be associated with administration of the test item, other changes such as diffuse, dark red discoloration of the collapsed lungs were regarded as agonal.

In all surviving rats, dosed at 550 or 2000 mg/kg bw and terminated on Day 14, no changes were observed at necropsy.

Gábor Boros, D.V.M.
Histopathologist

Date 25. March 2015.

APPENDIX 2 Certificate of Analysis

GLP Testing Facility WMU
Analytical Development &
Product Chemistry GS2131

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

Certificate of Analysis

A20183B
sedaxane/fludioxonil/imidacloprid FS
(050/025/350)
SMU4EP001

Batch Identification	**SMU4EP001**
Product Code	**A20183B**
Other Product Code	SYN524464/CGA173506/imidacloprid FS (050/025/350)

Chemical Analysis
(Active Ingredient Content)

– **Identity of the Active Ingredients***	**confirmed**
– **Content of sedaxane***	**4.17 % w/w corresponding to 50.2 g/l**
– **Content of fludioxonil***	**2.05 % w/w corresponding to 24.7 g/l**
– **Content of imidacloprid***	**28.9 % w/w corresponding to 348 g/l**

The Active Ingredients content is within the FAO limits.

Methodology used for Characterization / Recertification	HPLC, oscillating density meter

Physical Analysis

– **Appearance**	**red liquid**
– **Density***	**1205 kg/m^3**

Stability:

– **Storage Temperature**	**< 30°C**
– **Recertification Date**	**End of July 2017**

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:	CHMU140431
Study number(s) of batch recertification:	---

Authorization: 31 July 2014

Daniel Jenniches
Analytical Development & Product Chemistry

APPENDIX 3 GLP Certificate

GYEMSZI
National Institute for Quality- and Organizational Development in Healthcare and Medicines

National Institute of Pharmacy

Zrínyi utca 3., H-1051 Budapest
H-1372 Budapest, PO Box 450.
Tel.: +36 1 888 9300
Fax: +36 1 888 9460
E-mail: ogyi@ogyi.hu
Web: www.ogyi.hu

Ref. no: OGYI/38593-5/2012
Admin.: Urbin Magdolna Zita
Date: 3 December, 2012

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, 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: **8-11. October 2012.**

Dr. Kőszeginé Dr. Szalai Hilda
Deputy Director-General