charles river

Profenofos

Profenofos EC (A8591B) – Skin Sensitization Test (Local Lymph Node Assay)

Final Report

TEST GUIDELINE(S):	OECD 429 (2010) EC 640/2012, B.42 (2012)
AUTHOR(S):	Barbara Varga-Kanizsai, M.Sc.
COMPLETION DATE:	31 March 2022
PERFORMING LABORATORY:	Charles River Laboratories Hungary Kft. H-8200 Veszprém, Szabadságpuszta, hrsz. 028/1., Hungary
LABORATORY PROJECT ID:	Report Number: 21/201-037E Study Number: 21/201-037E Task Number: TK0612490
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 and its Amendments 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 etc.) of dosing formulation preparations was checked and revealed no abnormalities of consequences. 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: Varga-Kanizsai Barbara Date: 31 March 2022

Barbara Varga-Kanizsai, 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

This page is intentionally left blank. It will be replaced by an appropriate Flagging statement by the Sponsor.

QUALITY ASSURANCE STATEMENT

Study Number: 21/201-037E

Study Title: Profenofos EC (A8591B) – Skin Sensitization Test (Local Lymph Node Assay)

This Study has been audited by Quality Assurance in accordance with the applicable Good Laboratory Practice regulations. Audit reports were submitted in accordance with SOPs as follows:

Date of Inspection	Phase(s) Inspected/Audited	Date of report to	
		Management	Study Director
15 November 2021	Study Plan	15 November 2021	15 November 2021
05 January 2022	Treatment	05 January 2022	05 January 2022
11 January 2022	Formulation	11 January 2022	11 January 2022
13 January 2022	Clinical observations	13 January 2022	13 January 2022
24 January 2022	Amendment 1 to the Study Plan	24 January 2022	24 January 2022
11 February 2022	Body weight	11 February 2022	11 February 2022
10 March 2022	Draft Report	10 March 2022	10 March 2022
17 March 2022	Necropsy	17 March 2022	17 March 2022
29 March 2022	Final Report	29 March 2022	29 March 2022

In addition to the above-mentioned audits, (which may include study specific inspections and/or relevant process based inspections) routine facility inspections were also conducted.

The Final Report reflects the raw data and accurately and completely describes the methods and procedures of the study.

Signature: Kovács Márk

Márk Kovács, M.Sc.
On behalf of QA

Date: 31 March 2022

STATEMENT OF THE MANAGEMENT

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) – Skin Sensitization Test (Local Lymph Node Assay)" was performed, in compliance with the Principles of Good Laboratory Practice.

Signature: _____ Date: 31 March 2022

Balázs Tóth, Ph.D.
General Manager

GENERAL INFORMATION

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

Name	**Function**
Barbara Varga-Kanizsai, M.Sc.	Study Director
Nikoletta Szalóki, Ph.D.	Assistant Scientist
Balázs Tóth, Ph.D.	General Manager
David J. Esdaile, M.Sc.	Director of Science and Regulatory Affairs
Márk Kovács, M.Sc.	Quality Assurance
László Székelyhidi, D.V.M.	Veterinary Care
Tamás Mészáros, Ph.D.	Pharmacy
Mihály Schmidt	Technical Team Leader
Ferenc Szűcs	Animal Technical and Support Laboratory Operations
Carolina Vaccari, M.Sc.	Syngenta Study Manager

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

Study dates

Study Initiation Date	17 November 2021
Preliminary Experiments (I-IV.):	
Experimental Starting Date	17 November 2021
Experimental Completion Date	13 December 2021
Amendment 1 to the Study Plan	21 January 2022
Amendment 2 to the Study Plan	15 February 2022
Main Experiment I.:	
Experimental Starting Date	26 January 2022
Experimental Completion Date	01 February 2022
Main Experiment II.:	
Experimental Starting Date	17 February 2022
Experimental Completion Date	23 February 2022
Draft Report Date (non-audited)	28 February 2022
Draft Report date (audited)	10 March 2022
Final Report	31 March 2022
Receipt of Animals	28 October 2021 (Preliminary Experiment I., II., III.) / 25 November 2021 (Preliminary Experiment IV.) / 20 January 2022 (Main Experiment I.) / 03 February 2022 (Main Experiment II.)
Acclimatisation	20 days (Preliminary Experiment I.) / 27 days (Preliminary Experiment II.). / 34 days (Preliminary Experiment III.) 13 days (Preliminary Experiment IV.)

	6 days (Main Experiment I.) / 14 days (Main Experiment II.)
Treatment	17-18 November 2021 (Preliminary Experiment I.)* 24-25 November 2021 (Preliminary Experiment II.)* 01-03 December 2021 (Preliminary Experiment III.)* 08-10 December 2021 (Preliminary Experiment IV.) 26-28 January 2022 (Main Experiment I.) 17-19 February 2022 (Main Experiment II.)
Observation	17-18 November 2021 (Preliminary Experiment I.)* 24-25 November 2021 (Preliminary Experiment II.)* 01-03 December 2021 (Preliminary Experiment III.)* 08-13 December 2021 (Preliminary Experiment IV.) 26-31 January 2022 (Main Experiment I.) 17-22 February 2022 (Main Experiment II.)
Necropsy	No necropsy was performed in Preliminary Experiment I.; II. and III.* 13 December 2021 (Preliminary Experiment IV.) 31 January 2022 (Main Experiment I.) 22 February 2022 (Main Experiment II.)

*Note: Animals were found dead on Day 2 or Day 3.

Deviation from the Study Plan
Due to technical reasons, relative humidity values (minimum of 20%) outside the expected range of 30-70% were recorded occasionally during the study. Temperature values (maximum of 25.9°C) outside the expected range of 19-25°C were recorded occasionally during the study.

Due to an administrative error the Amendment 1 to the Study Plan included an incorrect text in the last paragraph on page 2 which stated that 1% (w/v) was considered an acceptable concentration for the main test, however 2.5% (w/v) was selected as highest dose for the main test. The report includes the correct text.

Due to scientific reason the report includes Positive control data of relevant studies from the last 12 months instead of last 6 months as it was indicated in the Study Plan or in the Amendment 1 to the study Plan to ensure that sufficient data are available for reporting purposes.

These deviations were considered not to adversely affect the results or integrity of the study.

Performing laboratory test substance reference number
210495

Other
The study documents and sample:
-study plan and amendments,
-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. 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

The object of this study was to determine the skin sensitisation potential of the test item, profenofos EC (A8591B) following topical application to the dorsal surface of the ear. The study is being performed with vertebrate animals. As the study is not being used for REACH submission or it does not fall under the scope of EU regulations, the *in vivo* testing is acceptable or is required by the regulatory agency concerned.

Based on the recommendations of the OECD No. 429 guideline [1] and in agreement with the Sponsor and preliminary compatibility test, the test item was formulated in 1% aqueous Pluronic® PE9200 (abbreviated as 1% Pluronic). The 100% (undiluted) was the highest concentration which was suitable for the test. The formulations at 50%, 25%, 10%, 2.5%, 1%, 0.5% and 0.25% (w/v) in 1% Pluronic were also suitable for treatment. The 50%, 25%, 10%, 2.5%, 1%, 0.5% and 0.25% (w/v) in 1% Pluronic formulations appeared to be white, homogeneous emulsion by visual examination.

In the Main Experiment II., twenty female CBA/CaOlaHsd mice were allocated five groups of four animals each:
- four groups received profenofos EC (A8591B) at 2.5%, 1%, 0.5% and 0.25%(w/v) (formulated in 1% Pluronic) concentrations respectively,
- the negative control group received the vehicle (1% Pluronic) only.

Note: The Main Experiment I. did not meet the validity due to the tritiated thymidine used. See further details in Section 4.1.

At the Sponsor's request a concurrent positive control group was not treated in this study, instead the evidence that the test performs as expected in this facility, is the positive control data (using 15% (w/v) Formaldehyde solution in 1% Pluronic) from similar LLNA studies carried out within 12 months of this study (Table 20).

The test item solutions were applied on the dorsal surface of ears of experimental animals (25 µL/ear) for three consecutive days (Days 1, 2 and 3). There was no treatment on Days 4, 5 and 6. The cell proliferation in the local lymph nodes was assessed by measuring disintegrations per minutes after incorporation of tritiated methyl thymidine (^{3}HTdR) and the values obtained were used to calculate stimulation indices (SI) in comparison with the control group.

1.2 Results

No mortality was observed during the Main experiment II. No test item residue was observed during the Main experiment II. There was no indication of local irritation at the site of application. Slight activity decreased was observed at 3 out of 4 animals at 2.5% (w/v) dose group on Day 3.

In the high dose group (2.5 % (w/v)) 4 out of 4 animals had more than 5% body weight loss which resulted in a -10.3% or -9.5% group mean on Day 2 or Day 3, and on Day 6, 1 out of 4 animals had more than 5% body weight loss (-11.5%) and a -4.2% group mean.

The observed reduction in body weight values is over the limit given in the relevant OECD guideline used to indicate systemic toxicity, hence the 2.5% (w/v) dose group was excluded from data analysis (although this has no effect on the study interpretation).

The appearance of the lymph nodes was normal in all animals of all dose groups. The appearance of the lymph nodes was normal in all animals of the negative control group.

A table summarising the sensitization results noted is found below:

	Mean DPN	Stimulation Index[1]
Group 1 - vehicle control	186.5	-
Group 2 – 2.5% (w/v) test item	432.9	2.3[Note]
Group 3 - 1% (w/v) test item	340.9	1.8
Group 4 – 0.5% (w/v) test item	260.6	1.4
Group 5 – 0.25% (w/v) test item	325.4	1.7

[1] The stimulation index is derived by dividing the DPN of each experimental group by the DPN of the vehicle control group. A stimulation index of greater than or equal to 3.0 generally indicates a positive response.
Note: The 2.5% (w/v) group was excluded as per the OECD guideline, based on the body weight data.

1.3 Conclusion

In conclusion, under the conditions of the present assay profenofos EC (A8591B) tested in a suitable vehicle, was shown to have no sensitization potential (non-sensitizer) in the Local Lymph Node Assay.

2.0 INTRODUCTION

2.1 Purpose

The basic principle underlying the Local Lymph Node Assay (LLNA) is that skin sensitizers induce proliferation of lymphocytes in the lymph nodes draining the site of chemical application.

Generally, under appropriate test conditions, this proliferation is proportional to the concentration applied, and provides a means of obtaining an objective, quantitative measurement of sensitization potential. The test measures cellular proliferation as a function of *in vivo* radioisotope incorporation into the DNA of dividing lymphocytes. The LLNA assesses proliferation in the draining auricular lymph nodes located in the cervical region at the bifurcation of the jugular vein. Lymphocyte proliferation in test groups is compared to that in the vehicle treated control. The ratio of the proliferation in test groups to that in the control, termed Stimulation Index (SI), is determined and must be at least equal or greater than three, for a test substance to be classified as a skin sensitizer.

The purpose of this study was to determine the skin sensitization potential of the test item, profenofos EC (A8591B) following dermal exposure in the Local Lymph Node Assay.

2.2 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*:	Profenofos content: 73.12% w/w corresponding to 969.03 g/L
Density:	1.3253 g/cm^3
Appearance:	Light yellow liquid
Recertification date:	06 May 2023
Storage conditions:	Room temperature (<30^{o}C)
Safety precautions:	Enhanced safety precautions (nitrile gloves, goggles, face mask (ABEK-P3-filter), lab coat).

*Note: No adjustment for active ingredient content of the test item was applied as agreed by 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 provided 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

In agreement with the Sponsor, 1% aqueous Pluronic® PE9200 (1% Pluronic) was used as vehicle for the test item in the study. The highest achievable concentration based on the regulatory requirements of OECD No. 429 and the physical characteristics of the test item was 100% (undiluted). As 1% Pluronic is one of the vehicles recommended by the relevant OECD guideline, it was selected for vehicle of the study. The formulation at 50%, 25%, 10%, 2.5% , 1%, 0.5% and 0.25% (w/v) in 1% Pluronic were also suitable for treatment. All formulations appeared to be white, homogenous emulsion by visual examination.

The test item was weighed and formulations prepared daily on a weight: volume basis (as % (w/v)) in the Pharmacy of Charles River Laboratories Hungary Kft.

Analytical determination of the test item concentration, stability and homogeneity was not performed because of the character and the short period of study.

3.2 Controls

3.2.1 Negative control

Animals assigned to the negative control group were treated with the vehicle only, concurrent to the test item treated groups. In agreement with the Sponsor 1% Pluronic solution was selected for vehicle of the study. Data of the chemicals used for preparation of the vehicle in the study are listed below:

1% Pluronic

Name:	Pluronic® PE 9200
Batch No.:	93048268E0
Manufacturer:	BTC Europe GmbH (BASF)
Expiry:	23 March 2025
Storage condition:	Room temperature

Name:	Distilled water
Batch No.:	11952Y25-2
Manufacturer:	B. Braun
Expiry:	30 April 2024
Storage condition:	Room temperature

3.2.2 Positive control

At the Sponsor's request a concurrent positive control group was not treated in this study. Positive control data of relevant studies (using 15% (w/v) Formaldehyde solution) from the last 12 months was used in this study using the same vehicle (1% Pluronic) instead of a concurrent positive control group.

Note: The use of FA 15% as the positive control was based on a scientific publication [9] as well as the local historic data.

3.3 Other Chemicals Used in the Study

The data of the chemicals used in the study are summarized in Table 1.

3.4 Instrument System

Name:	Tri-Carb 4910 TR Liquid Scintillation Analyzer
Manufacturer:	PerkinElmer
Serial Number:	SGLO01211479
Protocol:	09234244,10
Date of IQ/OQ (first):	08 February 2021
Date of OQ:	03 February 2022

IQ – Installation Qualification, OQ – Operational Qualification

3.5 Experimental Animals

Species and strain:	CBA/CaOlaHsd mice
Source:	Envigo RMS B.V. Postbus 553 5800 AN Venray Netherland
Hygienic level:	SPF at arrival; standard housing conditions during the study
Justification of strain:	On the basis of OECD Guideline, mice of CBA/Ca or CBA/J strain can be used. Females are used because the existing database is predominantly based on females.
Number of animals (Main Experiment II.):	4 animals / treatment group
Sex:	Female, nulliparous, non-pregnant
Age of animals at starting (Main Experiment II.)*:	Young adults, 10 weeks old
Body weight range at starting (Main Experiment II.):	17.1 – 22.2 grams (The weight variation in animals in the study did not exceed ± 20 % of the mean weight.)
Acclimatization time (Main Experiment II.):	At least 14 days

*Note: One mouse of 10 weeks of age (17.5 gram) was used in the Preliminary Experiment I., one mouse of 11 weeks of age (18.4 gram) was used in the Preliminary Experiment II., two mice of 12 weeks of age (17.7-18.4 grams) were used in the Preliminary Experiment III., two mice of 9 weeks of age (17.8 and 18.0 grams) were used in the Preliminary Experiment IV.

In the Main Experiment I. same number of animals was used as in the Main experiment II.

3.5.1 Husbandry

Animal health:	Only healthy animals were used for the study. Health status was certified by the veterinarian
Housing / Enrichment:	Group caging / mice were provided with glass tunnel-tubes
Cage type:	Type II. polypropylene / polycarbonate
Bedding/Nesting:	Bedding and nesting were available to animals during the study
Light:	12 hours daily, from 6.00 a.m. to 6.00 p.m.
Temperature:	20.0 – 25.9°C
Relative humidity:	20 - 65%
Ventilation:	15-20 air exchanges/hour

The temperature and relative humidity were recorded twice every day during the acclimatisation and experimental phases (for further details see ‘Deviation from the Study Plan’ section).

3.5.2 Food and feeding

Animals received ssniff® SM Rat/Mouse – Breeding and Maintenance, 15 mm, autoclavable "Complete feed for Rats and Mice – Breeding and Maintenance" (ssniff Spezialdiäten GmbH (Ferdinand-Gabriel-Weg 16, D-59494 Soest, Germany, batch number:53684829, expiry dates:31 May 2022), and Gel diet Transport (Scientific Animal Food & Engineering, Route de Saint Bris, 89290 Augy, France, batch number: 60210430030101, expiry date: 12 February 2022; batch number:21221001, expiry date: 09 August 2022), 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. The content of the standard diets and the test reports of the diets analyses, provided by the manufacturer are retained in the archives of Charles River Laboratories Hungary Kft.

3.5.3 Water supply

The animals received tap water, fit for human consumption, *ad libitum*, from 500 ml bottles. The water 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 at least once every three months and microbiological assessment is performed monthly by Local Public Health Laboratories. The quality control results are retained in the archives at Charles River Laboratories Hungary Kft.

3.5.4 Bedding and nesting

Bedding of certified wood chips especially designed to keep animals in the best natural environment was provided for animals during the study. SAFE 3/4-S Hygienic Animal Bedding produced by J. Rettenmaier & Söhne GmbH + Co.KG (D-73494 Rosenberg, Germany) was available to animals during the study. Certified nest building material was also provided for animals (SAFE crinklets natural produced by J. Rettenmaier & Söhne GmbH + Co.KG).

3.5.5 Identification and randomisation

A unique number written on the tail with a permanent marker was identify each animal. The animal number were assigned on the basis of Charles River Laboratories Hungary Kft.'s Master File. The cages were marked with identity cards with information including at least study code, cage number, and dose group, sex and individual animal number.

The animals were randomised and allocated to the experimental groups. The randomisation was checked by computer software (PROVANTIS v.9) according to the actual body weights, verifying the homogeneity and variability between the groups.

3.6 Administration of the Test Item

3.6.1 Dose selection and justification of dose selection

The Preliminary Irritation / Toxicity Test I. was started according to the Study Plan on CBA/CaOlaHsd mice and at the Sponsor's request using one dose (1 animal/dose) at test item concentration of 100% (undiluted). Since the animal was found dead in the first preliminary experiment an additional preliminary experiment was performed at lower dose level.

The Preliminary Irritation / Toxicity Test II. was performed on CBA/CaOlaHsd mice using one dose (1 animal/dose) at test item concentration of
50% (w/v) in 1% Pluronic. Since the animal was found dead in the second preliminary experiment an additional preliminary experiment was performed at lower dose levels.

The Preliminary Irritation / Toxicity Test III. was performed on CBA/CaOlaHsd mice using two doses (1 animal/dose) at test item concentrations of
25% and 10% (w/v) in 1% Pluronic. Since the animals were found dead in the third preliminary experiment an additional preliminary experiment was performed at lower dose level.

Based on the observed severe effects in the previous preliminary tests the next dose level was 2.5% (w/v) in 1% Pluronic instead of 5% (w/v) in 1% Pluronic in the Preliminary Irritation / Toxicity Test IV.

The additional preliminary experiment was performed on CBA/CaOlaHsd mice using two doses (1 animal/dose) at test item concentrations of 2.5% and 1% (w/v) in 1% Pluronic.

The Preliminary Experiments were conducted in a similar experimental manner to the main test, but it was terminated on Day 6 and the radioactive proliferation assay was not performed.

In the Preliminary Irritation / Toxicity Tests, all mice were observed daily for any clinical sign of systemic toxicity or local irritation at the application site. Both ears of each mouse were observed for erythema and scored using Table 2 [1, 2, 3]. Ear thickness was also measured using a thickness gauge on Day 1 (pre-dose), Day 3 (approximately 48 hours after the first dose) and Day 6. Additional quantification of the ear thickness was performed by ear punch weight determination after the euthanasia of the experimental animals.

The maximum concentration of test item in an acceptable vehicle was established according to OECD guideline 429. Based on the observation of the solubility test, the maximum available concentration was 100 % (undiluted).

Preliminary Irritation/Toxicity Test I.

Clinical observations

During the Preliminary Irritation / Toxicity Test I. the one animal of the 100% (undiluted) dose group was found dead on Day 2.
Clinical observations are summarized in Table 7.

Body weight

Body weight data are summarized in Table 5.

Measurement of the ears

The ear thickness values (2 per animal) are summarized in Table 6. Ear punches were not measured since the animal was found dead.

Lymph nodes

The draining auricular lymph nodes of the animal were not examined since the animal was found dead.

Preliminary Irritation/Toxicity Test II.

Clinical observations

During the Preliminary Irritation / Toxicity Test II. the one animal of the 50% (w/v) dose group was found dead on Day 2. Clinical observations are summarized in Table 10.

Body weight

Body weight data are summarized in Table 8.

Measurement of the ears

The ear thickness values (2 per animal) are summarized in Table 9. Ear punches were not measured since the animal was found dead.

Lymph nodes

The draining auricular lymph nodes of the animal were not examined since the animal was found dead.

Preliminary Irritation/Toxicity Test III.

Clinical observations

During the Preliminary Irritation / Toxicity Test III. additional observations were performed after the treatment. Slight amount of test item residue was observed on the ears of the animal of the 25% (w/v) dose group on Day 1 (after treatment). Activity decreased (slight) was observed in the animal of the 25% (w/v) dose group on Day 1 (after treatment). No indication of irritancy was observed in the animal of the 25% (w/v) dose group. The animal of the 25% (w/v) dose group was found dead on Day 2.

Ataxia (slight), activity decreased (slight or moderate), hunched back or lachrymation (both eyes) were observed in the animal of the 10% (w/v) dose group on Day 2 (after treatment). No indication of irritancy was observed in the animal of the 10% (w/v) dose group. The animal of the 10% (w/v) dose group was found dead on Day 3.
Clinical observations are summarized in Table 13.

Body weight
Additional body weight measurement was performed on Day 2 in case of the animal of the 10% (w/v) dose group. Marked body weight loss was observed (decrease >5%) on Day 2 compared to Day 1. The animal of the 25% (w/v) dose group was found dead on Day 2.
Body weight data are summarized in Table 11.

Measurement of the ears

The ear thickness values (2 per animal) are summarized in Table 12. Ear punches were not measured since the animal was found dead.

Lymph nodes

The draining auricular lymph nodes of the animals were not examined since the animals were found dead.

Preliminary Irritation/Toxicity Test IV.

Clinical observations

During the Preliminary Irritation / Toxicity Test IV. no mortality was observed in both dose groups. No clinical signs were observed in the animals of the 1% (w/v) dose group. Activity decreased (slight) was observed in the animal of the 2.5% (w/v) dose group on Days 2-3 (after treatment) and on Days 4-6, this slight effect was considered to be of equivocal relationship with treatment, since the group size was one.
No indication of irritancy was observed in the animals of the 2.5% (w/v) and 1% (w/v) dose groups. Clinical observations are summarized in Table 16.

Body weight

Additional body weight measurements were performed on Day 2 and Day 3.
Marked body weight loss was observed (decrease >5%) in the animal of the 2.5% (w/v) dose group. This body weight loss was considered as probably a systemic effect.
No marked body weight loss was observed in the animals of the 1% (w/v) dose group.
Body weight data are summarized in Table 14.

Measurement of the ears

Ear thickness of the animals was measured using by a thickness gauge on Days 1, 3 and 6, and by ear punch weight determination after the euthanasia of the experimental animals on Day 6. The ear thickness values and the weights of the ear punches (2 per animal) are summarized in Table 15.
The ear thickness values and ear punch weights of all animals were within the acceptable range.

Lymph nodes

The draining auricular lymph nodes of the animals were visually examined:
they were slightly smaller than normal for the animal of the 2.5% dose group and they were normal for the animal of the 1% (w/v) (w/v) dose group (subjective judgement by analogy with observations of former experiments).

3.6.2 Main study concentrations

No ear thickness measurement or ear biopsy was conducted in the Main experiment II.
Additional body weight measurements were performed on Day 2 and Day 3 in the Main experiment II. Additional observation time point was included in the Main experiment II.

In case of mice, the body weight can be variable; the body weight data of the single mouse at 2.5% (w/v) dose group, it is a good indication (following the trend at higher dose levels) but not certain that this is systemic toxicity. If in a main study, 2.5% (w/v) shows some indication of toxicity, and the SI values for all dose levels are <3, then the result is valid for regulatory submission.
Based on this the 2.5% (w/v) dose was selected as top dose for the Main experiment II.

At the Sponsor’s request and to ensure that there were properly analysable concentrations in the Main experiment II. an additional fourth dose group was also included in the Main Experiment II.

Preliminary experiments data is summarized in Table 3. Experimental groups and dose levels for the main experiment are summarized in Table 4.

3.6.3 Topical application

During the study, animals were topically dosed with 25 µL of the appropriate formulation using a pipette on the dorsal surface of each ear. Each animal was dosed once a day for three consecutive days (Days 1, 2 and 3). There was no treatment on Days 4, 5 and 6.

3.7 Proliferation Assay

3.7.1 Injection of tritiated thymidine (3HTdR)

On Day 6, animals were taken to the radioactive suite and each mouse was intravenously injected via the tail vein with 250 µL of sterile PBS (phosphate buffered saline) containing approximately 20 µCi of ^{3}HTdR using a gauge 25G x 1" hypodermic needle with 1 mL sterile syringe. Once injected, the mice were left for 5 hours (± 30 minutes).

3.7.2 Removal and preparation of draining auricular lymph nodes

Five hours (± 30 minutes) after intravenous injection the mice were euthanized by asphyxiation with ascending doses of carbon dioxide (deep anaesthesia was confirmed before making incision, death was confirmed before discarding carcasses).

The draining auricular lymph nodes were excised by making a small incision on the skin between the jaw and sternum, pulling the skin gently back towards the ears and exposing the lymph nodes. The nodes were then removed using forceps. The carcasses were discarded after cervical dislocation or after cutting through major cervical blood vessels.

Once removed, the nodes of mice were collected in separate Petri dishes containing a small amount (1-2 mL) of PBS to keep the nodes wet before processing. The nodes of each animal were processed individually.

3.7.3 Preparation of single cell suspension of lymph node cells

A single cell suspension (SCS) of lymph node cells (LNCs) was prepared and collected in disposable tubes by gentle mechanical disaggregating of the lymph nodes through a cell strainer using the plunger of a disposable syringe. The cell strainer was washed with PBS (up to 10 mL). LNCs were pelleted with a relative centrifugal force (RCF) of 190 x g (approximately) for 10 minutes at 4 °C. After centrifugation supernatants were discarded. Pellets were gently resuspended and 10 mL of PBS was added to the tubes. The washing step was repeated twice. This procedure was repeated for each group of lymph nodes for each individual animal.

3.7.4 Determination of incorporated 3HTdR

After the final washing step, supernatants were removed. Pellets were gently agitated resuspended and 3 mL of 5 % (w/v) TCA solution was added to the tubes for precipitation of macromolecules.

After overnight (approximately 18 hours) incubation at 2-8 °C, precipitates were centrifuged (approximately 190 x g for 10 minutes at 4°C), and supernatants were removed. Pellets were resuspended in 1 mL of 5 % (w/v) TCA solution and dispersed by using an ultrasonic bath. Samples were transferred into a suitable sized scintillation vial filled with 10 mL of scintillation liquid and thoroughly mixed. The vials were loaded into a β-scintillation counter and ^{3}HTdR incorporation was measured (10-minute measurement).

The β-counter expresses the ^{3}HTdR incorporation as the number of radioactive disintegrations per minute (DPM). Background level was also measured in duplicates by adding 1 mL of 5 % (w/v) TCA solution into a scintillation vial filled with 10 mL of scintillation liquid.

3.8 Observations

3.8.1 Clinical observations

During the study (Day 1 to Day 6) each animal was observed daily for any clinical signs, including local irritation and systemic toxicity. Clinical observations were performed at least twice a day (before and after treatments at additional observation points) on Days 1, 2 and 3 and twice daily on Days 4, 5 and once on Day 6. Individual records were maintained.

The principles and criteria summarized in the OECD Humane Endpoints Guidance Document No. 19 were taken into consideration.

3.8.2 Measurement of body weight

Individual body weights were recorded on Day 1 (beginning of the test), Day 2, Day 3, Day 4 and on Day 6 (prior to ^{3}HTdR injection) with a precision of 0.1 g.

3.9 Evaluation of the Results

The proliferative response of lymph node cells from the lymph nodes of each individual animal is expressed as radioactive disintegrations per minute (DPM) per animal. The average of the two measured DPM values of 5 % (w/v) TCA solutions was used as background DPM value.

The results were expressed as disintegrations per node (DPN = DPM divided by the number of lymph nodes) for each animal following the industry standard for data presentation.

Stimulation index (SI = mean DPN of treated group divided by mean DPN of the appropriate control group) for each treatment group was also calculated. A stimulation index of 3 or greater is an indication of a positive result.

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, as appropriate.

3.9.1 Interpretation of results

The test item is regarded as a sensitizer if both of the following criteria are fulfilled:
- That exposure to at least one concentration of the test item resulted in an incorporation of ^{3}HTdR at least 3-fold or greater than recorded in control mice, as indicated by the stimulation index.
- The data are compatible with a conventional dose response, although allowance must be made (especially at high topical concentrations) for either local toxicity or immunological suppression.

3.9.2 Acceptability of the test

The Local Lymph Node Assay is considered valid if it meets the following criteria:

- the DPN value of the negative (vehicle) control group falls within the range of historical laboratory control data,
- the positive control substance produces a significant lymphoproliferative response (SI>3),
- the positive control is chosen such that it does not normally cause excessive skin irritation or systemic toxicity and the induction is reproducible but not excessive (i.e. SI>20),
- each treated and control group includes at least 4 animals,
- the test item does not cause serious systemic toxicity or local irritation.

3.10 Permission of the IACUC

The conduct of the study was permitted by Institutional Animal Care and Use Committee (IACUC) on 17 November 2021.

3.11 Use of Radioactive Materials

Use of radioactive materials was recorded in the appropriate register. Regular decontamination of the working area with a verification of decontamination was carried out. Radioactive waste materials were processed according to normal laboratory standards.

3.12 Justifications and Guidelines

3.12.1 Justification of test system and number of animals

CBA/CaOlaHsd mice as a rodent is one of the standard strains of skin sensitisation assessment in accordance with the OECD No. 429 guideline and Commission Regulation (EC) No 440/2008 of 2008, B.42. The required number of animals was used in the study, that

was compatible with the Sponsor regulatory submission requirements and provided valid, interpretable data.

3.12.2 Justification of route and dose levels

The treatment volume and route of administration was specified by the relevant guideline(s) for testing skin sensitisation.

3.12.3 Guidelines for study

OECD Guidelines for Testing of Chemicals No.429, Skin Sensitization: Local Lymph Node Assay (Adopted 2010)
Commission Regulation (EC) No 440/2008 of 2008, B.42. "Skin Sensitization: Local Lymph Node Assay", (Official Journal L 142, 31/05/2008) as amended by Commission Regulation (EU) No 640/2012 of 2012 (Official Journal L 193, 2012)

4.0 RESULTS AND DISCUSSION

4.1 Reason for Main Experiment II

In the Main Experiment I, a very low measured DPM (radioactive disintegration per minute) was considered to have been caused by degradation of the radiolabelled thymidine; the experiment did not meet the validity requirements of the study and it was considered to be invalid. Therefore, an additional experiment (Main Experiment II.) was performed with a verified fresh batch of isotope to provide valid, interpretable data.

The experimental conditions, dose groups, ear thickness and biopsy measurements were the same as in the first main experiment. Results of the invalid experiment (Main Experiment I.) are not reported; however, all data are kept and archived in the raw data binder.

4.2 Clinical Observation

No mortality was observed during the Main experiment II. No test item residue was observed during the Main experiment II. There was no indication of local irritation at the site of application. Slight activity decreased was observed at 3 out of 4 animals at 2.5% (w/v) dose group on Day 3.

The results of the observations are summarized in Table 19.

4.3 Body Weight Measurement

In the high dose group (2.5 % (w/v)) 4 out of 4 animals had more than 5% body weight loss which resulted in a -10.3% or -9.5% group mean on Day 2 or Day 3 (additional measurement points) and on Day 6, 1 out of 4 animals had more than 5% body weight loss (-11.5%) which resulted in a -4.2% group mean. These values are over the limit of 5% given in the relevant OECD guideline used to indicate systemic toxicity.

Marked body weight loss was seen in some other individual animals, however the group mean body weight changes were below 5%.

The observed reduction in body weight values at 2.5% (w/v) is over the limit given in the relevant OECD guideline used to indicate systemic toxicity, hence the dose group was excluded from data analysis (although this has no effect on the study interpretation). Individual and mean body weights are given in Table 17.

4.4 Proliferation Assay

The results of the proliferation assay are summarized in Table 18 and Figure 1.

The appearance of the lymph nodes was normal in all animals of all dose groups. The appearance of the lymph nodes was normal in all animals of the negative control group.

The stimulation index values were (2.3), 1.8, 1.4 and 1.7 at concentrations of (2.5%), 1%, 0.5% and 0.25% (w/v), respectively.

4.5 Interpretation of Observations

The test item was liquid, which was formulated in 1% Pluronic. Based on the body weight effects at 2.5% (w/v) concentration, this concentration was excluded from the evaluation of the data analysis, as per the OECD guideline (although this has no effect on the study interpretation). The resulting stimulation index observed under these test conditions at 1% (w/v) concentration was considered to be good evidence that profenofos EC (A8591B) is non-sensitizer (Figure 1).

4.6 Reliability of the Test

The positive control group animals from relevant studies from the last 12 months, treated with 15% (w/v) Formaldehyde solution in 1% Pluronic is presented in Table 20. The positive control material performs reliably in this facility. The positive control substance for these studies were chosen according to the OECD No. 429 guideline [1].

In these studies, no mortality, cutaneous reactions or signs of toxicity were observed in the positive control group. Significant lymphoproliferative responses were noted for FA in these experiments. The results of the positive control groups in these studies demonstrated the appropriate performance of the assay at the Test Facility.

The group DPN values observed for the vehicle control in the Main Assay were in harmony with the historical control range.

Historical control data for the positive and negative control substances are shown in Table 21.

5.0 CONCLUSIONS

In conclusion, under the conditions of the present assay profenofos EC (A8591B) tested in a suitable vehicle, was shown to have no sensitization potential (non-sensitizer) in the Local Lymph Node Assay.

6.0 REFERENCES

1. OECD Guidelines for Testing of Chemicals No.429, Skin Sensitisation: Local Lymph Node Assay (Adopted 2010)
2. Commission Regulation (EC) No 440/2008 of 2008, B.42. “Skin Sensitisation: Local Lymph Node Assay”, (Official Journal L 142, 2008)
3. Commission Regulation (EU) No 640/2012 of 2012 amending for the purpose of its adaptation to technical progress, Regulation (EC) No 440/2008 (Official Journal L 193, 2012)
4. 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
5. OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring, Number 1, OECD Principles of Good Laboratory Practice (as revised in 1997) ENV/MC/CHEM (98)17, 1998
6. G.F.: Examination of a vehicle for use with water soluble materials in the murine local lymph node assay. Food and Chem. Tox. 40 (2002), 1719-1725
7. Boverhof, D.R., Wiescinski, C.M., Botham, P., Lees, D., Debruyne, E., Repetto-Larsay, M., Ladics, G., Hoban, D., Gamer, A., Remmele, M., Wang-Fan, W., Ullmann, L.G., Mehta, J., Billington, R., Woolhiser, M.R.: Interlaboratory validation of 1 % Pluronic L92 surfactant as a suitable , aqueous vehicle for testing pesticide formulations using the murine local lymph node assay. Toxicological Sciences 105 (2008), 79-85
8. OECD Guidance Document No. 19, Guidance Document on the Recognition, Assessment and Use of Clinical Signs as Humane Endpoints for Experimental Animals Used in Safety Evaluation, Organisation for Economic Co-Operation and Development, Paris, 2000
9. Globally Harmonised System of Classification and Labelling of Chemicals (ninth revised edition), United Nations, New York and Geneva, 2021
10. Commission Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006 (Official Journal L 353, 1-1355, 2008)

7.0 DISTRIBUTION OF THE FINAL REPORT

Sponsor: 1x PDF file and a Word file will be uploaded to the collaborative website
OECD summary will be uploaded to the collaboration website.
Archive: 1x original, bound

TABLES SECTION

TABLE 1 Chemicals Used in the Experiments

Chemical	Manufacturer / Supplier	Batch Number	Expiry date
Aqua purificata	Magilab Kft.	2111-8166	25 May 2022
Phosphate buffered saline	Sigma-Aldrich Co.	SLCL4813	October 2022
Trichloroacetic acid (Abbreviation: TCA)	Sigma-Aldrich Co.	BCCG1278	30 July 2024
[Methyl-3H]-Thymidine	American Radiolabeled Chemicals Inc.	220107	-
OptiPhase HiSafe 3	PerkinElmer	152-21011	01 August 2022

TABLE 2 Erythema Scoring

Observation	Score
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beef redness) to eschar formation preventing grading of erythema	4

Note: Excessive local skin irritation is indicated by an erythema score ≥ 3 and/or an increase in ear thickness of ≥ 25 % on any day of measurement.

TABLE 3 Summary of Preliminary Test Data

Preliminary Concentrations	Physical Formulation	Clinical Observations	Body Weight	Erythema	Ear Thickness	Ear Biopsy weight
100% (undiluted)	A	U	N/A	N/A	N/A	N/A
50% (w/v)	A	U	N/A	N/A	N/A	N/A
25% (w/v)	A	U	N/A	N/A	N/A	N/A
10% (w/v)	A	U	N/A	N/A	N/A	N/A
2.5% (w/v)	A	E	U*	A	A	A
1% (w/v)	A	A	A	A	A	A

Notes: A=Acceptable; U=Unacceptable, N/A=Not applicable (the results were not interpretable, there were not enough data due to the death of the animal); E=Equivocal

*Note: The body weight loss was considered probably unacceptable for this single mouse, however the normal protocol includes 2 preliminary mice to allow better evaluation of the significance of findings in single animals due to the large variability in individual mouse body weight losses (hence the uncertainty for acceptability of 2.5% in the main study).

TABLE 4 Experimental Groups and Treatments

Groups	Test item concentration (% w/v)	No. of animals
Negative (vehicle) control (1% Pluronic)	-	4
Profenofos EC (A8591B) in 1% Pluronic	2.5	4
Profenofos EC (A8591B) in 1% Pluronic	1	4
Profenofos EC (A8591B) in 1% Pluronic	0.5	4
Profenofos EC (A8591B) in 1% Pluronic	0.25	4

TABLE 5 Individual Body Weights for all Animals with Group Means (Preliminary Irritation/Toxicity Test I.)

Identity Number	Animal Number	Test Group Name	Initial Body Weight (g)	Terminal Body Weight* (g)	Change# (%)
1	950	100% (undiluted)	17.5	--	--

Notes:
#: = (Terminal Body Weight – Initial Body Weight) / Initial Body Weight x 100
*: Terminal body weights were measured on Day 6.

TABLE 6 Individual Ear Thickness for all Animals (Preliminary Irritation/Toxicity Test I.)

Identity Number	Animal Number	Test Group Name	Ear Thickness on Day 1 (mm)		Ear Thickness on Day 3 (mm)*		Ear Thickness on Day 6 (mm)*		Biopsy weight** on Day 6 (mg)
			Right	Left	Right	Left	Right	Left	
1	950	100% (undiluted)	0.21	0.22	--	--	--	--	--

Notes:
1.*: For ear thickness values, an irritant response is considered when result is ≥25% above the Day 1 value.
2.**: Historical control range: 12.50-21.30 mg. Positive response is over 26.63 mg (≥25%).

TABLE 7 Summarized Clinical Observations (Preliminary Irritation/Toxicity Test I.)

Period	Group	Identity No.	Animal No.	Clinical observations
DAY 1	100% (undiluted)	1	950	Before treatment: symptom-free, ES: 0 After treatment: symptom-free, ES: 0
DAY 2	100% (undiluted)	1	950	Before treatment: Found dead After treatment: --

Notes:
1. The clinical observation of animals on the first day was performed simultaneously with the body weight measurements.
2. ES: Erythema score (0 – No erythema)

TABLE 8 Individual Body Weights for all Animals with Group Means (Preliminary Irritation/Toxicity Test II.)

Identity Number	Animal Number	Test Group Name	Initial Body Weight (g)	Terminal Body Weight* (g)	Change# (%)
1	958	50% (w/v)	18.4	--	--

Notes:
#: = (Terminal Body Weight – Initial Body Weight) / Initial Body Weight x 100
*: Terminal body weights were measured on Day 6.

TABLE 9 Individual Ear Thickness for all Animals (Preliminary Irritation/Toxicity Test II.)

Identity Number	Animal Number	Test Group Name	Ear Thickness on Day 1 (mm)		Ear Thickness on Day 3 (mm)*		Ear Thickness on Day 6 (mm)*		Biopsy weight** on Day 6 (mg)
			Right	Left	Right	Left	Right	Left	
1	958	50% (w/v)	0.22	0.22	--	--	--	--	--

Notes:
1.*: For ear thickness values, an irritant response is considered when result is ≥25% above the Day 1 value.
2.**: Historical control range: 12.50-21.30 mg. Positive response is over 26.63 mg (≥25%).

TABLE 10 Summarized Clinical Observations (Preliminary Irritation/Toxicity Test II.)

Period	Group	Identity No.	Animal No.	Clinical observations
DAY 1	50% (w/v)	1	958	Before treatment: symptom-free, ES: 0 After treatment: symptom-free, ES: 0
DAY 2	50% (w/v)	1	958	Before treatment: Found dead After treatment: --

Notes:
1. The clinical observation of animals on the first day was performed simultaneously with the body weight measurements.
2. ES: Erythema score (0 – No erythema)

TABLE 11 Individual Body Weights for all Animals with Group Means (Preliminary Irritation/Toxicity Test III.)

Identity Number	Animal Number	Test Group Name	Initial Body Weight (g)	Day 2 Body Weight (g)	Terminal Body Weight* (g)	Change# (Day 1 to Day 2)
1	952	25% (w/v)	17.7	--	--	--
2	951	10% (w/v)	18.4	15.2	--	-17.4

Notes:

1. #: = (Day 2 Body Weight – Initial Body Weight) / Initial Body Weight x 100
2. *: Terminal body weights were measured on Day 6.

TABLE 12 Individual Ear Thickness for all Animals (Preliminary Irritation/Toxicity Test III.)

Identity Number	Animal Number	Test Group Name	Ear Thickness on Day 1 (mm)		Ear Thickness on Day 3 (mm)*		Ear Thickness on Day 6 (mm)*		Biopsy weight** on Day 6 (mg)
			Right	Left	Right	Left	Right	Left	
1	952	25% (w/v)	0.22	0.22	--	--	--	--	--
2	951	10% (w/v)	0.21	0.22	--	--	--	--	--

Notes:

1.*: For ear thickness values, an irritant response is considered when result is ≥25% above the Day 1 value.
2.**: Historical control range: 12.50-21.30 mg. Positive response is over 26.63 mg (≥25%).

TABLE 13 Summarized Clinical Observations (Preliminary Irritation/Toxicity Test III.)

Period	Group	Identity No.	Animal No.	Clinical observations
DAY 1	25% (w/v)	1	952	Before treatment: Symptom-free, ES: 0 After treatment #1: Symptom-free, ES: 0, ** After treatment #2: Activity decreased (slight), ES: 0 After treatment #3: Activity decreased (slight), ES: 0
	10% (w/v)	2	951	Before treatment: Symptom-free, ES: 0 After treatment #1: Symptom-free, ES: 0 After treatment #2: Symptom-free, ES: 0 After treatment #3: Symptom-free, ES: 0
DAY 2	25% (w/v)	1	952	Before treatment: Found dead After treatment: --
	10% (w/v)	2	951	Before treatment: Ataxia (slight), hunched back, ES: 0 After treatment #1: Ataxia (slight), hunched back, lachrymation, both eyes (2), ES: 0 After treatment #2: Ataxia (slight), hunched back, ES: 0 After treatment #3: Ataxia (slight), hunched back, activity decreased (slight), ES: 0 After treatment #4: Ataxia (slight), hunched back, activity decreased (moderate), ES: 0 After treatment #5: Ataxia (slight), hunched back, activity decreased (moderate), ES: 0
DAY 3	10% (w/v)	2	951	Before treatment: Found dead After treatment: --

Notes:

1. The clinical observation of animals on the first day was performed simultaneously with the body weight measurements.
2. ES: Erythema score (0 – No erythema)
3. **: Slight amount of test item residue
4. Additional observations were performed after the treatment.
5. Lachrymation score 2: Discharge with moistening of the lids and hairs just adjacent to lids).

TABLE 14 Individual Body Weights for all Animals with Group Means (Preliminary Irritation/Toxicity Test IV.)

Animal Number	Identity Number	Test Group Name	Initial Body Weight (g)	Day 2 Body Weight (g)	Day 3 Body Weight (g)	Terminal Body Weight* (g)	Change# (Day 1 to Day 2) (%)	Change# (Day 1 to Day 3) (%)	Change# (Day 1 to Day 6)
1048	1	2.5% (w/v)	18.0	15.5	16.2	16.4	-13.9	-10.0	-8.9
1049	2	1% (w/v)	17.8	18.1	17.8	17.3	1.7	0.0	-2.8

Notes:
1. #: = (Terminal / Day 2 / Day 3 Body Weight – Initial Body Weight) / Initial Body Weight x 100
2. *: Terminal body weights were measured on Day 6.
3. Additional body weight measurements were performed on Day 2 and Day 3.

TABLE 15 Individual Ear Thickness for all Animals (Preliminary Irritation/Toxicity Test IV.)

Animal Number	Identity Number	Test Group Name	Ear Thickness on Day 1 (mm)		Ear Thickness on Day 3 (mm)*		Ear Thickness on Day 6 (mm)*		Biopsy weight** on Day 6 (mg)
			Right	Left	Right	Left	Right	Left	
1048	1	2.5% (w/v)	0.22	0.21	0.20	0.19	0.20	0.21	12.1
1049	2	1% (w/v)	0.21	0.22	0.20	0.21	0.21	0.20	17.2

Notes:
1.*: For ear thickness values, an irritant response is considered when result is ≥25% above the Day 1 value.
2.**: Historical control range: 12.50-21.30 mg. Positive response is over 26.63 mg (≥25%).

TABLE 16 Summarized Clinical Observations (Preliminary Irritation/Toxicity Test IV.)

Period	Group	Identity No.	Animal No.	Clinical observations
DAY 1	2.5% (w/v)	1	1048	Before treatment: Symptom-free, ES: 0 After treatment #1: Symptom-free, ES: 0 After treatment #2: Symptom-free, ES: 0 After treatment #3: Symptom-free, ES: 0 After treatment #4: Symptom-free, ES: 0
	1% (w/v)	2	1049	Before treatment: Symptom-free, ES: 0 After treatment #1: Symptom-free, ES: 0 After treatment #2: Symptom-free, ES: 0 After treatment #3: Symptom-free, ES: 0 After treatment #4: Symptom-free, ES: 0
DAY 2	2.5% (w/v)	1	1048	Before treatment: Symptom-free, ES: 0 After treatment #1: Symptom-free, ES: 0 After treatment #2: Symptom-free, ES: 0 After treatment #3: Symptom-free, ES: 0 After treatment #4: Activity decreased (slight), ES: 0
	1% (w/v)	2	1049	Before treatment: Symptom-free, ES: 0 After treatment #1: Symptom-free, ES: 0 After treatment #2: Symptom-free, ES: 0 After treatment #3: Symptom-free, ES: 0 After treatment #4: Symptom-free, ES: 0
DAY 3	2.5% (w/v)	1	1048	Before treatment: Activity decreased (slight), ES: 0 After treatment #1: Activity decreased (slight), ES: 0 After treatment #2: Activity decreased (slight), ES: 0 After treatment #3: Activity decreased (slight), ES: 0 After treatment #4: Activity decreased (slight), ES: 0
	1% (w/v)	2	1049	Before treatment: Symptom-free, ES: 0 After treatment #1: Symptom-free, ES: 0 After treatment #2: Symptom-free, ES: 0 After treatment #3: Symptom-free, ES: 0 After treatment #4: Symptom-free, ES: 0
DAY 4	2.5% (w/v)	1	1048	Observation #1: Activity decreased (slight), ES: 0 Observation #2: Activity decreased (slight), ES: 0 Observation #3: Activity decreased (slight), ES: 0
	1% (w/v)	2	1049	Observation #1: Symptom-free, ES: 0 Observation #2: Symptom-free, ES: 0 Observation #3: Symptom-free, ES: 0

Notes:

1. The clinical observation of animals on the first day was performed simultaneously with the body weight measurements.
2. ES: Erythema score (0 – No erythema)
3. Additional observations were performed from Day 1 to Day 5.

TABLE 17 Individual Body Weights for all Animals with Group Means

Animal Number	Identity Number	Test Group Name	Initial Body Weight (g)	Day 2 Weight (g)*	Day 3 Weight (g)*	Terminal Body Weight** (g)	Change# (%) (Day 1 to Day 2)	Change# (%) (Day 1 to Day 3)	Change## (%) Day 1 to Day 6
1376	1	Negative (vehicle) control	17.4	16.6	17.1	17.2	-4.6	-1.7	-1.1
1381	2	1% Pluronic	20.9	20.4	20.8	19.7	-2.4	-0.5	-5.7
1389	3		18.1	17.7	17.6	18.3	-2.2	-2.8	1.1
1394	4		20.0	19.6	19.9	19.7	-2.0	-0.5	-1.5
		Mean	**19.1**	**18.6**	**18.9**	**18.9**	**-2.8**	**-1.4**	**-1.8**
1385	5	Profenofos EC (A8591B) 2.5% (w/v) in 1% Pluronic	17.4	16.5	16.1	17.4	-5.2	-7.5	0.0
1378	6		20.0	17.7	18.9	19.9	-11.5	-5.5	-0.5
1380	7		20.1	17.4	18.0	19.1	-13.4	-10.4	-5.0
1393	8		19.1	17.0	16.3	16.9	-11.0	-14.7	-11.5
		Mean	**19.2**	**17.2**	**17.3**	**18.3**	**-10.3**	**-9.5**	**-4.2**
1390	9	Profenofos EC (A8591B) 1% (w/v) in 1% Pluronic	17.3	16.6	16.9	16.9	-4.0	-2.3	-2.3
1377	10		21.1	21.1	20.6	19.9	0.0	-2.4	-5.7
1384	11		19.1	19.2	19.8	19.1	0.5	3.7	0.0
1382	12		17.6	16.9	16.9	17.0	-4.0	-4.0	-3.4
		Mean	**18.8**	**18.5**	**18.6**	**18.2**	**-1.9**	**-1.2**	**-2.9**
1388	13	Profenofos EC (A8591B) 0.5% (w/v) in 1% Pluronic	17.6	17.6	17.6	17.4	0.0	0.0	-1.1
1386	14		19.5	18.7	19.0	19.2	-4.1	-2.6	-1.5
1387	15		18.4	19.3	18.9	20.5	4.9	2.7	11.4
1379	16		20.6	20.7	20.8	19.2	0.5	1.0	-6.8
		Mean	**19.0**	**19.1**	**19.1**	**19.1**	**0.3**	**0.3**	**0.5**
1392	17	Profenofos EC (A8591B) 0.25% (w/v) in 1% Pluronic	17.1	17.2	16.9	17.2	0.6	-1.2	0.6
1383	18		22.2	21.8	20.1	17.5	-1.8	-9.5	-21.2
1391	19		18.1	18.1	17.4	21.3	0.0	-3.9	17.7
1375	20		19.7	19.6	18.7	19.7	-0.5	-5.1	0.0
		Mean	**19.3**	**19.2**	**19.2**	**18.9**	**-0.4**	**-4.9**	**-0.7**

Notes:

*: = Additional body weights were measured on Day 2 or Day 3.

**: = Terminal body weights were measured on Day 6.

#: = (Day 2 / Day 3 body Weight - Initial Body Weigh) / Initial Body Weight x 100

##: = (Terminal Body Weight – Initial Body Weight) / Initial Body Weight x 100

TABLE 18 DPN and Stimulation Index Values for all Groups

Test Group Name	Animal Number	Measured DPM	Total DPM	No. Of Nodes	DPN	Mean DPN	Stimulation Index Values
Background (5 (w/v) % TCA)	-	38.5	-	-	-	-	-
Negative (vehicle) control (1% Pluronic)	1376	496	457.5	2	228.8	186.5	1.0
	1381	208	169.5	2	84.8		
	1389	311	272.5	2	136.3		
	1394	631	592.5	2	296.3		
Profenofos EC (A8591B) 2.5% (w/v) in 1% Pluronic	1385	990	951.5	2	475.8	432.9	2.3[Note]
	1378	677	638.5	2	319.3		
	1380	1240	1201.5	2	600.8		
	1393	710	671.5	2	335.8		
Profenofos EC (A8591B) 1% (w/v) in 1% Pluronic	1390	1352	1313.5	2	656.8	340.9	1.8
	1377	521	482.5	2	241.3		
	1384	385	346.5	2	173.3		
	1382	623	584.5	2	292.3		
Profenofos EC (A8591B) 0.5% (w/v) in 1% Pluronic	1388	723	684.5	2	342.3	260.6	1.4
	1386	541	502.5	2	251.3		
	1387	538	499.5	2	249.8		
	1379	437	398.5	2	199.3		
Profenofos EC (A8591B) 0.25% (w/v) in 1% Pluronic	1392	1039	1000.5	2	500.3	325.4	1.7
	1383	587	548.5	2	274.3		
	1391	716	677.5	2	338.8		
	1375	415	376.5	2	188.3		

Notes:
1. DPM (Disintegrations Per Minute)
2. Total DPM = Measured DPM – Background DPM
3. DPN (Disintegrations Per Node) = DPM divided by the number of lymph nodes.
4. Stimulation Index = DPN of a treated group divided by DPN of the appropriate control group.
5. Background is the mean of 45 and 32.

Note: The 2.5% (w/v) group was excluded as per the OECD guideline, based on the body weight data.

TABLE 19 Summarized Clinical Observations

Group	Animal No.	CLINICAL OBSERVATIONS					
		DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6
Negative (vehicle) control (1% Pluronic)	1376	BT: Symptom-free, ES:0 AT#1: Symptom-free, ES:0 AT#2: Symptom-free, ES:0	BT: Symptom-free, ES:0 AT#1: Symptom-free, ES:0 AT#2: Symptom-free, ES:0	BT: Symptom-free, ES:0 AT#1: Symptom-free, ES:0 AT#2: Symptom-free, ES:0	#1: Symptom-free, ES:0 #2: Symptom-free, ES:0	#1: Symptom-free, ES:0 #2: Symptom-free, ES:0	Symptom-free, ES:0
	1381	BT: Symptom-free. ES:0 AT#1: Symptom-free, ES:0 AT#2: Symptom-free, ES:0	BT: Symptom-free. ES:0 AT#1: Symptom-free, ES:0 AT#2: Symptom-free, ES:0	BT: Symptom-free. ES:0 AT#1: Symptom-free, ES:0 AT#2: Symptom-free, ES:0	#1: Symptom-free, ES:0 #2: Symptom-free, ES:0	#1: Symptom-free, ES:0 #2: Symptom-free, ES:0	Symptom-free, ES:0
	1389	BT: Symptom-free, ES:0 AT#1: Symptom-free, ES:0 AT#2: Symptom-free, ES:0	BT: Symptom-free, ES:0 AT#1: Symptom-free, ES:0 AT#2: Symptom-free, ES:0	BT: Symptom-free, ES:0 AT#1: Symptom-free, ES:0 AT#2: Symptom-free, ES:0	#1: Symptom-free, ES:0 #2: Symptom-free, ES:0	#1: Symptom-free, ES:0 #2: Symptom-free, ES:0	Symptom-free, ES:0
	1394	BT: Symptom-free, ES:0 AT#1: Symptom-free, ES:0 AT#2: Symptom-free, ES:0	BT: Symptom-free, ES:0 AT#1: Symptom-free, ES:0 AT#2: Symptom-free, ES:0	BT: Symptom-free, ES:0 AT#1: Symptom-free, ES:0 AT#2: Symptom-free, ES:0	#1: Symptom-free, ES:0 #2: Symptom-free, ES:0	#1: Symptom-free, ES:0 #2: Symptom-free, ES:0	Symptom-free, ES:0
Profenofos EC (A8591B) 2.5% (w/v) in 1% Pluronic	1385	BT: Symptom-free, ES:0 AT#1: Symptom-free, ES:0 AT#2: Symptom-free, ES:0	BT: Symptom-free, ES:0 AT#1: Symptom-free, ES:0 AT#2: Symptom-free, ES:0	BT: Activity decreased. slight, ES:0 AT#1: Activity decreased. slight, ES:0 AT#2: Activity decreased. slight, ES:0	#1: Symptom-free, ES:0 #2: Symptom-free, ES:0	#1: Symptom-free, ES:0 #2: Symptom-free, ES:0	Symptom-free, ES:0
	1378	BT: Symptom-free. ES:0 AT#1: Symptom-free, ES:0 AT#2: Symptom-free, ES:0	BT: Symptom-free. ES:0 AT#1: Symptom-free, ES:0 AT#2: Symptom-free, ES:0	BT: Symptom-free. ES:0 AT#1: Symptom-free, ES:0 AT#2: Symptom-free, ES:0	#1: Symptom-free, ES:0 #2: Symptom-free, ES:0	#1: Symptom-free, ES:0 #2: Symptom-free, ES:0	Symptom-free, ES:0
	1380	BT: Symptom-free, ES:0 AT#1: Symptom-free, ES:0 AT#2: Symptom-free, ES:0	BT: Symptom-free, ES:0 AT#1: Symptom-free, ES:0 AT#2: Symptom-free, ES:0	BT: Symptom-free, ES:0 AT#1: Symptom-free, ES:0 AT#2: Activity decreased. slight, ES:0	#1: Symptom-free, ES:0 #2: Symptom-free, ES:0	#1: Symptom-free, ES:0 #2: Symptom-free, ES:0	Symptom-free, ES:0
	1393	BT: Symptom-free, ES:0 AT#1: Symptom-free, ES:0 AT#2: Symptom-free, ES:0	BT: Symptom-free, ES:0 AT#1: Symptom-free, ES:0 AT#2: Symptom-free, ES:0	BT: Symptom-free, ES:0 AT#1: Activity decreased. slight, ES:0 AT#2: Activity decreased. slight, ES:0	#1: Symptom-free, ES:0 #2: Symptom-free, ES:0	#1: Symptom-free, ES:0 #2: Symptom-free, ES:0	Symptom-free, ES:0

Notes:

1. BT: before treatment, AT: after treatment
2. ES: Erythema score (0 – No erythema)
3. #1; #2: Additional observation points from Day 1 to Day 5.

Group	Animal No.	CLINICAL OBSERVATIONS					
		DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6
Profenofos EC (A8591B) 1% (w/v) in 1% Pluronic	1390	BT: Symptom-free, ES:0 AT#1: Symptom-free, ES:0 AT#2: Symptom-free, ES:0	BT: Symptom-free, ES:0 AT#1: Symptom-free, ES:0 AT#2: Symptom-free, ES:0	BT: Symptom-free, ES:0 AT#1: Symptom-free, ES:0 AT#2: Symptom-free, ES:0	#1: Symptom-free, ES:0 #2: Symptom-free, ES:0	#1: Symptom-free, ES:0 #2: Symptom-free, ES:0	Symptom-free, ES:0
	1377	BT: Symptom-free. ES:0 AT#1: Symptom-free, ES:0 AT#2: Symptom-free, ES:0	BT: Symptom-free. ES:0 AT#1: Symptom-free, ES:0 AT#2: Symptom-free, ES:0	BT: Symptom-free. ES:0 AT#1: Symptom-free, ES:0 AT#2: Symptom-free, ES:0	#1: Symptom-free, ES:0 #2: Symptom-free, ES:0	#1: Symptom-free, ES:0 #2: Symptom-free, ES:0	Symptom-free, ES:0
	1384	BT: Symptom-free, ES:0 AT#1: Symptom-free, ES:0 AT#2: Symptom-free, ES:0	BT: Symptom-free, ES:0 AT#1: Symptom-free, ES:0 AT#2: Symptom-free, ES:0	BT: Symptom-free, ES:0 AT#1: Symptom-free, ES:0 AT#2: Symptom-free, ES:0	#1: Symptom-free, ES:0 #2: Symptom-free, ES:0	#1: Symptom-free, ES:0 #2: Symptom-free, ES:0	Symptom-free, ES:0
	1382	BT: Symptom-free, ES:0 AT#1: Symptom-free, ES:0 AT#2: Symptom-free, ES:0	BT: Symptom-free, ES:0 AT#1: Symptom-free, ES:0 AT#2: Symptom-free, ES:0	BT: Symptom-free, ES:0 AT#1: Symptom-free, ES:0 AT#2: Symptom-free, ES:0	#1: Symptom-free, ES:0 #2: Symptom-free, ES:0	#1: Symptom-free, ES:0 #2: Symptom-free, ES:0	Symptom-free, ES:0
Profenofos EC (A8591B) 0.5% (w/v) in 1% Pluronic	1388	BT: Symptom-free, ES:0 AT#1: Symptom-free, ES:0 AT#2: Symptom-free, ES:0	BT: Symptom-free, ES:0 AT#1: Symptom-free, ES:0 AT#2: Symptom-free, ES:0	BT: Symptom-free, ES:0 AT#1: Symptom-free, ES:0 AT#2: Symptom-free, ES:0	#1: Symptom-free, ES:0 #2: Symptom-free, ES:0	#1: Symptom-free, ES:0 #2: Symptom-free, ES:0	Symptom-free, ES:0
	1386	BT: Symptom-free. ES:0 AT#1: Symptom-free, ES:0 AT#2: Symptom-free, ES:0	BT: Symptom-free. ES:0 AT#1: Symptom-free, ES:0 AT#2: Symptom-free, ES:0	BT: Symptom-free. ES:0 AT#1: Symptom-free, ES:0 AT#2: Symptom-free, ES:0	#1: Symptom-free, ES:0 #2: Symptom-free, ES:0	#1: Symptom-free, ES:0 #2: Symptom-free, ES:0	Symptom-free, ES:0
	1387	BT: Symptom-free, ES:0 AT#1: Symptom-free, ES:0 AT#2: Symptom-free, ES:0	BT: Symptom-free, ES:0 AT#1: Symptom-free, ES:0 AT#2: Symptom-free, ES:0	BT: Symptom-free, ES:0 AT#1: Symptom-free, ES:0 AT#2: Symptom-free, ES:0	#1: Symptom-free, ES:0 #2: Symptom-free, ES:0	#1: Symptom-free, ES:0 #2: Symptom-free, ES:0	Symptom-free, ES:0
	1379	BT: Symptom-free, ES:0 AT#1: Symptom-free, ES:0 AT#2: Symptom-free, ES:0	BT: Symptom-free, ES:0 AT#1: Symptom-free, ES:0 AT#2: Symptom-free, ES:0	BT: Symptom-free, ES:0 AT#1: Symptom-free, ES:0 AT#2: Symptom-free, ES:0	#1: Symptom-free, ES:0 #2: Symptom-free, ES:0	#1: Symptom-free, ES:0 #2: Symptom-free, ES:0	Symptom-free, ES:0

Notes:

1. BT: before treatment, AT: after treatment
2. ES: Erythema score (0 – No erythema)
3. #1; #2: Additional observation points from Day 1 to Day 5.

Group	Animal No.	CLINICAL OBSERVATIONS					
		DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6
Profenofos EC (A8591B) 0.25% (w/v) in 1% Pluronic	1392	BT: Symptom-free, ES:0 AT#1: Symptom-free, ES:0 AT#2: Symptom-free, ES:0	BT: Symptom-free, ES:0 AT#1: Symptom-free, ES:0 AT#2: Symptom-free, ES:0	BT: Symptom-free, ES:0 AT#1: Symptom-free, ES:0 AT#2: Symptom-free, ES:0	#1: Symptom-free, ES:0 #2: Symptom-free, ES:0	#1: Symptom-free, ES:0 #2: Symptom-free, ES:0	Symptom-free, ES:0
	1383	BT: Symptom-free. ES:0 AT#1: Symptom-free, ES:0 AT#2: Symptom-free, ES:0	BT: Symptom-free. ES:0 AT#1: Symptom-free, ES:0 AT#2: Symptom-free, ES:0	BT: Symptom-free. ES:0 AT#1: Symptom-free, ES:0 AT#2: Symptom-free, ES:0	#1: Symptom-free, ES:0 #2: Symptom-free, ES:0	#1: Symptom-free, ES:0 #2: Symptom-free, ES:0	Symptom-free, ES:0
	1391	BT: Symptom-free, ES:0 AT#1: Symptom-free, ES:0 AT#2: Symptom-free, ES:0	BT: Symptom-free, ES:0 AT#1: Symptom-free, ES:0 AT#2: Symptom-free, ES:0	BT: Symptom-free, ES:0 AT#1: Symptom-free, ES:0 AT#2: Symptom-free, ES:0	#1: Symptom-free, ES:0 #2: Symptom-free, ES:0	#1: Symptom-free, ES:0 #2: Symptom-free, ES:0	Symptom-free, ES:0
	1375	BT: Symptom-free, ES:0 AT#1: Symptom-free, ES:0 AT#2: Symptom-free, ES:0	BT: Symptom-free, ES:0 AT#1: Symptom-free, ES:0 AT#2: Symptom-free, ES:0	BT: Symptom-free, ES:0 AT#1: Symptom-free, ES:0 AT#2: Symptom-free, ES:0	#1: Symptom-free, ES:0 #2: Symptom-free, ES:0	#1: Symptom-free, ES:0 #2: Symptom-free, ES:0	Symptom-free, ES:0

Notes:

1. BT: before treatment, AT: after treatment
2. ES: Erythema score (0 – No erythema)
3. #1; #2: Additional observation points from Day 1 to Day 5.

TABLE 20 Positive control data from relevant studies from last 12 months conducted with 1% Pluronic as vehicle (15% (w/v) FA)

Study code	Study occasions (start of experiments and end of experiments)	DPN of the Positive control group	SI of the Positive control group
21/235-037E	10-16 November 2021	5285.8	22.0*
21/121-037E	21-27 July 2021	6669.8	18.8
21/103-037E	16-22 June 2021	4720	27.8*
20/263-037E	17-23 March 2021	3401.2	11.6
20/286-037E	03-09 March 2021	3939.8	17.5

*Note: The stimulation index value was slightly above the normal range; however, the DPN results were in harmony with the historical control range and they were considered acceptable.

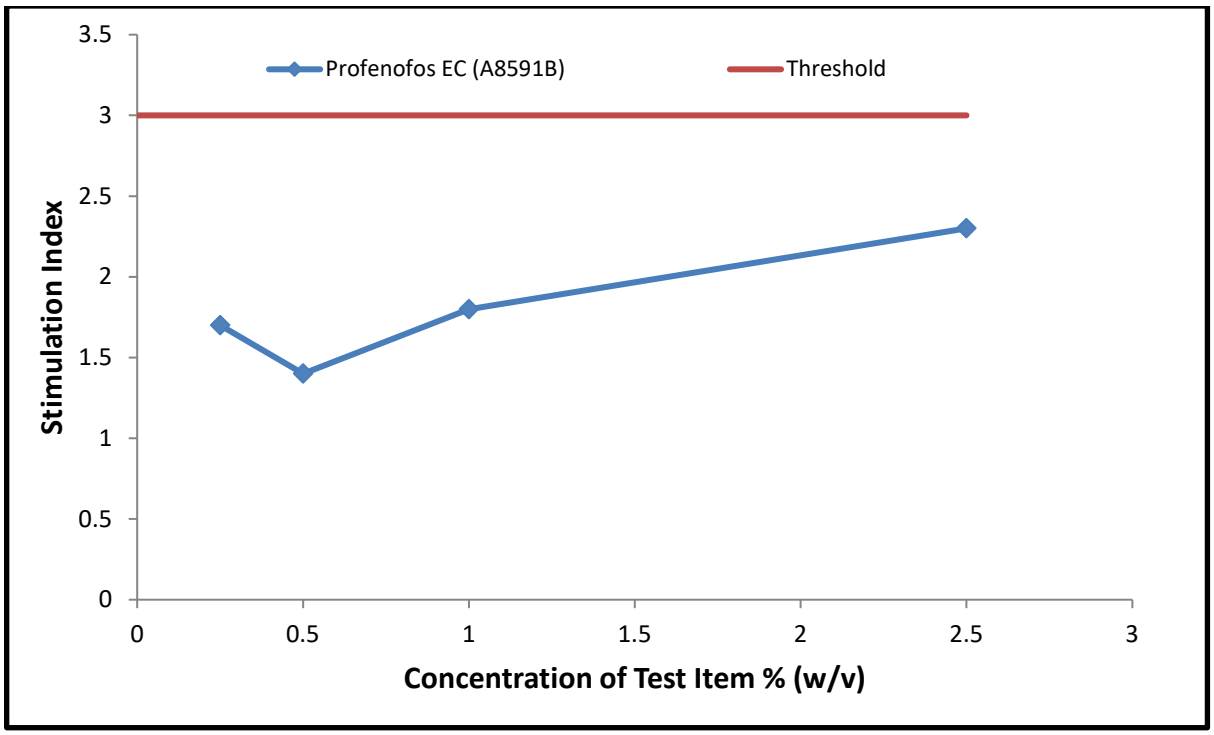
TABLE 21 Historical Control Data of the Positive and Negative Controls for CBA/CaOlaHsd mice

		Vehicle		
		1% Pluronic PE9200 in water		
		DPN values		SI value
		Control	FA 15%	FA 15%
Average:		268.5	4128.1	15.8
Standard deviation:		146.2	1114.8	5.0
Confidence interval (95%)	*Low end:*	216.1	3729.2	14.0
	High end:	320.8	4527.0	17.5
***Range**:*	*Minimum value:*	58.3	1382.8	6.5
	Maximum value:	609.0	6670.0	25.3
Number of cases:		30	30	30

FA 15% = Formaldehyde 15% (w/v)
SI (Stimulation Index)= mean DPN of the positive control group divided by the mean DPN of the negative (vehicle) control group (in case of the individual approach, individual SI values were similarly calculated).
DPN (Disintegrations Per Node) = DPM (Disintegrations Per Minute) divided by the number of lymph nodes.
Updated: 21 January 2021 (period: September 2019-February 2020)

FIGURES SECTION

FIGURE 1 Test Item Stimulation Index Values



APPENDICES SECTION

APPENDIX 1 Study Schedule

PRELIMINARY EXPERIMENTS		17 November-13 December 2021
	Study Day	Date
PRE-EXPERIMENTAL PERIOD:		
Animal receipt:	Day (-14)	03 February 2022
Veterinary control and acclimatisation:	from Day (-14) to Day (1)	from 03 February2022 to 17 February 2022
Animal identification:	Day 1	17 February 2022
Randomisation:	Day 1	17 February 2022
EXPERIMENTAL PERIOD:		
Treatment days:	Day 1	17 February 2022
	Day 2	18 February 2022
	Day 3	19 February 2022
Body weight measurement:	Day 1 (beginning of the test)	17 February 2022
	and Day 6 (prior to ^{3}HTdR injection)	22 February 2022
Clinical observation:	daily from Day 1 to Day 6	from 17 February 2022 to 22 February 2022
Injection of ^{3}HTdR:	Day 6	22 February 2022
Preparation of LNC:	Day 6	22 February 2022
Sample measurement:	Day 7	23 February 2022

APPENDIX 2 Copy of the 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/cm^3
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

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

APPENDIX 3 Copy of the GLP Certificate

OGYÉI Országos Gyógyszerészeti és Élelmezés-egészségügyi Intézet

Hatósági Ellenőrzési Főosztály

1051 Budapest, Zrínyi utca 3.
Levélcím: 1372 Postafiók 450
Tel.: +36 1 886 9300, Fax: +36 1 886 9460
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”).