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Difenoconazole/Fludioxonil/Metalaxyl-M/Cyclobutrifluram

Difenoconazole/Fludioxonil/Metalaxyl-M/Cyclobutrifluram FS (A23793B) - Acute Inhalation Toxicity Study (Nose-Only) in Rats

Final Report

TEST GUIDELINE(S): OECD 403 (2009)
EPA 870.1300 (1998)
EC 440/2008, B.2 (2008)

AUTHOR(S): Imre Biró, M.Sc.

COMPLETION DATE: 29 June 2022

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

LABORATORY PROJECT ID: Report Number: 21/245-004P
Study Number: 21/245-004P
Task Number: TK0518483

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

STATEMENT OF DATA CONFIDENTIALITY CLAIMS

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410 Swing Road
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Greensboro, NC 27419-8300 USA

Submitter: _____ Date: _____

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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.

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: 29 June 2022

Imre Biró, 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: 21/245-004P

Study Title: Difenoconazole/Fludioxonil/Metalaxyl-M/Cyclobutrifluram FS (A23793B) - Acute Inhalation Toxicity Study (Nose-Only) in Rats

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
27 October 2021	Study Plan	27 October 2021	27 October 2021
17 November 2021	Amendment 1 to the Study Plan	17 November 2021	17 November 2021
30 November 2021	Amendment 2 to the Study Plan	30 November 2021	30 November 2021
01 December 2021	Treatment	01 December 2021	01 December 2021
28 January 2022	Draft Report	28 January 2022	28 January 2022
03 May 2022	Final Report	03 May 2022	03 May 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: Schleicher Ivett Date: 29 June 2022

Ivett Schleicher, Ph.D.
On behalf of QAU

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 "Difenoconazole/Fludioxonil/Metalaxyl-M/Cyclobutrifluram FS (A23793B) - Acute Inhalation Toxicity Study (Nose-Only) in Rats" was performed in compliance with the Principles of Good Laboratory Practice.

Signature: _____ Date: 29 June 2022

Balázs Tóth, Ph.D.
General Manager

GENERAL INFORMATION

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

Name	Function
Imre Biró, M.Sc.	Study Director
Nóra Krajcs, Ph.D.	Assistant Scientist
Ágnes Rédl, M.Sc.	Quality Assurance (during in life phase and reporting)
Ivett Schelicher, Ph.D.	Quality Assurance (Final report)
László Székelyhidi, D.V.M.	Veterinary Control
Tamás Mészáros, Ph.D.	Pharmacy
Ferenc Szűcs	Animal Service Laboratories
András Bálint	Technical Team Leader, Inhalation
Carolina Vaccari, M.Sc.	Syngenta Study Manager

Study dates

Study Initiation Date:	27 October 2021
Experimental Starting Date:	18 November 2021
Experimental Completion Date:	15 December 2021

Sighting Exposure – Group 0.1

Receipt of Animals:	28 October 2021
Inhalation Exposure (Day 0):	18 November 2021
Observation:	18 November – 02 December 2021
Necropsy:	02 December 2021

Main Study – Group 1

Receipt of Animals:	11 November 2021
Inhalation Exposure (Day 0):	01 December 2021
Observation:	01 – 15 December 2021
Necropsy:	15 December 2021

Performing laboratory test substance reference number
210533

Deviations from the guidelines
No deviations from the guidelines occurred during the study.

Deviations from the Study Plan
Due to technical reasons, relative humidity (minimum of 20%) were outside the expected range of 30-70% were recorded occasionally in the animal room during the study. This deviation was considered to have no impact on the outcome of the study and interpretation of the results.

Other
The study documents and samples:
- Study Plan and amendments,
- all raw data,
- sample of the test item,
- 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.

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1.0 EXECUTIVE SUMMARY

1.1 Study Design

This study was performed to assess the acute inhalation toxicity of difenoconazole/fludioxonil/metalaxyl-M/cyclobutrifluram FS (A23793B) following a 4-hour exposure to 5 male and 5 female rats.

The maximum achievable concentration was tested during the animal exposures following the OECD test guideline number 403.

A Sighting Exposure was performed prior to the Main Study with 2 male and 2 female rats at a target concentration of 2.55 mg/L.

In the Main Study group, 10 (5 males and 5 females) Crl:WI Wistar rats, were exposed to a target concentration of 2.48 mg/L difenoconazole/fludioxonil/metalaxyl-M/cyclobutrifluram FS (A23793B).

The animals were exposed for 4 hours using a nose-only exposure system, followed by a 14-day observation period. The day of exposure was designated as Day 0. Aerosol concentrations were measured gravimetrically. The particle size distribution of the test aerosol was determined regularly during the exposure period. Clinical observations and bodyweights were recorded throughout the study, at/until death and at the end of the scheduled period the surviving animals were euthanised and all animals were subjected to a gross examination *post mortem*.

1.2 Results

Atmosphere

Sighting Exposure (Group 0.1):
The maximum achievable mean atmosphere concentration was 2.55 mg/L. The MMAD (Mass Median Aerodynamic Diameter) was 3.87 µm with a GSD (Geometric Standard Deviation) of 2.15.

Main Study (Group 1):
The maximum achievable mean atmosphere concentration was 2.48 mg/L. The MMAD was 3.53 µm with a GSD of 2.11.

Mortality

No mortality occurred in the Sighting Study.
One out of five female animals was found dead on Day 0 during the exposure in the Main Study.

Clinical observations

Group 0.1 (Sighting Exposure – 2.55 mg/L)
In the male animals, laboured respiration (slight), noisy respiration (slight), fur staining by test item (on the head, whole body and on the first third of animal) and wet fur (on the whole body) were observed on Day 0-3. All the animals were symptom-free from Day 4.

In the female animals, laboured respiration (slight), fur staining by test item (on the head, whole body and on the first third of animal) and wet fur (on the whole body) were observed on Days 0-3. All the animals were symptom-free from Day 4.

Group 1 (Main Exposure – 2.48 mg/L)
In the male animals, laboured respiration (slight), fur staining by test item (on the whole body and/or first third of animal and on the head) and wet fur (on the whole body) were noted on Days 0-3. All the animals were symptom-free from Day 4.

In the surviving female animals, laboured respiration (slight), fur staining by test item (on the whole body and/or first third of animal) and wet fur (on the whole body) were observed on Days 0-4. These animals were symptom free from Day 5.

Wet fur and fur staining (as chromodacryorrhea) in the animals were considered to be related to the restraint and exposure procedures but not to be toxicologically significant.

Bodyweight

Group 0.1 (Sighting Exposure – 2.55 mg/L)
In case of male animals, slight body weight losses were noted on Days 0-1. The body weight gains were normal on Days 1-14.
In the female animals, no body weight loss was noted. The body weight gains were normal in both animals on Days 0-14.

Group 1 (Main Exposure – 2.48 mg/L)
In the male animals, slight body weight losses were observed on Days 0-1. The body weight gains were normal in all male animals on Days 1-14.

In case of the surviving female animals, slight body weight losses were observed in three out of four animals on Days 0-1 and in two out of four animals on Days 3-7. The body weight gains were normal in one animal on Days 0-14, in two out of four animals on Day 1-3 and on Days 7-14 and in case of one female animal on Days 1-14.

Necropsy

Group 0.1 (Sighting Exposure – 2.55 mg/L)
No macroscopic observations were seen on scheduled necropsy Day 14.

Group 1 (Main Exposure – 2.48 mg/L)
In the found dead female animal, diffuse red discoloration of all lobes of the non-collapsed lungs and multifocal red discoloration of thymus were observed. No macroscopic observations were seen on scheduled necropsy Day 14 in the surviving animals.

1.3 Conclusion

Under the experimental conditions of this study, one mortality occurred in a group of 10 rats when exposed to 2.48 mg/L of Difenoconazole/Fludioxonil/Metalaxyl-M/Cyclobutrifluram FS (A23793B) for 4 hours. The acute inhalation median lethal concentration of Difenoconazole/Fludioxonil/Metalaxyl-M/Cyclobutrifluram FS (A23793B) in Crl:WI Wistar rats is therefore considered to be above 2.48 mg/L.

2.0 INTRODUCTION

2.1 Purpose

This study was performed to assess the acute inhalation toxicity of Difenoconazole/Fludioxonil/Metalaxyl-M/Cyclobutrifluram FS (A23793B) following a 4-hour nose-only exposure to male and female Crl:WI rats.

2.2 Regulatory Test Guidelines

The study was designed to meet or exceed the regulatory guidelines shown below:

- OECD Guidelines for the Testing of Chemicals No. 403 "Acute Inhalation Toxicity" (adopted: 2009)
- US Environmental Protection Agency Health Effects Division Test Guideline, OPPTS 870.1300, Acute Inhalation Toxicity (1998)
- Council Regulation (EC) No 440/2008, Annex Part B, B.2: "Acute Toxicity (Inhalation)", Official Journal of the European Union No. L 142, (2008)

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 authorised the conduct of the study.

3.0 MATERIALS AND METHODS

3.1 Test Item

The following information was provided by the Sponsor.

Name:	Difenoconazole/Fludioxonil/Metalaxyl M/Cyclobutrifluram FS (A23793B)
Batch number:	1200767
Design code:	A23793B
Active ingredient content*:	Difenoconazole 5.45 % w/w 64.0 g/L, fludioxonil 4.37 % w/w 51.3 g/L, metalaxyl-M 4.31 % w/w 50.6 g/L, cyclobutrifluram 21.0 % w/w 247 g/L
Appearance:	Red liquid
Recertification date:	31 August 2024
Storage conditions:	Room temperature (<30ºC)
Safety precautions:	Routine safety precautions (gloves, goggles, face mask, lab coat) for unknown materials were applied to assure personnel health and safety.

*No adjustment for active ingredient content was applied.
The copy of the Certificate of Analysis is presented in Appendix 4.

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

3.1.1 Identification and receipt

Information relating to the identity, purity and stability of the test item was provided by the Sponsor and identification of the test item on receipt by the Pharmacy Department of Charles River Laboratories Hungary Kft., was made on the basis of these data.

3.1.2 Preparation

During the Technical Trials the undiluted test item and its different formulations (from 60% up to 80% (w/w) aqueous solution) were tested to achieve the maximum attainable atmosphere concentration. Based on the results of these trials, the test item was used as a 70% (w/w) aqueous (Aqua Purificata, Batch number: 2109-8099, Expiry date: 14 March 2022, Manufacturer: MAGILAB Kft., Hungary) formulation. Details of the concentration selection is presented in Appendix 5.

3.1.3 Other materials

Name:	Vaseline
Batch number:	STBJ1482

Expiry date:	31 March 2022
Manufacturer:	Sigma Aldrich
Storage conditions:	Room temperature

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:	Sighting Study: 2 animals / sex Main Study: 5 animals / sex
Sex:	Males and females (nulliparous and non-pregnant)
Age of animals when treated:	Sighting Study: 10 weeks old Main Study: 9 weeks old
Body weight at exposure:	Sighting Study: males: 393 g; females: 204-277 g Main Study: males: 317-391 g; females: 212-263 g
Identification:	The animals were identified by numbers written on the tail with an indelible marker. The cages were marked with individual identity cards with information about study number, sex, cage number, dose group and individual animal numbers.
Randomization:	PROVANTIS v.10 software was used in order to verify homogeneity/variation within groups based on actual body weight.
Acclimatization time:	Sighting Study: 21 days; Main Study: 20 days

3.2.2 Husbandry

Animal health:	Only healthy animals were used for the test. The health status was certified by the Veterinarian.
Housing:	Group caging (2 or 3 animals by sex/cage)
Cage type:	Polypropylene solid floor cages (type II or III) with stainless steel mesh lids
Enrichment:	Rodents were housed with deep wood sawdust bedding to allow digging and other normal rodent activities. Cardboard tunnels produced by LBS (Serving Biotechnology) Ltd., UK was also available to animals during the study. Copies of the Certificate of Analysis are retained in the Archive at Charles River Laboratories Hungary Kft.
Bedding and nesting:	SAFE 3/4-S Hygienic Animal Bedding and nest building material (SAFE crinklets natural) produced by J. Rettenmaier &

	Söhne GmbH+Co. KG, Holzmühle 1, 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:	20.0-23.2°C
Relative humidity:	20-64%
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

The animals were provided with ssniff SM R/M "Autoclavable Complete Feed for Rats and Mice – Breeding and Maintenance" (ssniff Spezialdiäten GmbH, D-59494 Soest, Germany) *ad libitum*. The content of the standard diet and the test report of the diet analysis, provided by the manufacturer are retained in the archives of Charles River Laboratories Hungary Kft. The food was considered not to contain any contaminants that could reasonably be expected to affect the purpose or integrity of the study.

3.2.4 Water supply and quality control

Animals received tap water from the municipal supply from a 400 mL or 500 mL bottles *ad libitum*. The water was considered not to contain any contaminants that could reasonably be expected to affect the purpose or integrity of the study.

The quality control analysis is performed once every three months and microbiological assessment is performed monthly, by Veszprém County Institute of State Public Health and Medical Officer Service (H-8200 Veszprém, József Attila utca 36, Hungary). Copies of the relevant Certificates of Analysis are retained in the archive of Charles River Laboratories Hungary Kft.

3.3 Inhalation Exposure

3.3.1 Technical trials

Prior to animal exposures, test item atmospheres were generated within the exposure chamber. During these Technical Trials, test item input rates were varied to achieve the required aerosol concentration of particles with a mass median aerodynamic diameter (MMAD) between 1 to 4 µm and a geometric standard deviation (GSD) in the range of 1.5 to 3.0. Measurements of aerodynamic particle size were performed from the animal's breathing zone using a cascade impactor (Details are presented in Appendix 5.).

3.3.2 Atmosphere generation

The test item formulation was aerosolised using a stainless steel concentric jet nebuliser (TSE Systems GmbH, Bad Homburg, Germany) located at the top of the exposure chamber. The rate of test item use was controlled by a syringe pump. Compressed air was supplied by means of an oil-free compressor passed through a suitable filter system prior to introduction to the nebuliser.

3.3.3 Animal exposure system

The animals were exposed, nose-only, to an atmosphere of the test item using a TSE Rodent Exposure System (TSE Systems GmbH, Bad Homburg, Germany). This system comprised of 2 concentric anodised aluminium chambers and a computer control system incorporating pressure detectors and mass flow controllers.

Fresh aerosol from the generation system was constantly supplied to the inner plenum (distribution chamber) of the exposure system from where, under positive pressure, it was distributed to the individual exposure ports. The animals were held in polycarbonate restraint tubes located around the chamber which allowed only the animal's nostrils to enter the exposure port. After passing through the animal's breathing zone, used aerosol entered the outer cylinder from where it was exhausted through a suitable filter system. Atmosphere generation was therefore dynamic. A schematic diagram of the exposure system is presented in Figure 1.

Airflows and relative pressures within the system were constantly monitored and controlled by the computer system thus ensuring a uniform distribution and constant flow of fresh aerosol to each exposure port (breathing zone). The flow of air through each port was at least 0.5 L/min. This flow rate was considered adequate to minimise re-breathing of the test atmosphere as it is about twice the respiratory minute volume of a rat.

Homogeneity of the test atmosphere within the test chamber and amongst the exposure ports was not specifically determined during this study. However, chambers of this design have been fully validated and have shown to produce evenly distributed atmospheres in the animals' breathing zones (Ref. 1).

3.3.4 Sighting exposure

Sighting Exposure was performed with 2 male and 2 female rats in order to estimate the test item's inhalation toxicity, identify sex differences in susceptibility and assist in selecting exposure concentration levels for the Main Study.

3.3.5 Main study

Based on the results of the Sighting Exposure a Limit Test was performed with 5 males and 5 females to assess the acute inhalation toxicity of the test item.

3.3.6 Exposure procedure

Each rat was individually held in a tapered, polycarbonate restraining tube fitted onto a single tier of the exposure chamber. Only the nose of each animal was exposed to the test atmosphere.

Following an equilibration period of at least the theoretical chamber equilibration time (T_{99}) (Ref. 2), a sighting group of 4 rats (2 males and 2 females) were exposed to the maximum attainable mean concentration for a period 4 hours. Based on the results of this Sighting Study, a Limit Test was performed, in which a main group of 10 rats (5 males and 5 females) were exposed to the maximum achievable mean concentration for a period 4 hours.

Before the animal exposures, the hairs across the closed eye surface of each animal was wiped with Vaseline (see details in 3.1.3), to reduce the test item getting into the eyes while they were in the restraint tube. No remaining Vaseline was noted in the eyes of the animals at the end of the exposures.

No control animals were used in the study.

3.4 Exposure Monitoring

3.4.1 Test atmosphere concentrations

Prior to atmosphere generation, the non-volatile component of the test material was determined by adding a small, known amount of the material to glass fibre filters (Type GF/C, Whatman, GE Healthcare UK Limited UK, Lot No. 17160519). The filters were then dried at atmospheric pressure in a desiccator at room temperature for at least 24 hours and weighed again. The difference in the two weights was taken as the volatile content of the test material and the non-volatile component was calculated as a percentage. The mean non-volatile content of the batch used for the animals' exposure was found to be 62.15% (n = 10) with a standard deviation 0.82 %.

The test atmosphere was sampled at regular intervals during the exposure period. Samples were taken from an unoccupied exposure port (representing the animal’s breathing zone) by pulling a suitable, known volume of test atmosphere through weighed GF10 glass fibre filters (Type GF10, Whatman, GE Healthcare UK Limited UK, Lot Numbers: A29518736).

After sampling, the filters were dried (under the same conditions as those previously described) and weighed again 24 hours later. The difference in the pre and post sampling weights, corrected by mean of non-volatile content (62.15%) and divided by the volume of atmosphere sampled, was equal to the actual achieved test atmosphere concentration.

Filter samples were collected at the breathing zone (approximately every 10-20 minutes) during each 4-hour exposure period and analysed.

The nominal concentration was calculated by dividing the mass of test material disseminated into the chamber by the total volume of air that went through the chamber during the same period.

3.4.2 Particle size analysis

The particle size of the test atmosphere was determined three times during the exposure period using a 7-stage impactor of Mercer style (TSE Systems GmbH, Bad Homburg, Germany). Such devices employ an inertial separation technique to isolate particles in the discrete aerodynamic size ranges. Samples were taken from an unoccupied exposure port (representing the animal's breathing zone).

The collection substrates and the backup filter were weighed before and after sampling and the weight of test item, collected at each stage, calculated by this difference.

The total amount collected for each stage was used to determine the cumulative amount below each cut-off point size. In this way, the proportion (%) of aerosol less than 0.550, 0.550; 0.960, 1.550, 2.105, 3.555, 6.655 and 10.550 µm was calculated.
These are considered to be valid when the particle distribution approximates to a 'normal curve' distribution.
From these data, using software supplied with the impactor (TSE Systems GmbH, Bad Homburg, Germany), the Mass Median Aerodynamic Diameter (MMAD), and Geometric Standard Deviation (GSD) were calculated. These are considered to be valid when particle distribution approximates to a 'normal curve' distribution. In addition, the proportion (%) of aerosol less than 4µm (considered to be the respirable portion) was determined.

3.4.3 Chamber environmental conditions

The following variables were monitored continuously and recorded during each exposure period by the monitoring system integrated into the exposure system:

- Chamber airflow rates
- Test atmosphere temperature
- Test atmosphere relative humidity

Summaries of the data are presented in Table 3.

3.5 Observations

3.5.1 Clinical observations

All animals were observed for clinical signs at hourly intervals during exposure whilst the animals were still restrained. Following exposure, clinical observations were performed twice on the day of exposure (following removal from the restrainer and approximately one hour after completion of the exposure) and subsequently once daily for 14 days or until death.

Observations included changes in the skin and fur, eyes and mucous membranes and also respiratory, circulatory, autonomic and central nervous system, somatomotor activity and behaviour pattern. Particular attention was directed to observation of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma.

3.5.2 Bodyweight

Individual bodyweights were recorded prior to treatment on the day of exposure (Day 0) and on Days 1, 3, 7 and 14 or at death.

3.6 *Post Mortem* Investigation

All animals were subjected to macroscopic examination. All surviving animals were exsanguinated under pentobarbital anaesthesia (Euthanimal 40% injection) (details in 3.6.1). After examination of the external appearance, the thoracic and abdominal cavities were opened and the appearance of the tissues and organs were observed. Any gross macroscopic changes were recorded. Special attention was given to the respiratory tract for macroscopic signs of irritancy or local toxicity.

3.6.1 Materials used for euthanasia

Name:	Euthanimal 40% (400 mg/mL sodium pentobarbital)
Batch No.:	2001004-06
Expiry Date:	31 January 2023
Produced by:	Alfasan Nederland BV, The Netherlands

3.7 Evaluation of Data

Data evaluations included the relationship, if any, between the animals' exposure to the test item and the incidence and severity of all abnormalities including mortality, behavioural or clinical changes, bodyweight changes, macroscopic abnormalities or any other toxicological effects.

Data were collected using the software PROVANTIS v.10 or were recorded on data collection sheets taken from the relevant SOPs, then tabulated using PROVANTIS v.10, Microsoft Office Word and/or Excel, as appropriate.

Only a Limit Test was performed, the four-hour inhalation LC_{50} was not calculated.

4.0 RESULTS AND DISCUSSION

4.1 Test Atmosphere Concentration

The test atmosphere concentration was sampled at approximately equal intervals during the exposure and the actual concentration of the test item calculated. The mean values obtained were:

Group	Mean achieved concentration (mg/L)	Standard Deviation	Nominal Concentration (mg/L)
0.1 (Sighting exposure)	2.55	0.23	82.92
1 (Main Study)	2.48	0.15	85.82

The individual data are presented graphically in Figure 2 and detailed in Table 1.

4.2 Particle Size Analysis

The particle size distribution of the test atmosphere was as follows:

Group	Mean achieved concentration (mg/L)	Mean Mass Median Aerodynamic Diameter (MMAD) (μm)	Geometric Standard Deviation (GSD)	Respirable Fraction (% < 4μm)
0.1 (Sighting exposure)	2.55	3.87	2.15	51.7
1 (Main Study)	2.48	3.53	2.11	56.7

The particle size analysis showed that the distribution was approximately 'normal', hence the calculations were considered to be valid.

The data are presented graphically in Figure 3 and detailed in Table 2.

4.3 Mortality Rates

No mortality occurred in the Sighting Study.
One out of five female animals was found dead on Day 0 during the exposure in the Main Study.

Mortality data are detailed in Table 4.

4.4 Clinical Observations

Group 0.1 (Sighting Exposure – 2.55 mg/L)
In the male animals, laboured respiration (slight), noisy respiration (slight), fur staining by test item (on the head, whole body and on the first third of animal) and wet fur (on the whole body) were observed on Day 0-3. All the animals were symptom-free from Day 4.

In the female animals, laboured respiration (slight), fur staining by test item (on the head, whole body and on the first third of animal) and wet fur (on the whole body) were observed on Days 0-3. All the animals were symptom-free from Day 4.

Group 1 (Main Exposure – 2.48 mg/L)
In the male animals, laboured respiration (slight), fur staining by test item (on the whole body and/or first third of animal and on the head) and wet fur (on the whole body) were noted on Days 0-3. All the animals were symptom-free from Day 4.

In the surviving female animals, laboured respiration (slight), fur staining by test item (on the whole body and/or first third of animal) and wet fur (on the whole body) were observed on Days 0-4. These animals were symptom free from Day 5.

Wet fur and fur staining (as chromodacryorrhea) in the animals were considered to be related to the restraint and exposure procedures but not to be toxicologically significant.

Individual clinical observations are presented in Appendix 1.

4.5 Bodyweight

Group 0.1 (Sighting Exposure – 2.55 mg/L)
In case of male animals, slight body weight losses were noted on Days 0-1. The body weight gains were normal on Days 1-14.
In the female animals, no body weight loss was noted. The body weight gains were normal in both animals on Days 0-14.

Group 1 (Main Exposure – 2.48 mg/L)
In the male animals, slight body weight losses were observed on Days 0-1. The body weight gains were normal in all male animals on Days 1-14.

In case of the surviving female animals, slight body weight losses were observed in three out of four animals on Days 0-1 and in two out of four animals on Days 3-7. The body weight gains were normal in one animal on Days 0-14, in two out of four animals on Day 1-3 and on Days 7-14 and in case of one female animal on Days 1-14.

Individual data, together with bodyweight changes, are presented in Appendix 2.

4.6 Necropsy

Necropsy

Group 0.1 (Sighting Exposure – 2.55 mg/L)
No macroscopic observations were seen on scheduled necropsy Day 14.

Group 1 (Main Exposure – 2.48 mg/L)
In the found dead female animal, diffuse red discoloration of all lobes of the non-collapsed lungs and multifocal red discoloration of thymus were observed. No macroscopic observations were seen on scheduled necropsy Day 14 in the surviving animals.

Individual necropsy data are presented in Appendix 3.

5.0 CONCLUSIONS

Under the experimental conditions of this study, one mortality occurred in a group of 10 rats when exposed to 2.48 mg/L of Difenoconazole/Fludioxonil/Metalaxyl-M/Cyclobutrifluram FS (A23793B) for 4 hours. The acute inhalation median lethal concentration of Difenoconazole/Fludioxonil/Metalaxyl-M/Cyclobutrifluram FS (A23793B) in Crl:WI Wistar rats is therefore considered to be above 2.48 mg/L.

6.0 REFERENCES

1. Pauluhn J. (1994): Validation of an Improved Nose-Only Exposure System for Rodents. J. App. Tox. 14 (1), 55-62
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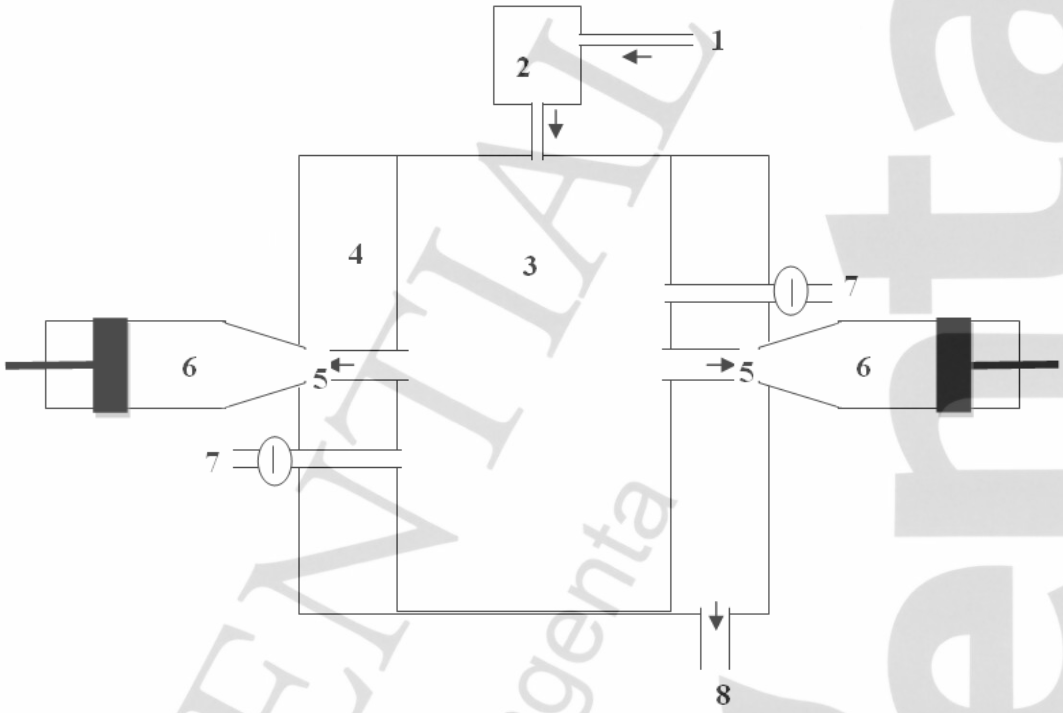
5. OECD Guidance Document on the Recognition, Assessment and Use of Clinical Signs as Humane Endpoints for Experimental Animals Used in Safety Evaluation, Environmental Health and Safety Monograph Series on testing and assessment No.19 (2000)
6. US Environmental Protection Agency (EPA) Health Effects Test Guidelines, OPPTS 870.1300, Acute Inhalation Toxicity (1998)
7. Council Regulation (EC) No 440/2008, Annex Part B, B.2: "Acute Toxicity (Inhalation)", Official Journal of the European Union No. L 142, (2008)
8. Finney, D. J. (1985): The median Lethal Dose and its Estimation; Archives of Toxicology, 56, 215-218

FIGURES SECTION

FIGURE 1 Schematic Diagram of the Exposure System

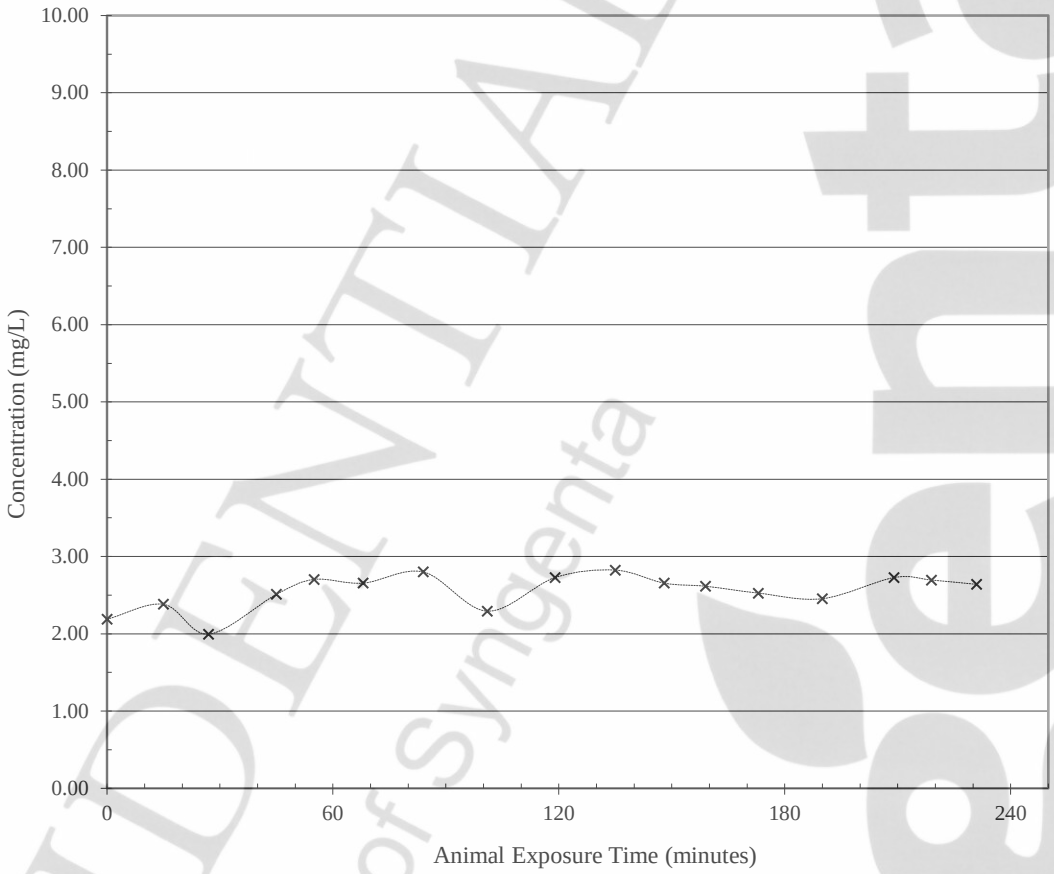


KEY:

1:	Metered Air Supply	5:	Animal Exposure Port
2:	Aerosol Generation System	6:	Animal Restraint Tube
3:	Central Plenum	7:	Sample Ports (not used)
4:	Outer Cylinder	8:	Metered Exhaust to Filters

FIGURE 2 Achieved Atmosphere Concentrations

Sighting Exposure – Group 0.1



Main Study – Group 1

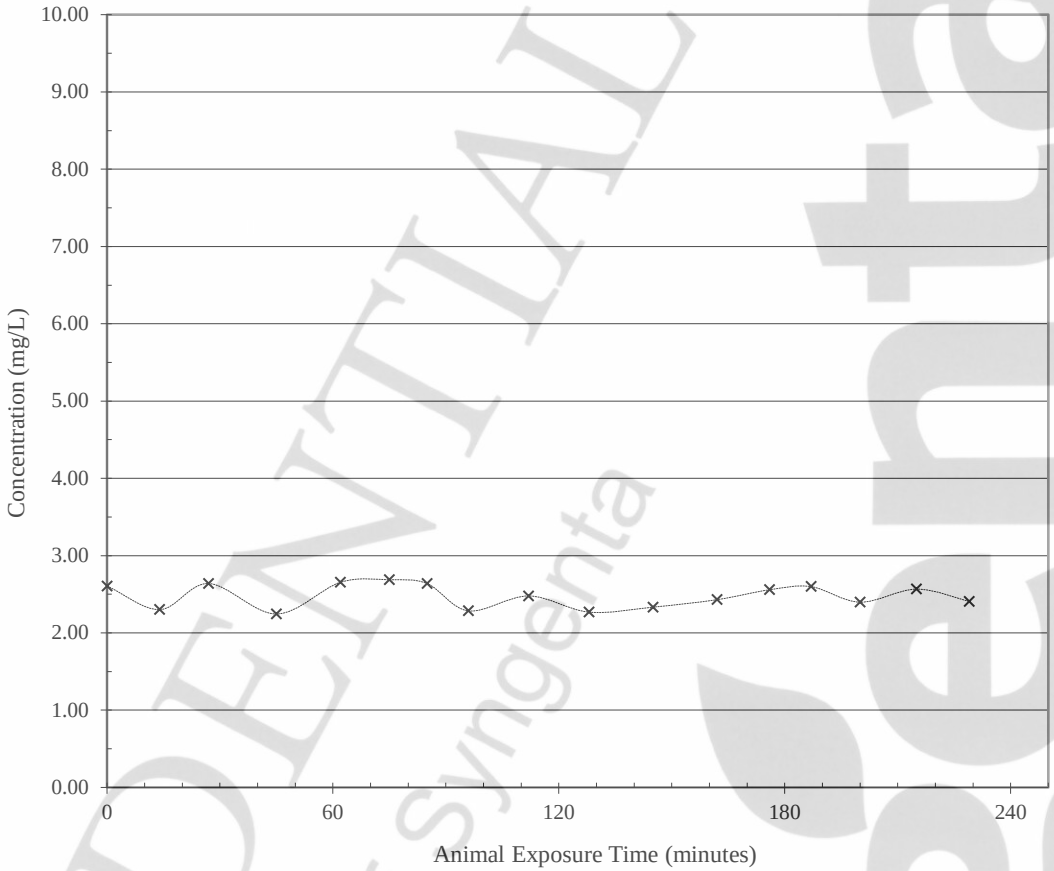
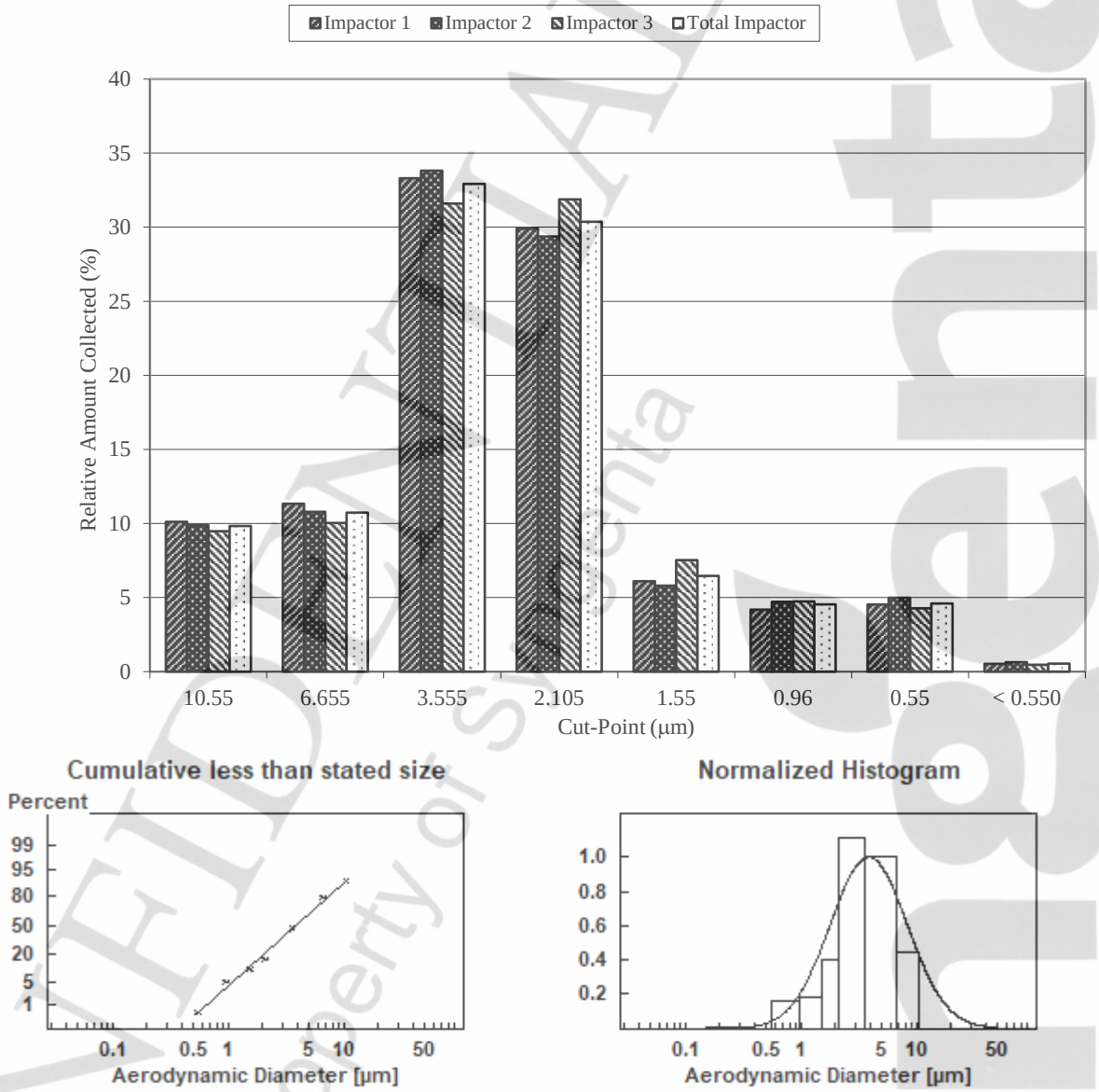


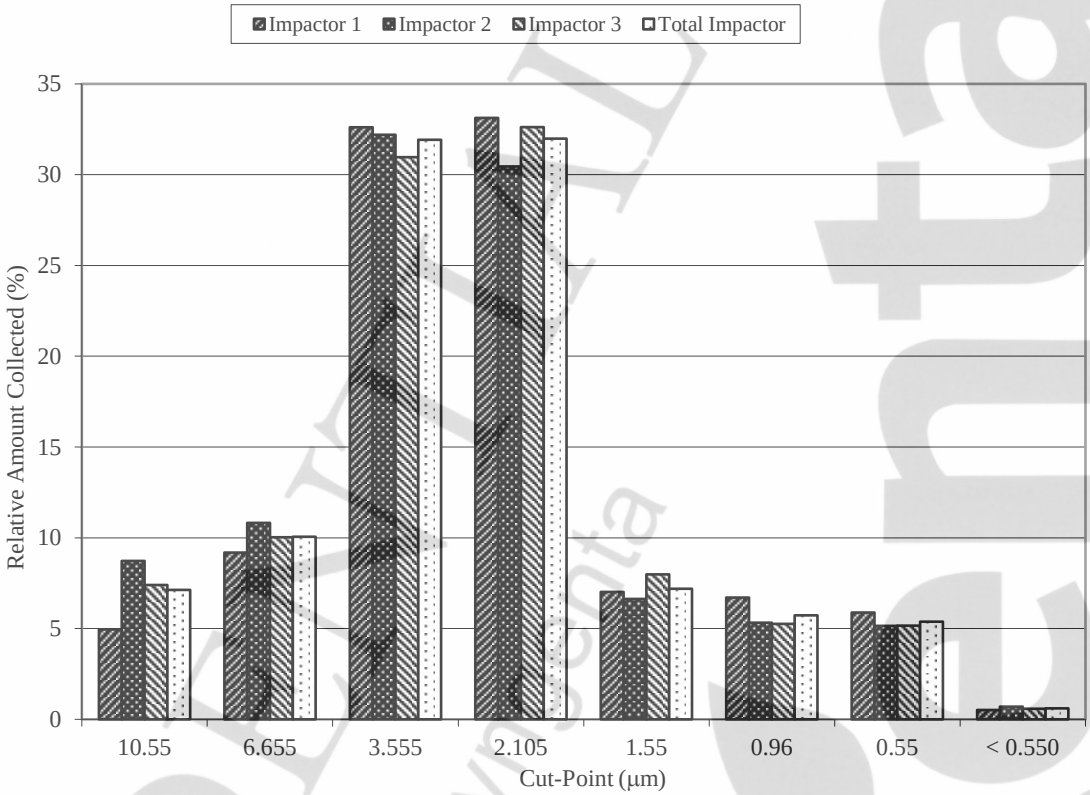
FIGURE 3 Particle Size Distribution

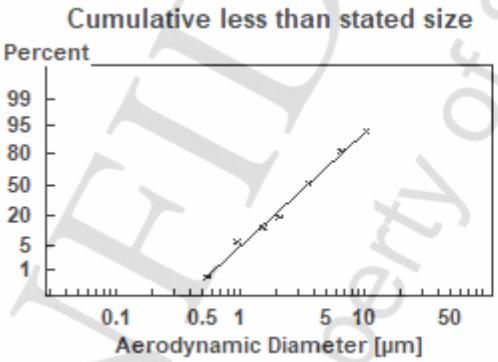
Sighting Exposure – Group 0.1

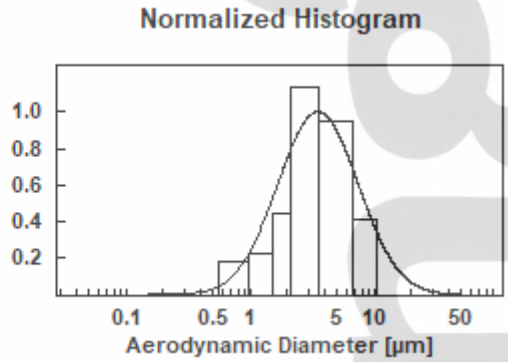


The data distribution was considered by the Study Director to be adequately 'normal' for the automated data describing the atmosphere characteristice to be valid.

Main Study – Group 1







The data distribution was considered by the Study Director to be adequately 'normal' for the automated data describing the atmosphere characteristice to be valid.

TABLES SECTION

TABLE 1 Test Atmosphere Concentrations

Sighting Exposure – Group 0.1

Exposure Duration (minutes)	Sample Volume (L)	Amount of Non Volatiles Collected* (mg)	Equivalent Test Item Amount (mg)	Atmospheric Concentration of the Test Item (mg/L)
0	2.0	2.72	4.38	2.19
15	2.0	2.96	4.76	2.38
27	2.0	2.48	3.99	2.00
45	2.0	3.12	5.02	2.51
55	2.0	3.36	5.41	2.70
68	2.0	3.30	5.31	2.65
84	2.0	3.48	5.60	2.80
101	2.0	2.85	4.59	2.29
119	2.0	3.39	5.45	2.73
135	2.0	3.51	5.65	2.82
148	2.0	3.30	5.31	2.65
159	2.0	3.25	5.23	2.61
173	2.0	3.14	5.05	2.53
190	2.0	3.05	4.91	2.45
209	2.0	3.39	5.45	2.73
219	2.0	3.35	5.39	2.70
231	2.0	3.28	5.28	2.64

Mean achieved concentration = 2.55 mg/L
Standard Deviation = 0.23

Amount of Test Item Used (g): 624.40
Total Volume of Air Used (L): 7530
Nominal Concentration (mg/L): 82.92

* = non-volatile content of Difenoconazole/Fludioxonil/Metalaxyl-M/Cyclobutrifluram FS (A23793B) was 62.15%

Main Study – Group 1

Exposure Duration (minutes)	Sample Volume (L)	Amount of Non Volatiles Collected* (mg)	Equivalent Test Item Amount (mg)	Atmospheric Concentration of the Test Item (mg/L)
0	2.0	3.24	5.21	2.61
14	2.0	2.86	4.60	2.30
27	2.0	3.28	5.28	2.64
45	2.0	2.79	4.49	2.24
62	2.0	3.30	5.31	2.65
75	2.0	3.34	5.37	2.69
85	2.0	3.28	5.28	2.64
96	2.0	2.84	4.57	2.28
112	2.0	3.08	4.96	2.48
128	2.0	2.82	4.54	2.27
145	2.0	2.90	4.67	2.33
162	2.0	3.02	4.86	2.43
176	2.0	3.18	5.12	2.56
187	2.0	3.23	5.20	2.60
200	2.0	2.98	4.79	2.40
215	2.0	3.19	5.13	2.57
229	2.0	2.99	4.81	2.41

Mean achieved concentration = 2.48 mg/L
Standard Deviation = 0.15

Amount of Test Item Used (g): 646.23
Total Volume of Air Used (L): 7530
Nominal Concentration (mg/L): 85.82

* = non-volatile content of Difenoconazole/Fludioxonil/Metalaxyl-M/Cyclobutrifluram FS (A23793B) was 62.15%

TABLE 2 Test Atmosphere Particle Size Distribution Data

Sighting Exposure – Group 0.1

Stage Number	Cut Point (µm)	Amount Collected (mg)			Total Collected per Stage (mg)
		Sample 1	Sample 2	Sample 3	
1	10.550	1.16	1.09	1.02	3.27
2	6.655	1.30	1.19	1.08	3.57
3	3.555	3.82	3.73	3.40	10.95
4	2.105	3.43	3.24	3.43	10.10
5	1.550	0.70	0.64	0.81	2.15
6	0.960	0.48	0.52	0.51	1.51
7	0.550	0.52	0.55	0.46	1.53
Filter	< 0.550	0.06	0.07	0.05	0.18
				Total Amount Collected (mg)	33.26

Size Range (µm)	Total Mass/stage (mg)	Cumulative Mass (%)
< 0.550	0.18	0.54
0.550 - 0.960	1.53	5.14
0.960 - 1.550	1.51	9.68
1.550 - 2.105	2.15	16.15
2.105 – 3.555	10.10	46.51
3.555 - 6.655	10.95	79.43
6.655 – 10.550	3.57	90.17
> 10.550	3.27	100.00

Mean achieved concentration = 2.55 mg/L

Mean Mass Median Aerodynamic Diameter (MMAD) = 3.87 µm
Geometric Standard Deviation (GSD) = 2.15
Respirable Fraction (% < 4µm) = 51.7 %

Main Study – Group 1

Stage Number	Cut Point (µm)	Amount Collected (mg)			Total Collected per Stage (mg)
		Sample 1	Sample 2	Sample 3	
1	10.550	0.48	1.00	0.76	2.24
2	6.655	0.89	1.24	1.03	3.16
3	3.555	3.16	3.69	3.18	10.03
4	2.105	3.21	3.49	3.35	10.05
5	1.550	0.68	0.76	0.82	2.26
6	0.960	0.65	0.61	0.54	1.80
7	0.550	0.57	0.59	0.53	1.69
Filter	< 0.550	0.05	0.08	0.06	0.19
				Total Amount Collected (mg)	31.42

Size Range (µm)	Total Mass/stage (mg)	Cumulative Mass (%)
< 0.550	0.19	0.60
0.550 - 0.960	1.69	5.98
0.960 - 1.550	1.80	11.71
1.550 - 2.105	2.26	18.91
2.105 – 3.555	10.05	50.89
3.555 - 6.655	10.03	82.81
6.655 – 10.550	3.16	92.87
> 10.550	2.24	100.00

Mean achieved concentration = 2.48 mg/L

Mean Mass Median Aerodynamic Diameter (MMAD) = 3.53 µm
Geometric Standard Deviation (GSD) = 2.11
Respirable Fraction (% < 4µm) = 56.7%

TABLE 3 Test Chamber Environmental and Equilibration Data

Sighting Exposure – Group 0.1

Measurement	Mean Value	Minimum	Maximum
Air Flow In (Inner Plenum) (L/min)	30.0	25.3	30.1
Air Flow Out (Outer Cylinder) (L/min)	31.0	30.8	31.4
Temperature* **(°C)**	23.9	23.6	24.2
Relative Humidity† **(%)**	73.3	68.7	81.6

Theoretical Chamber Equilibration Time (T_{99}):

$T_{99} = (4.605 \times (^{\text{Chamber Volume}}/_{\text{Chamber Flow rate}})$ (Silver, 1946)
Chamber volume (inner plenum) = 3.85 L (Pauluhn, 1994)
T_{99} (Minimum Acceptable Equilibration Time) = 1 minute
Actual equilibration time allowed = 12 minutes

* Temperature was measured by handheld thermometer.
† The chamber humidity was measured by handheld hygrometer.

Main Study – Group 1

Measurement	Mean Value	Minimum	Maximum
Air Flow In (Inner Plenum) (L/min)	30.1	29.6	32.2
Air Flow Out (Outer Cylinder) (L/min)	31.0	30.7	31.3
Temperature* **(°C)**	23.2	23.1	23.3
Relative Humidity† **(%)**	70.9	69.3	72.7

Theoretical Chamber Equilibration Time (T_{99}):

$T_{99} = (4.605 \times (^{\text{Chamber Volume}}/_{\text{Chamber Flow rate}})$ (Silver, 1946)
Chamber volume (inner plenum) = 3.85 L (Pauluhn, 1994)
T_{99} (Minimum Acceptable Equilibration Time) = 1 minute
Actual equilibration time allowed = 12 minutes

* Temperature was measured by handheld thermometer.
† The chamber humidity was measured by handheld hygrometer.

TABLE 4 Mortality Data

Day Number	Number of Deaths			
	Group 0.1		Group 1	
	Male	Female	Male	Female
0 (During Exposure)	0	0	0	1
0 (After Exposure)	0	0	0	0
1	0	0	0	0
2	0	0	0	0
3	0	0	0	0
4	0	0	0	0
5	0	0	0	0
6	0	0	0	0
7	0	0	0	0
8 – 14	0	0	0	0
Total Deaths	0/2	0/2	0/5	1/5
Grand Total Deaths	0/4		1/10	

APPENDICES SECTION

APPENDIX 1 Individual Clinical Observations

SIGHTING EXPOSURE
DOSE GROUP: **0.1**
CONCENTRATION: **2.55 mg/L**

SEX: MALE

Animal number	Observations	Days of study: 0 (exposure) 0	during 1h	during 2h	during 3h	after 4h	after 5h	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Frequency	
1587♂	Normal	/	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	11	/19
	Fur staining by test item - Head	/	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	/19
	Fur staining by test item - Whole body	/	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	2	/19
	Fur staining by test item - First third of animal	/	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	1	/19
	Laboured respiration	/	Sl	Sl	Sl	Sl	-	Sl	-	-	-	-	-	-	-	-	-	-	-	-	-	5	/19
	Noisy respiration	/	-	-	-	-	-	Sl	-	-	-	-	-	-	-	-	-	-	-	-	-	1	/19
	Wet fur- Whole body	/	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	/19
1598♂	Normal	/	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	11	/19
	Fur staining by test item - Head	/	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	/19
	Fur staining by test item - Whole body	/	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	2	/19
	Fur staining by test item - First third of animal	/	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	1	/19
	Laboured respiration	/	Sl	Sl	Sl	Sl	-	Sl	-	-	-	-	-	-	-	-	-	-	-	-	-	5	/19
	Noisy respiration	/	-	-	-	-	-	Sl	-	-	-	-	-	-	-	-	-	-	-	-	-	1	/19
	Wet fur- Whole body	/	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	/19

Standard footnotes:
+ = present - = absent
h = hour (s) ' = minute
# = Found dead M = Moribund / = No clinical observation (was) done at 0
Frequency of observation = number of occurrence of observation / total number of observations

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

APPENDIX 1 Individual Clinical Observations

SIGHTING EXPOSURE
DOSE GROUP: **0.1**
CONCENTRATION: **2.55 mg/L**

SEX: FEMALE

Animal number	Observations	Days of study: 0 (exposure) during 0	1h	2h	3h	after 4h	5h	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Frequency	
1632♀	Normal	/	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	11	/19
	Fur staining by test item - Head	/	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	/19
	Fur staining by test item- Whole body	/	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	2	/19
	Fur staining by test item- First third of animal	/	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	2	/19
	Laboured respiration	/	Sl	Sl	Sl	Sl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	/19
	Wet fur- Whole body	/	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	/19
1626♀	Normal	/	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	12	/19
	Fur staining by test item - Head	/	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	4	/19
	Laboured respiration	/	Sl	Sl	Sl	Sl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	/19
	Wet fur- Whole body	/	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	/19

Standard footnotes:
+ = present - = absent
h = hour (s) ' = minute
# = Found dead M = Moribund / = No clinical observation (was) done at 0
Frequency of observation = number of occurrence of observation / total number of observations

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

APPENDIX 1 Individual Clinical Observations

MAIN STUDY
DOSE GROUP: **1**
CONCENTRATION: **2.48 mg/L** SEX: MALE

Animal number	Observations	Days of study: 0 (exposure) during 0	1h	2h	3h	after 4h	5h	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Frequency
1875♂	Normal	/	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	12 /19
	Fur staining by test item - Whole body	/	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	2 /19
	Fur staining by test item - First third of animal	/	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2 /19
	Laboured respiration	/	Sl	Sl	Sl	Sl	Sl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5 /19
	Wet fur - Whole body	/	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2 /19
1878♂	Normal	/	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	12 /19
	Fur staining by test item - Whole body	/	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	1 /19
	Fur staining by test item - First third of animal	/	-	-	-	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	3 /19
	Laboured respiration	/	Sl	Sl	Sl	Sl	Sl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5 /19
	Wet fur - Whole body	/	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2 /19
1881♂	Normal	/	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	11 /19
	Fur staining by test item - Head	/	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	2 /19
	Fur staining by test item - Whole body	/	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	1 /19
	Fur staining by test item - First third of animal	/	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2 /19
	Laboured respiration	/	Sl	Sl	Sl	Sl	Sl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5 /19
	Wet fur - Whole body	/	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2 /19

Standard footnotes:
+ = present - = absent
h = hour (s) ' = minute
# = Found dead M = Moribund / = No clinical observation (was) done at 0
Frequency of observation = number of occurrence of observation / total number of observations

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

APPENDIX 1 Individual Clinical Observations

MAIN STUDY
DOSE GROUP: **1**
CONCENTRATION: **2.48 mg/L** SEX: MALE

Animal number	Observations	Days of study: 0 (exposure) during 0	1h	2h	3h	after 4h	5h	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Frequency	
1886♂	Normal	/	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	11	/19
	Fur staining by test item - Head	/	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	2	/19
	Fur staining by test item - First third of animal	/	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	3	/19
	Laboured respiration	/	Sl	Sl	Sl	Sl	Sl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5	/19
	Wet fur - Whole body	/	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	/19
1888♂	Normal	/	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	11	/19
	Fur staining by test item - Head	/	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	1	/19
	Fur staining by test item - Whole body	/	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	1	/19
	Fur staining by test item - First third of animal	/	-	-	-	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	3	/19
	Laboured respiration	/	Sl	Sl	Sl	Sl	Sl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5	/19
	Wet fur - Whole body	/	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	/19

Standard footnotes:
+ = present - = absent
h = hour (s) ' = minute
# = Found dead M = Moribund / = No clinical observation (was) done at 0
Frequency of observation = number of occurrence of observation / total number of observations

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

APPENDIX 1 Individual Clinical Observations

MAIN STUDY
DOSE GROUP: **1**
CONCENTRATION: **2.48 mg/L**

SEX: FEMALE

Animal number	Observations	Days of study: 0 (exposure) during 0	1h	2h	3h	after 4h	5h	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Frequency	
1931♀	Normal	/	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	10	/19
	Fur staining by test item - First third of animal	/	-	-	-	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	6	/19
	Laboured respiration	/	Sl	Sl	Sl	Sl	Sl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5	/19
	Wet fur - Whole body	/	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	/19
1929♀	Normal	/	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	10	/19
	Fur staining by test item - Whole body	/	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	3	/19
	Fur staining by test item - First third of animal	/	-	-	-	+	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	3	/19
	Laboured respiration	/	Sl	Sl	Sl	Sl	Sl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5	/19
	Wet fur - Whole body	/	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	/19
1928♀	Normal	/	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	10	/19
	Fur staining by test item - First third of animal	/	-	-	-	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	6	/19
	Laboured respiration	/	Sl	Sl	Sl	Sl	Sl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5	/19
	Wet fur - Whole body	/	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	/19
1938♀#	Found dead	+																				-	
1924♀	Normal	/	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	10	/19
	Fur staining by test item - Whole body	/	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	4	/19
	Fur staining by test item - First third of animal	/	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	/19
	Laboured respiration	/	Sl	Sl	Sl	Sl	Sl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5	/19
	Wet fur - Whole body	/	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	/19

Standard footnotes:
+ = present - = absent
h = hour (s) ' = minute
# = Found dead M = Moribund / = No clinical observation (was) done at 0
Frequency of observation = number of occurrence of observation / total number of observations

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

APPENDIX 2 Individual Bodyweight Data

SIGHTING EXPOSURE
DOSE GROUP: 0.1
CONCENTRATION: 2.55 mg/L SEX: MALE

Animal Number	Body weight (g) on days					Day/B.W. (g) Death	Body weight gain (g) between days				
	0	1	3	7	14		0-1	1-3	3-7	7-14	0-14
1587♂	393	390	390	421	455	-	-3	0	31	34	62
1598♂	393	386	390	405	431	-	-7	4	15	26	38
Mean:	393.0	388.0	390.0	413.0	443.0	-	-5.0	2.0	23.0	30.0	50.0
Standard deviation:	0.0	2.8	0.0	11.3	17.0	-	2.8	2.8	11.3	5.7	17.0

DOSE GROUP: 0.1
CONCENTRATION: 2.55 mg/L SEX: FEMALE

Animal Number	Body weight (g) on days					Day/B.W. (g) Death	Body weight gain (g) between days				
	0	1	3	7	14		0-1	1-3	3-7	7-14	0-14
1632♀	277	283	285	287	309	-	6	2	2	22	32
1626♀	204	210	220	229	240	-	6	10	9	11	36
Mean:	240.5	246.5	252.5	258.0	274.5	-	6.0	6.0	5.5	16.5	34.0
Standard deviation:	51.6	51.6	46.0	41.0	48.8	-	0.0	5.7	4.9	7.8	2.8

MAIN STUDY
DOSE GROUP: 1
CONCENTRATION: 2.48 mg/L SEX: MALE

Animal Number	Body weight (g) on days					Day/B.W. (g) Death	Body weight gain (g) between days				
	0	1	3	7	14		0-1	1-3	3-7	7-14	0-14
1875♂	343	326	338	359	383	-	-17	12	21	24	40
1878♂	391	375	385	408	439	-	-16	10	23	31	48
1881♂	317	314	328	341	368	-	-3	14	13	27	51
1886♂	328	321	336	347	367	-	-7	15	11	20	39
1888♂	386	369	377	398	425	-	-17	8	21	27	39
Mean:	353.0	341.0	352.8	370.6	396.4	-	-12.0	11.8	17.8	25.8	43.4
Standard deviation:	33.7	28.7	26.2	30.5	33.5	-	6.6	2.9	5.4	4.1	5.7

DOSE GROUP: 1
CONCENTRATION: 2.48 mg/L SEX: FEMALE

Animal Number	Body weight (g) on days					Day/B.W. (g) Death	Body weight gain (g) between days				
	0	1	3	7	14		0-1	1-3	3-7	7-14	0-14
1931♀	250	247	277	257	279	-	-3	30	-20	22	29
1929♀	212	216	228	233	237	-	4	12	5	4	25
1928♀	263	256	275	276	294	-	-7	19	1	18	31
1938♀#	260	-	-	-	-	0/253	-	-	-	-	-
1924♀	237	231	242	241	261	-	-6	11	-1	20	24
Mean:	244.4	237.5	255.5	251.8	267.8	-	-3.0	18.0	-3.8	16.0	27.3
Standard deviation:	20.8	17.7	24.4	19.0	24.5	-	5.0	8.8	11.1	8.2	3.3

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

APPENDIX 3 Individual Necropsy Findings

SIGHTING EXPOSURE
CONCENTRATION: 2.55 mg/L **SEX: MALE**

Dose group	Animal Number	Necropsy Day	External Observations	Internal Observations	Organ/Tissue
0.1	1587♂	Day 14	No external observations recorded	No internal observations recorded	Not applicable
	1598♂	Day 14	No external observations recorded	No internal observations recorded	Not applicable

CONCENTRATION: 2.55 mg/L **SEX: FEMALE**

Dose group	Animal Number	Necropsy Day	External Observations	Internal Observations	Organ/Tissue
0.1	1632♀	Day 14	No external observations recorded	No internal observations recorded	Not applicable
	1626♀	Day 14	No external observations recorded	No internal observations recorded	Not applicable

MAIN STUDY
CONCENTRATION: 2.48 mg/L **SEX: MALE**

Dose group	Animal Number	Necropsy Day	External Observations	Internal Observations	Organ/Tissue
1	1875♂	Day 14	No external observations recorded	No internal observations recorded	Not applicable
	1878♂	Day 14	No external observations recorded	No internal observations recorded	Not applicable
	1881♂	Day 14	No external observations recorded	No internal observations recorded	Not applicable
	1886♂	Day 14	No external observations recorded	No internal observations recorded	Not applicable
	1888♂	Day 14	No external observations recorded	No internal observations recorded	Not applicable

CONCENTRATION: 2.48 mg/L **SEX: FEMALE**

Dose group	Animal Number	Necropsy Day	External Observations	Internal Observations	Organ/Tissue
1	1931♀	Day 14	No external observations recorded	No internal observations recorded	Not applicable
	1929♀	Day 14	No external observations recorded	No internal observations recorded	Not applicable
	1928♀	Day 14	No external observations recorded	No internal observations recorded	Not applicable
	1938♀#	Day 0	Cause of Death: Undetermined	Discoloration; red, diffuse, all lobes	Lungs
				Non collapsed	
				Discoloraton; red, multifocal	Thymus
	1924♀	Day 14	No external observations recorded	No internal observations recorded	Not applicable

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

APPENDIX 4 Copy of the Certificate of Analysis

syngenta

Syngenta Crop Protection, LLC
Analytical and Product Chemistry
Greensboro, NC 27409

Certificate of Analysis

A23793B

Batch ID 1200767 (GP210610)

Test Substance Name:	CGA169374/CGA173506/CGA329351/SYN549522 FS (062.51/049.93/050.05/250.08)
Common Name:	Difenoconazole/Fludioxonil/Metalaxyl-M/Cyclobutrifluram FS (062.51/049.93/050.05/250.08)
Material ID:	A23793B
Batch ID:	1200767
Other ID:	GP210610
Source:	Syngenta Crop Protection LLC., 410 Swing Road, Greensboro, NC 27409, US

Chemical Analysis

AI	% w/w	g/L
Difenoconazole	5.45	64.0
Fludioxonil	4.37	51.3
Metalaxyl-M	4.31	50.6
Cyclobutrifluram	21.0	247

Identity of the Active Ingredients: Confirmed

Methodology Used for Characterization: LC, mass spectrometry, oscillating density meter.

The Active Ingredient(s) content is within the FAO limits.

Isomer Assay

Analyte	Isomer	% w/w
CGA329351	D-alanine, N-(2,6-dimethylphenyl)-N-(methoxyacetyl)-, Methyl Ester	4.15
CGA351920	L-alanine, N-(2,6-dimethylphenyl)-N-(methoxyacetyl)-, Methyl Ester	0.15

COA Number: USGR210208

APPENDIX 4 Copy of the Certificate of Analysis

Physical Analysis

Analyte	Value	Units
Density	1.174	g/cm3

Appearance: red liquid

Storage Temperature: <30°C

Re-certification Date:End of Aug/2024

If stored under the conditions given above, this test substance can be considered stable until the recertification date is reached.

The stability of this test substance will be determined concurrently through reanalysis of material held in inventory under GLP conditions at Syngenta Crop Protection, LLC, Greensboro, NC.

This Certificate of Analysis is summarizing data from a study that has been performed in compliance with Good Laboratory Practices per 40 CFR Part 160. Raw data, documentation, protocols, any amendments to study protocols and reports pertaining to this study are maintained in the Syngenta Crop Protection Archives in Greensboro, NC.

Study Number: USGR210208

Authorization: Sherry Perine

Sherry C Perine

Sherry Perine

Analytical and Product Chemistry Department

Aug 24, 2021

Date

COA Number: USGR210208

Page 2 of 2

APPENDIX 5 Attempts to Achieve the Maximum Concentration

Technical Trial	Test Item Concentration (% w/w)	Test Item Flow (mL/hr)	Air Flow In (set) (L/min)	Achieved Test Atmosphere Concentration (mg/L)	MMAD (µm)	GSD
1	100	200	30	0.69-1.12	-	-
1	80	200	30	1.68-2.44	4.97;4.24	2.17;2.22
2	70	200	30	1.85-2.49	3.58;3.79	2.15;2.22
2	60	200	30	1.32-2.02	2.77;3.33	2.19;2.30
3*	**70**	**200**	**30**	**2.09-2.65**	**3.55;3.71**	**2.20;2.10**

*Note: This setting was used for animal exposures.

APPENDIX 6 Good Laboratory Practice (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 Élelmezésegészségügyi Intézet") - ("National Institute of Pharmacy and Nutrition"); ("Hatósági Ellenőrzési Főosztály") - (Inspectorate Division); and at the signature: ("Digitálisan aláírta") - (Digitally signed); ("Dátum") - ("Date").

APPENDIX 6 Good Laboratory Practice (GLP) Certificate

OGYÉI Országos Gyógyszerészeti és Élelmezés-egészségügyi Intézet

1135 Budapest, Szabolcs utca 33.
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/28510-6/2022
Admin.: Dr. Szaller Zoltán

GOOD LABORATORY PRACTICE (GLP) CERTIFICATE

It is hereby certified that the test facility

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H-8200 Veszprém, Szabadságpuszta

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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 till 11th of August, 2022.

Dr. Lukács Ferenc József
Digitálisan aláírta: Dr. Lukács Ferenc József
Dátum: 2022.06.21 12:42:42 +02'00'

Dr. Ferenc Lukács
Head of Inspectorate

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