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Profenofos

Profenofos EC (A8591B) – Acute Oral Toxicity Study in Rats (Up and Down Procedure)

Final Report

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

AUTHOR(S): Balázs Mráz, M.Sc.

COMPLETION DATE: 08 December 2021

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

LABORATORY PROJECT ID: Report Number: 21/201-001P
Study Number: 21/201-001P
Task Number: TK0612492

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: 08 December 2021

Balázs Mráz, M.Sc.
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: 21/201-001P

Study Title: Profenofos EC (A8591B) - Acute Oral Toxicity Study in Rats (Up and Down Procedure)

Test Item: Profenofos EC (A8591B)

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
27 September 2021	Study Plan	27 September 2021	27 September 2021
30 September 2021	Treatment	30 September 2021	30 September 2021
19 November 2021	Draft Report	19 November 2021	19 November 2021
07 December 2021	Final Report	07 December 2021	07 December 2021

Signature: Nyitrai Ildikó Date: 08 December 2021

Ildikó Nyitrai, M.Sc.
On behalf of QA

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 "Profenofos EC (A8591B) - Acute Oral Toxicity Study in Rats (Up and Down Procedure)" has been performed in compliance with the Principles of Good Laboratory Practice.

Signature: Balázs Tóth Date: 09 December 2021

Balázs Tóth, Ph.D.
General Manager

GENERAL INFORMATION

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

Name	Function or Department
Balázs Mráz, M.Sc.	Study Director
Nikoletta Szalóki, Ph.D.	Assistant Scientist
Ildikó Nyitrai, M.Sc.	Quality Assurance
László Székelyhidi, D.V.M.	Veterinary Care
Tamás Mészáros, Ph.D.	Pharmacy
Ferenc Szűcs	Animal Service Laboratories
Carolina Vaccari, M.Sc.	Syngenta Study Manager

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

Study dates

Study Initiation Date	27 September 2021
Experimental Starting Date	30 September 2021
Experimental Completion Date	28 October 2021
Draft Report	19 November 2021
Final Report	08 December 2021
Receipt of Animals	09 September 2021, 14 October 2021
Treatment	30 September 2021 (female no. 1218) 05 October 2021 (female no. 1219) 08 October 2021 (female no. 1220) 12 October 2021 (female no. 1221) 14 October 2021 (female no. 1225) 19 October 2021 (female no. 1447)
Observation	30 September - 14 October 2021 (female no. 1218) 05 October - 06 October 2021 (female no. 1219) 08 October - 22 October 2021 (female no. 1220) 12 October - 13 October 2021 (female no. 1221) 14 October - 28 October 2021 (female no. 1225) 19 October 2021 (female no. 1447)
Necropsy	14 October 2021 (female no. 1218) 06 October 2021 (female no. 1219) 22 October 2021 (female no. 1220) 13 October 2021 (female no. 1221) 28 October 2021 (female no. 1225) 19 October 2021 (female no. 1447)

Deviation from the guideline and Study Plan
Due to technical error, the relative humidity values (minimum of 25.0%) outside the expected range of 30-70% were recorded occasionally in the animal rooms during the study. These deviations have no effect on the outcome of the study.

Performing laboratory test substance reference number
210495

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 hrsz. 028/1., 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, 6 female Crl:WI rats were given a single oral (gavage) dose of profenofos EC (A8591B) at dose level of 550 and 2000 mg/kg body weight (bw), followed by a 14 day observation period. 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 550 mg/kg bw. The dose selection for the next animals followed the recommendation of AOT425StatPgm software, based on available results.

Animals were observed individually at 30 minutes, 40 minutes (only one animal), 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 (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.

1.2 Results

No mortality was observed at the dose level of 550 mg/kg bw during the study.
Two females given 2000 mg/kg bw were preterminal euthanised on Day 1 of the experiment due to animal welfare reasons. One animal was found dead 4 hours after dosing.

In the 550 mg/kg bw dose group, one animal showed hunched back (1/3 animals) from Day 0 to Day 2. The animal was symptom-free from Day 3. The two remaining animals were symptom-free during the study.

Slight to extreme decreased activity (3/3), hunched back (3/3), slight ataxia (1/3), piloerection (2/3), red discharge from both eyes (1/3), decreased respiratory rate (2/3), continuous tremors on whole body (3/3), liquid faeces (1/3), gasping respiration (1/3), splayed gait (1/3), recumbency (2/3), moderate salivation (1/3), noisy respiration (1/3), prostration (1/3) and rooting of bedding (1/3) was observed on 2000 mg/kg bw dose group from Day 0 to termination (Day 1).

There was no test item related effect on body weight or body weight gain at 550 mg/kg bw. Body weights were within the range commonly recorded for this strain and age.

A single oral gavage of profenofos EC (A8591B) to Crl:WI female rats at dose level of 550 mg/kg bw was not associated with any gross observations at necropsy.

At 2000 mg/kg bw, mortality of all three females (3/3) occurred on the first day; necropsy revealed macroscopic changes in the stomach, indicating local irritation (3/3), and red discoloured eyes (1/3).

1.3 Conclusion

Under the conditions of this study, based on the outcome of AOT425StatPgm program, the estimated acute oral median lethal dose (LD50) of the test item profenofos EC (A8591B) 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 profenofos EC (A8591B) 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 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:	Profenofos EC (A8591B)
Other name:	CGA15324 EC (960)
Batch number:	RAN001-099-019
Design code:	A8591B
Active ingredient content*:	Profofenos content: 73.12% w/w corresponding to 969.3 g/L;
Density:	1.3253 g/cm³
Appearance:	Light yellow liquid
Recertification date:	06 May 2023
Storage conditions:	Room temperature (15-25°C, ≤70% relative humidity)
Safety precautions:	Enhanced safety precautions (nitrile gloves, goggles, face mask (ABEK-P3-filter), lab coat.

**Note: No adjustment for active ingredient content was applied.*

The Certificate of Analysis is presented in Appendix 2.

3.1.1 Identification and receipt

The test item of a suitable active ingredient content together with all precautions required in the handling and disposal of the test item were supplied by the Sponsor. The identification of the test item was made in the Pharmacy of Charles River Laboratories Hungary Kft. on the basis of the information provided by the Sponsor.

3.1.2 Formulation

The test item was undiluted and used as supplied by the Sponsor.

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:	6 (1 animal/step)
Sex:	Female rats, nulliparous and non-pregnant
Age when treated:	Young adult rats, 8-10-11-12 weeks old
Body weight (at dosing):	212 – 233 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 5 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. Additional enrichment (hiding tunnels) were also used during the study.
Bedding / Nesting:	SAFE 3/4 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:	21.7 - 24.6°C
Relative humidity:	25 - 66%
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" (Batch no.: 187 76795 Expiry date: 31 October 2021) produced by ssniff Spezialdiäten GmbH, *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. 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 at the request of the Sponsor.

No mortality was observed at the dose level of 550 mg/kg bw during the study. At the dose level of 2000 mg/kg bw, two out of three animals had preterminal euthanasia due to animal welfare reasons on Day 1, and one out of three animals was found dead on Day 0.

The animals were treated with a single oral (gavage) dose of profenofos EC (A8591B) at the dose levels of 550 mg/kg bw and 2000 mg/kg bw. The individual dose volumes used are shown below.

Animal Number	Dose [mg/kg body weight]	Volume Dosed [mL]	Bodyweight [g]	Mortality
1218	550	0.10	228	Survived
1219	2000	0.35	233	Preterminal
1220	550	0.09	226	Survived
1221	2000	0.33	219	Preterminal
1225	550	0.10	233	Survived
1447	2000	0.32	212	Found dead

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 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, 40 minutes (only one animal) 1, 2, 3, 4 and 6 hours after dosing, then once each day for 14 days. 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 weight was also measured before preterminal euthanasia, when it was necessary.

3.5 *Post Mortem* Investigations

All animals were subjected to gross macroscopic evaluation. All 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.

3.5.1 Material used for euthanasia

Name:	Euthanimal 40% (sodium pentobarbital)
Lot No.:	2001004-06
Expiry Date:	31 January 2023
Produced by:	Alfasan Nederland BV, Kuipersweg 9, Woerden, The Netherlands

3.6 Data Evaluation

Type, severity, and duration of clinical observations are described in the tables and results of this report. Body weight and body weight changes are summarised in tabular form. Necropsy findings are described and summarised in tabular form.

Data were recorded on the appropriate forms from the relevant SOPs of Charles River Laboratories Hungary Kft., and then tabulated using the Microsoft Office Word and/or Excel or collected using the software PROVANTIS v.9.

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.
At the dose level of 2000 mg/kg bw, two out of three animals had preterminal euthanasia due to animal welfare reasons on Day 1, and one out of three animals was found dead on Day 0.

4.2 Clinical Signs

In the 550 mg/kg bw dose group, one animal showed hunched back (1/3) from Day 0 to Day 2. The animal was symptom-free from Day 3. The two remaining animals were symptom-free during the study.

Slight to extreme decreased activity (3/3), hunched back (3/3), slight ataxia (1/3), piloerection (2/3), red discharge from eyes (1/3), decreased respiratory rate (2/3), continuous tremors on whole body (3/3), liquid faeces (1/3), gasping respiration (1/3), splayed gait (1/3), recumbency (2/3), moderate salivation (1/3), noisy respiration (1/3), prostration (1/3) and rooting of bedding (1/3) was observed on 2000 mg/kg bw dose group from Day 0 to Day 1.

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

4.3 Body Weights

There was no test item related effect on body weight or body weight gain at 550 mg/kg bw. Body weights were within the range commonly recorded for this strain and age.

Individual body weights are presented in Table 2.

4.4 Macroscopic Findings

A single oral gavage of profenofos EC (A8591B) to Crl:WI female rats at dose level of 550 mg/kg bw was not associated with any gross observations at necropsy.

At 2000 mg/kg bw, dark red focal discoloration in the stomach glandular mucosa was observed at necropsy in case of all the animals, indicating local irritation. In case of one animal, red diffuse discoloration of the eyes was recorded.

Macroscopic findings are presented in Table 3.

5.0 CONCLUSIONS

Under the conditions of this study, based on the outcome of AOT425StatPgm program, the estimated acute oral median lethal dose (LD50) of the test item profenofos EC (A8591B) 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	0							1	2	3	4	5	6	7	8	9	10	11	12	13	14	Frequency
			30'	40'	1h	2h	3h	4h	6h															
1	1218	Symptom Free	+	/	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	20/20
3	1220	Symptom Free	+	/	+	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	16/20
		Hunched back	-	/	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	4/20
5	1225	Symptom Free	+	/	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	20/20

Standard Footnotes:

+ = present
- = absent
h = hour (s)
" = minute
= Found dead
'M = Moribund
PE = Preterminal Euthanasia
Frequency of observation = number of occurrence of observation / total number of observations
Comment Present: Faeces coloured - yellowish white

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	0							1	2	3	4	5	6	7	8	9	10	11	12	13	14	Frequency
			30'	40'	1h	2h	3h	4h	6h															
2	1219PE	Symptom Free	+	/	-	-	-	-	-	-														1/7
		Activity decreased	-	/	-	-	-	-	-	Mo														1/7
		Hunched back	-	/	+	+	+	+	+	+														6/7
		Piloerection	-	/	-	-	-	-	-	+														1/7
		Red discharge - Both Eyes	-	/	-	-	-	-	-	+														1/7
		Respiratory rate decreased	-	/	-	-	-	-	-	Sl														1/7
		Tremors Continous - Whole body	-	/	-	-	-	-	-	+														1/7
		Preterminal Euthanasia	-	/	-	-	-	-	-	+														-
4	1221PE	Activity decreased	Sl	/	Mo	Ex	Ex	Ex	Ex	Ex														7/7
		Ataxia	Sl	/	Sl	-	-	-	-	-														2/7
		Faeces liquid	-	/	-	-	-	-	+	-														1/7
		Gasping respiration	-	/	-	-	-	-	+	+														2/7
		Hunched back	+	/	+	+	+	+	+	+														7/7
		Piloerection	-	/	-	-	-	-	+	+														2/7
		Recumbency	-	/	-	+	+	+	+	+														5/7
		Splayed gait	-	/	-	-	-	-	-	+														1/7
		Tremors Continous - Whole body	-	/	+	+	+	+	-	-														4/7
		Preterminal Euthanasia	-	/	-	-	-	-	-	+														-

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

Cage No.	Animal Number	Observations	0							1	2	3	4	5	6	7	8	9	10	11	12	13	14	Frequency
			30'	40'	1h	2h	3h	4h	6h															
6	1447#	Activity decreased	-	-	Mo	Ex	Ex																	3/5
		Hunched back	-	-	+	+	-																	2/5
		Increased Salivation	Mo	Mo	Mo	-	-																	3/5
		Noisy respiration	-	-	-	-	Sl																	1/5
		Prostration	-	+	+	+	+																	4/5
		Recumbency	-	+	+	+	+																	4/5
		Respiratory rate decreased	-	-	-	-	Mo																	1/5
		Rooting of bedding	+	+	-	-	-																	2/5
		Tremors Continous - Whole body	-	-	+	+	+																	3/5
		Found dead	-	-	-	-	-	+																-

Standard Footnotes:

+ = present
- = absent
h = hour (s)
" = minute
= Found dead
'M = Moribund
PE = Preterminal Euthanasia
Frequency of observation = number of occurrence of observation / total number of observations
Comment Present: Faeces coloured - yellowish white

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/B.W. (g) Death	Body Weight Gain (g)		
		-1	0	7	14		0-7	7- 14	0 - 14
1	1218	243	228	239	257	-	11	18	29
3	1220	241	226	244	267	-	18	23	41
5	1225	245	233	246	259	-	13	13	26
Mean:		243.0	229.0	243.0	261.0	-	14.0	18.0	32.0
Standard deviation:		2.0	3.6	3.6	5.3	-	3.6	5.0	7.9

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

Cage No.	Animal Number	Body weight (g) Days				Day/B.W. (g) Death	Body Weight Gain (g)		
		-1	0	7	14		0-7	7- 14	0 - 14
2	1219PE	251	233	-	-	1/224	-	-	-
4	1221PE	229	219	-	-	1/188	-	-	-
6	1447#	228	212	-	-	0/212	-	-	-
Mean:		236.0	221.3	-	-	-	-	-	-
Standard deviation:		13.0	10.7	-	-	-	-	-	-

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	1218	Day 14	No external observations recorded	No internal observations recorded	Not applicable
3	1220	Day 14	No external observations recorded	No internal observations recorded	Not applicable
5	1225	Day 14	No external observations recorded	No internal observations recorded	Not applicable

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

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	1219PE	Day 1	No external observations recorded	Discoloration; red, diffuse, bilateral	Eye
				Discoloration; dark red, focal, mucosa; glandular	Stomach
4	1221PE	Day 1	No external observations recorded	Discoloration; dark red, focal, mucosa; glandular	Stomach
6	1447#	Day 0	No external observations recorded	Discoloration; dark red, focal, mucosa; glandular	Stomach

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

APPENDICES SECTION

APPENDIX 1 AOT 425 Report (Main Test)

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

Date/Time: 2021. november 3., szerda, 8:57:18
Data file name: 21201001P.dat
Last modified: 2021. 11. 03. 8:57:14

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	1218	550	O	O
2	1219	2000	X	X
3	1220	550	O	O
4	1221	2000	X	X
5	1225	550	O	O
6	1447	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 2 Certificate of Analysis

ALS Laboratórios LS Ltda.
Rua Fábia, 59 – CEP: 05051-030
São Paulo, SP - Brazil

SYNGENTA PROTEÇÃO DE CULTIVOS Ltda.
Rua Doutor Rubens Gomes Bueno nº 691,
11º andar, Torre Sigma
CEP 04730-000 – Bairro Várzea de Baixo
São Paulo-SP – Brazil

Certificate of Analysis

A8591B
Profenofos EC (960)
RAN001-099-019

Batch Identification	RAN001-099-019
Product Code	A8591B
Other Product Code(s)	A8591; CGA15324 EC (960); EXF23490E
EUP number	514/2020 Expiry date: 26/02/2023
Received on	12 May 2021
Source	Syngenta Proteção de Cultivos Ltda. Rodovia Professor Zeferino Vaz, SP 332, s/nº, km 127,5 – Bairro Santa Terezinha, CEP 13148-915 – Paulínia – SP – Brasil

Chemical Analysis
(Active Ingredients Content)

– **Content of Profenofos ***	**73.12 % w/w corresponding to 969.03 g/L**

The Active Ingredient content is within the FAO limits.
Methodology used for Characterization: CG-FID (SF-1135/1)

Physical Analysis

– **Density ***	1.3253 g/cm³

Stability:

– **Storage Temperature**	**<30°C**
– **Recertification Date**	**06 May 2023**

If stored under the conditions given above, this test item 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. All original raw data, including any storage medium for electronically recorded data, documentation, the signed study plan, the protocol amendments, the final report and a sample of the test item will be retained in the GLP Archives at ALS Laboratórios LS Ltda.

Study number of batch characterization: 25926/2021CC

Authorization: 26 May 2021

V. Victor F.G. da Silva
Victor Ferreira Gomes da Silva
ALS Laboratórios LS Ltda.

APPENDIX 3 GLP Certificate

OGYÉI Országos Gyógyszerészeti és Élelmezés-egészségügyi Intézet
Hatósági Ellenőrzési Főosztály

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Levélcím: 1372 Postafiók 450
Tel.: +36 1 886 9300, Fax: +36 1 886 9450
E-mail: ogyei@ogyei.gov.hu
Web: www.ogyei.gov.hu

Ref. no: OGYÉI/-29520-2/2021
Admin.: Dr. Szaller Zoltán

GOOD LABORATORY PRACTICE (GLP) CERTIFICATE

It is hereby certified that the test facility

Charles River Laboratories Hungary Kft.

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.

This certificate is valid up to 11th of May, 2022.

Dr. Lukács Ferenc József
Digitálisan aláírta: Dr. Lukács Ferenc József
Dátum: 2021.05.06 13:04:14 +02'00'

Dr. Ferenc Lukács
Head of Inspectorate

Note: Translation of the text of the certificate in the header: ("Országos Gyógyszerészeti és Élelmezés-egészségügyi Intézet") - ("National Institute of Pharmacy and Nutrition"); (“Hatósági Ellenőrzési Főosztály”) - (Inspectorate Division) and at the signature: (“Digitálisan aláírta”) - (Digitally signed); (“Dátum”) - (“Date”).