

**Isocycloseram/Emamectin Benzoate**

**Isocycloseram/Emamectin Benzoate SC (A23220A) –  
Acute Inhalation Toxicity Study  
(Nose-Only) in Rats**

**Final Report**

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

**AUTHOR(S):** Nóra Krajcs, Ph.D.

**COMPLETION DATE:** 17 December 2020

**PERFORMING LABORATORY:** Charles River Laboratories Hungary Kft.  
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Hungary

**LABORATORY PROJECT ID:** Report Number: 20/080-004P  
Study Number: 20/080-004P  
Task Number: TK0416697

**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).

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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 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: 17 December 2020  
Nóra Krajcs, Ph.D.  
Study Director

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## **FLAGGING STATEMENT**

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## QUALITY ASSURANCE STATEMENT

Study Number: 20/080-004P

Study Title: Isocycloseram/Emamectin Benzoate SC (A23220A) -  
Acute Inhalation Toxicity Study (Nose-Only) in Rats

Test Item: Isocycloseram/emamectin benzoate SC (A23220A)

This study has been inspected, and this report audited by the Quality Assurance Unit in compliance with the Principles of Good Laboratory Practice. As far as it can be reasonably established, the methods described and the results incorporated in this report accurately reflect the raw data produced during this study.


All inspections, data reviews and the report audit were reported in written form to the Study Director and to Management. The dates of such inspections and of the report audit are given below:

| Date of Inspection | Phase(s) Inspected/Audited    | Date of report to |                  |
|--------------------|-------------------------------|-------------------|------------------|
|                    |                               | Management        | Study Director   |
| 19 June 2020       | Study Plan                    | 19 June 2020      | 19 June 2020     |
| 28 July 2020       | Amendment 1 to the Study Plan | 28 July 2020      | 28 July 2020     |
| 05 August 2020     | Amendment 2 to the Study Plan | 05 August 2020    | 05 August 2020   |
| 05 August 2020     | Treatment                     | 05 August 2020    | 05 August 2020   |
| 14 October 2020    | Draft Report                  | 14 October 2020   | 14 October 2020  |
| 07 December 2020   | Final Report                  | 07 December 2020  | 07 December 2020 |

Signature:  Date: 17 December 2020  
 Agnes Rédl, M.Sc.  
 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 "Isocycloseram/Emamectin Benzoate SC (A23220A) – Acute Inhalation Toxicity Study (Nose-Only) in Rats" was performed in compliance with the Principles of Good Laboratory Practice.

Signature:  Date: 17 December 2020  
David J. Esdaile, M.Sc.  
Director of Science and Regulatory Affairs

## GENERAL INFORMATION

### Contributors

The following contributed to this report in the capacities indicated:

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### Study dates

|                               |                |
|-------------------------------|----------------|
| Study Initiation Date:        | 26 June 2020   |
| Experimental Starting Date:   | 30 June 2020   |
| Experimental Completion Date: | 19 August 2020 |

#### *Sighting Exposure – Group 0.1*

|                              |                          |
|------------------------------|--------------------------|
| Receipt of Animals:          | 23 July 2020             |
| Inhalation Exposure (Day 0): | 31 July 2020             |
| Observation:                 | 31 July – 14 August 2020 |
| Necropsy:                    | 14 August 2020           |

#### *Main Study – Group 1*

|                              |                            |
|------------------------------|----------------------------|
| Receipt of Animals:          | 23 July 2020               |
| Inhalation Exposure (Day 0): | 05 August 2020             |
| Observation:                 | 05 August – 19 August 2020 |
| Necropsy:                    | 19 August 2020             |

### Performing laboratory test substance reference number

200164

### Deviations from the guidelines

No deviations from the guidelines occurred during the study.

### **Deviations from the Study Plan**

Due to technical reasons, chamber relative humidity (maximum of 74%) outside the expected range of 30-70% were recorded occasionally in the exposure chamber during the animal exposures. This deviation was considered to have no impact on the outcome of the study and interpretation of the results.

Due to technical oversight, oxygen and carbon dioxide concentrations were not measured. The inhalation system is operated with tempered and filtered ambient air and there was no reason to believe that oxygen concentration was lower than 19% and carbon dioxide concentration exceeded 1%. 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 isocycloseram/emamectin benzoate SC (A23220A) following a 4-hour exposure to 5 male and 5 female rats.

Since the target concentration of 5 mg/L was not technically feasible, the maximum achievable concentration was tested during the animal exposures following the OECD TG 403.

A Sighting Exposure was performed prior to the Main Study with 2 male and 2 female rats at a maximum achievable mean concentration of 2.75 mg/L.

In the Main Study group, 10 (5 males and 5 females) CrI:WI Wistar rats, were exposed to a maximum achievable mean concentration of 2.58 mg/L isocycloseram/emamectin benzoate SC (A23220A).

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 and at the end of the scheduled period the animals were euthanised and were subjected to a gross examination *post mortem*.

### **1.2 Results**

#### **Atmosphere**

##### *Sighting Exposure (Group 0.1):*

The maximum achievable mean atmosphere concentration was 2.75 mg/L. The MMAD (Mass Median Aerodynamic Diameter) was 3.31 µm with a GSD (Geometric Standard Deviation) of 2.22.

##### *Main Study (Group 1):*

The maximum achievable mean atmosphere concentration was 2.58 mg/L. The MMAD was 3.67 µm with a GSD of 2.40.

#### **Mortality**

No mortality occurred in the Sighting Exposure (Group 0.1, 0/4). In the Main Study (Group 1), one female animal (1/10) was pre-terminally euthanized on Day 4 due to its clinical conditions.

## **Clinical observations**

### *Group 0.1 (Sighting Exposure – 2.75 mg/L)*

In the male animals, decreased activity (slight), laboured respiration (slight), noisy respiration (slight to moderate), lack of grooming, fur staining by test item (on the nose), red-brown staining (on the head) and wet fur (on the whole body) were observed from Day 0 up to Day 4. Both male animals were symptom-free from Day 5.

In the female animals, laboured respiration (slight), noisy respiration (slight), lack of grooming, continuous tremors (whole body), heightened startle response, red-brown staining (on the head), fur staining by test item (on the nose) and wet fur (on the whole body) were observed from Day 0 up to Day 5. Both female animals were symptom-free from Day 6.

### *Group 1 (Main Exposure – 2.58 mg/L)*

In the male animals, laboured respiration (slight), noisy respiration (slight to moderate), decreased activity (slight), lack of grooming, red-brown staining (on the head), fur staining by test item (on the nose) and wet fur (on the whole body) were observed from Day up to Day 4. All male animals were symptom-free from Day 5.

In the female animals, laboured respiration (slight), noisy respiration (slight to moderate), continuous tremors (whole body), decreased activity (slight to moderate), hunched back, lack of grooming, red-brown-staining (on the head), fur staining by test item (on the nose) and wet fur (on the whole body) were observed from Day 0 up to Day 6. All surviving female animals were symptom-free from Day 7.

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

## **Bodyweight**

In the male animals, slight body weight losses were noted on Day 0-1. The body weight gain was normal from Day 1.

In the female animals, slight body weight losses were noted on Day 0-3. The body weight gain was normal from Day 3.

### *Group 1 (Main Exposure – 2.58 mg/L)*

In the male animals, slight body weight losses were noted on Day 0-1. Slight body weight losses were observed in 1 out of the 5 animals on Day 1-7. The body weight gain of the other animals was normal from Day 1.

In the pre-terminally euthanized female animal (animal number: 5624) body weight loss of 44 g was observed before death.

In the surviving female animals, slight body weight losses were noted on Day 0-3. The body weight gain was normal from Day 3.

## **Necropsy**

### *Group 0.1 (Sighting Exposure – 2.75 mg/L)*

No macroscopic observations were seen at necropsy on Day 14.

### *Group 1 (Main Exposure – 2.58 mg/L)*

In the male animals, no test item-related macroscopic changes were observed at necropsy on Day 14.

In three out of four female animals, no test item-related macroscopic changes were observed at necropsy on Day 14. In one out of four female animal, dark red discoloration of the lungs was observed. In the pre-terminally euthanized female animal, discoloration of the liver (on the left lateral lobe and on the right medial lobe), small spleen and small thymus were observed.

## **1.3 Conclusion**

Under the experimental conditions of this study, mortality of one female animal occurred in a group of 10 rats when exposed to 2.58 mg/L of isocycloseram/emamectin benzoate SC (A23220A) for 4 hours. The acute inhalation median lethal concentration of isocycloseram/emamectin benzoate SC (A23220A) in CrI:WI Wistar rats is therefore considered to be above 2.58 mg/L (maximum achievable mean concentration).

## **2.0 INTRODUCTION**

### **2.1 Purpose**

This study was performed to assess the acute inhalation toxicity of isocycloseram/emamectin benzoate SC (A23220A) 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: 07 September 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:                       | Isocycloseram/emamectin benzoate SC (A23220A)                                      |                            |          |
| Batch number:               | TSC002-041-001   |                            |          |
| Active Ingredient Content*: | Isocycloseram  | 17.5% w/w corresponding to | 201 g/L  |
|                             | emamectin benzoate   | 4.18% w/w corresponding to | 48.1 g/L |
| Density (predicted):        | 1150 kg/m <sup>3</sup>   |                            |          |
| Appearance:                 | Brown liquid   |                            |          |
| Recertification date:       | 31 January 2023  |                            |          |
| Storage conditions:         | Room temperature (<30°C)   |                            |          |
| Safety precautions:         | Appropriate safety precautions were applied to assure personnel health and safety. |                            |          |

\*No correction for active ingredient content was applied.

The copy of the Certificate of Analysis is presented in Appendix 5.

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 (100% (w/w)) and its different formulations (80, 70, 65 and 60% (w/w) aqueous dilutions) were tested to achieve the maximum attainable atmosphere concentration with acceptable MMAD- and GSD-values. Based on the results of these trials, the test item was used as a 70% (w/w) aqueous (Aqua Purificata, Batch numbers: 202001013, Expiry date: 23 July 2020 and 202003032, Expiry date: 11 September 2020, Manufacturer: MAGILAB Kft., Hungary) formulation. Details of the concentration selection is presented in Appendix 6.

### 3.1.3 Other materials

Name: Vaseline  
Batch number: STBH9261  
Expiry date: 30 November 2022  
Manufacturer: Sigma Aldrich  
Storage conditions: Room temperature

#### *Vehicle:*

Name: Distilled water (Aqua purificata)  
Batch number: 202001013 / 202003032  
Expiry date: 23 July 2020 / 11 September 2020  
Manufacturer: MAGILAB Kft.  
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 and Main Study: 9-10 weeks old  
Body weight at exposure: Sighting Study: males: 368-376 g; females: 229-231 g  
Main Study: males: 385-397 g; females: 246-264 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.9 software was used in order to verify homogeneity/variation within groups based on actual body weight.  
Acclimatization time: Sighting Study: 8 days; Main Study: 13 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. |
| 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. |
| Light:               | 12 hours daily, from 6.00 a.m. to 6.00 p.m.  |
| Temperature:         | 21.2-24.6°C  |
| Relative humidity:   | 44-74%   |
| 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 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 (ÁNTSZ, H-8201 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 6.).

#### **3.3.2 Atmosphere generation**

The test item 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 achievable 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, Germany, Lot No. 9648838). 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 39.90% ( $n = 10$ ) with a standard deviation 0.15%.

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, Germany, Lot Numbers: A28861276).

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 (39.90%) 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, 0.960, 1.550, 2.105, 3.555, 6.655 and 10.550  $\mu\text{m}$  was calculated.

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. In addition, the proportion (%) of aerosol less than 4 $\mu\text{m}$  (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.

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 on the day of death.

## **3.6 *Post Mortem* Investigation**

All animals were subjected to macroscopic examination. All 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.:   | 1811347-03                                      |
| Expiry Date: | 31 December 2021                                |
| 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.9 or were recorded on data collection sheets taken from the relevant SOPs, then tabulated using PROVANTIS v.9, Microsoft Office Word and/or Excel, as appropriate.

Only a Limit Test was performed, the four-hour inhalation LC<sub>50</sub> 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                      | Maximum achievable mean concentration (mg/L) | Standard Deviation | Nominal Concentration (mg/L) |
|----------------------------|--|--------------------|------------------------------|
| 0.1<br>(Sighting exposure) | 2.75   | 0.22               | 62.54                        |
| 1<br>(Main Study)          | 2.58   | 0.25               | 61.96                        |

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                      | Maximum achievable mean concentration (mg/L) | Mean Mass Median Aerodynamic Diameter (MMAD) ( $\mu\text{m}$ ) | Geometric Standard Deviation (GSD) | Respirable Fraction (% < 4 $\mu\text{m}$ ) |
|----------------------------|--|--|------------------------------------|--|
| 0.1<br>(Sighting exposure) | 2.75   | 3.31   | 2.22                               | 59.3                                       |
| 1<br>(Main Study)          | 2.58   | 3.67   | 2.40                               | 53.8                                       |

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

### 4.3 Mortality Rates

No mortality occurred in the Sighting Exposure (Group 0.1, 0/4).

One female animal (5624) in the Main Study (Group 1) was euthanized pre-terminally on Day 4 due to animal welfare reasons.

Mortality data are detailed in Table 4.

#### **4.4 Clinical Observations**

##### *Group 0.1 (Sighting Exposure – 2.75 mg/L)*

In the male animals, decreased activity (slight), laboured respiration (slight), noisy respiration (slight to moderate), lack of grooming, fur staining by test item (on the nose), red-brown staining (on the head) and wet fur (on the whole body) were observed from Day 0 up to Day 4. Both male animals were symptom-free from Day 5.

In the female animals, laboured respiration (slight), noisy respiration (slight), lack of grooming, tremors continuous (whole body), heightened startle response, red-brown staining (on the head), fur staining by test item (on the nose) and wet fur (on the whole body) were observed from Day 0 up to Day 5. Both female animals were symptom-free from Day 6.

##### *Group 1 (Main Exposure – 2.58 mg/L)*

In the male animals, laboured respiration (slight), noisy respiration (slight to moderate), decreased activity (slight), lack of grooming, red-brown staining (on the head), fur staining by test item (on the nose) and wet fur (on the whole body) were observed from Day up to Day 4. All male animals were symptom-free from Day 5.

In the female animals, laboured respiration (slight), noisy respiration (slight to moderate), tremors continuous (whole body), decreased activity (slight to moderate), hunched back, lack of grooming, red-brown-staining (on the head), fur staining by test item (on the nose) and wet fur (on the whole body) were observed from Day 0 up to Day 6. All surviving female animals were symptom-free from Day 7.

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

Individual clinical observations are presented in Appendix 1.

#### **4.5 Bodyweight**

##### *Group 0.1 (Sighting Exposure – 2.75 mg/L)*

In the male animals, slight body weight losses were noted on Day 0-1. The body weight gain was normal from Day 1.

In the female animals, slight body weight losses were noted on Day 0-3. The body weight gain was normal from Day 3.

##### *Group 1 (Main Exposure – 2.58 mg/L)*

In the male animals, slight body weight losses were noted on Day 0-1. Slight body weight losses were observed in 1 out of the 5 animals on Day 1-7. The body weight gain of the other animals was normal from Day 1.

In the pre-terminally euthanized female animal (animal number: 5624) body weight loss of 44 g was observed before death.

In the surviving female animals, slight body weight losses were noted on Day 0-3. The body weight gain was normal from Day 3.

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

## **4.6 Necropsy**

*Group 0.1 (Sighting Exposure – 2.75 mg/L)*

No macroscopic observations were seen at necropsy on Day 14.

*Group 1 (Main Exposure – 2.58 mg/L)*

In the male animals, no test item-related macroscopic changes were observed at necropsy on Day 14.

In three out of four female animals, no test item-related macroscopic changes were observed at necropsy on Day 14. In one out of four female animal, dark red discoloration of the lungs was observed. In the pre-terminal euthanized female animal, discoloration of the liver (on the left lateral lobe and on the right medial lobe), small spleen and small thymus were observed.

Individual necropsy data are presented in Appendix 3 and the Pathology Report is presented in Appendix 4.

## **5.0 CONCLUSIONS**

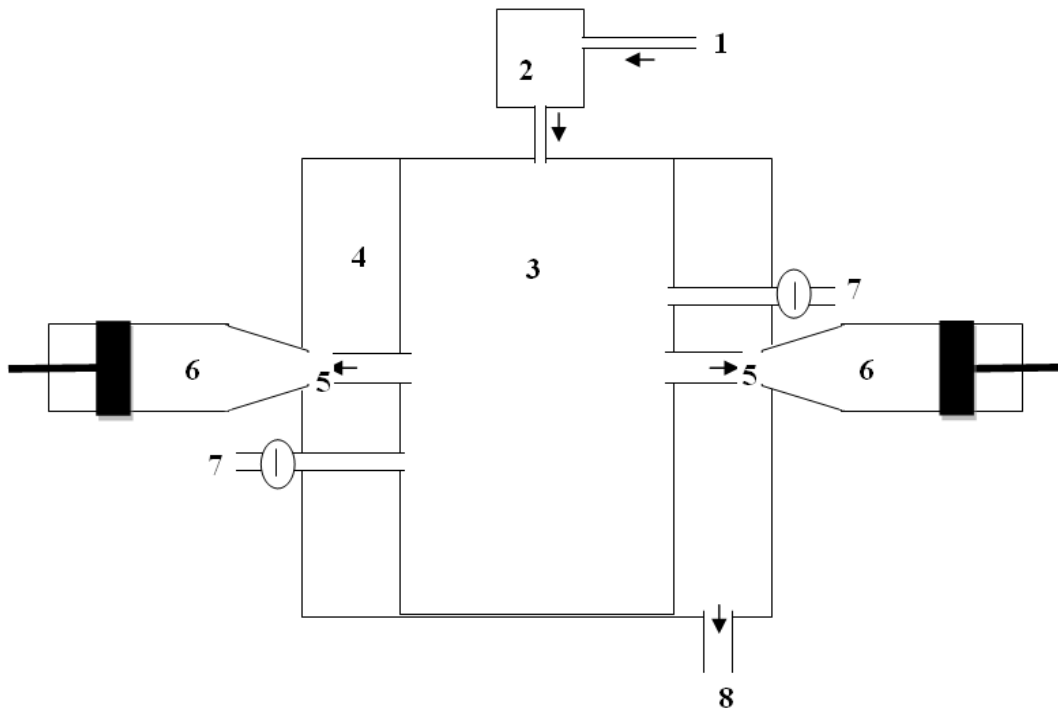
Under the experimental conditions of this study, mortality of one female animal occurred in a group of 10 rats when exposed to 2.58 mg/L of isocycloseram/emamectin benzoate SC (A23220A) for 4 hours. The acute inhalation median lethal concentration of isocycloseram/emamectin benzoate SC (A23220A) in Crl:WI Wistar rats is therefore considered to be above 2.58 mg/L (maximum achievable mean concentration).

## 6.0 REFERENCES

1. Pauluhn J. (1994): Validation of an Improved Nose-Only Exposure System for Rodents. *J. App. Tox.* 14 (1), 55-62
2. Silver S. D. (1946): Constant flow gassing chambers: Principles influencing design and operation. *J. Lab. Clin. Med.* 31, 1153-1161
3. 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.
4. OECD Guidelines for the Testing of Chemicals No. 403 "Acute Inhalation Toxicity", adopted 07 September 2009
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 (August 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)

## FIGURES SECTION

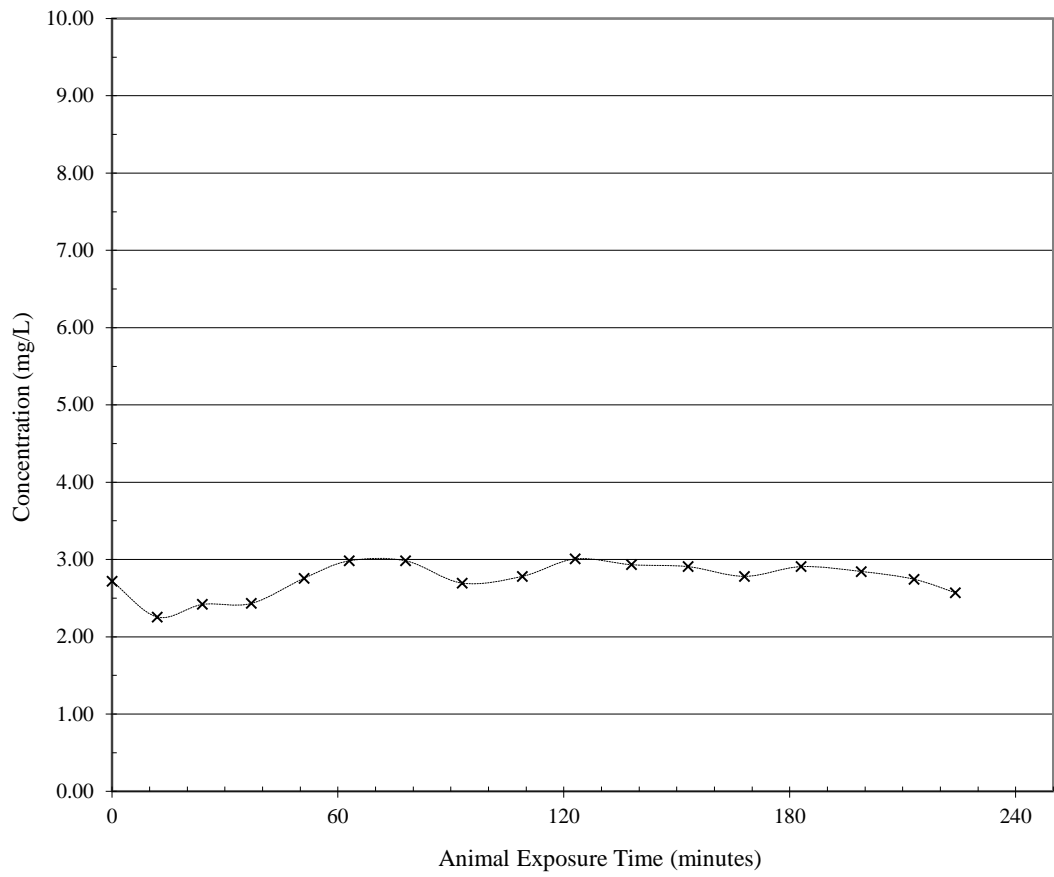
**FIGURE 1 Schematic Diagram of the Exposure System**



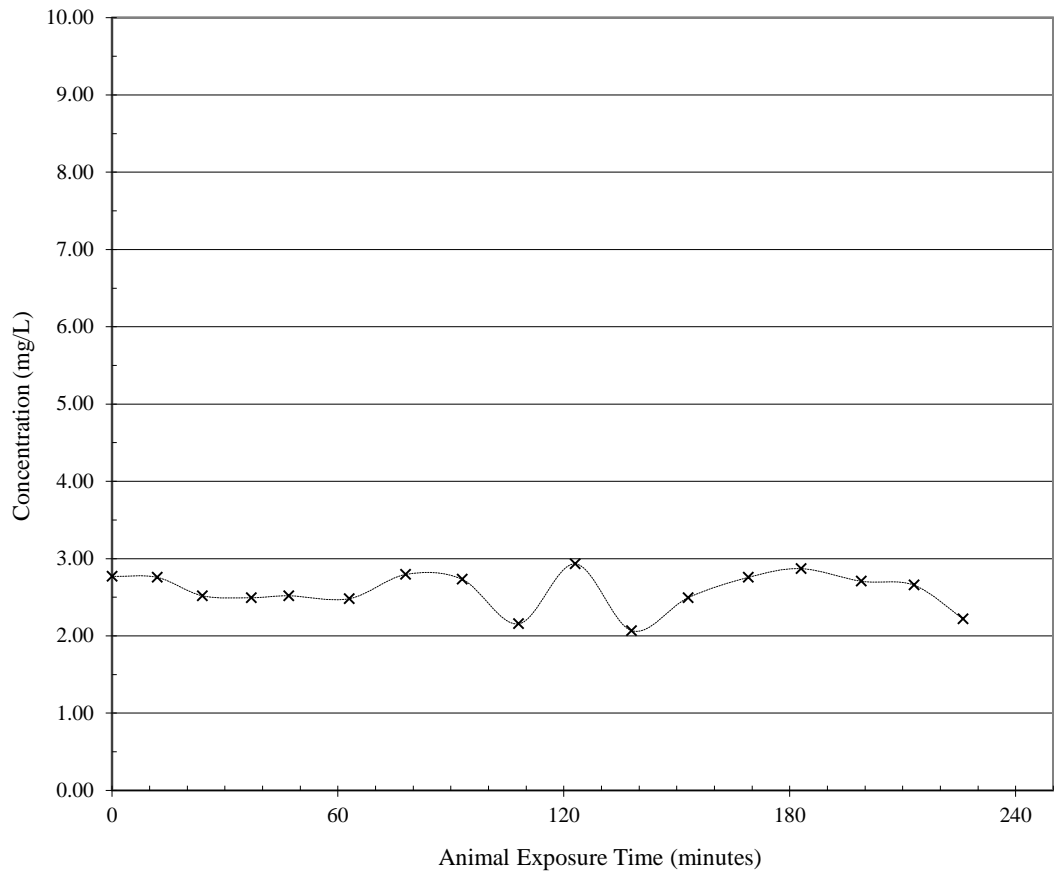
| KEY: |                           |    |                            |
|------|---------------------------|----|----------------------------|
| 1:   | Metered Air Supply        | 5: | Animal Exposure Port       |
| 2:   | Aerosol Generation System | 6: | Animal Restraint Tube      |
| 3:   | Central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

**FIGURE 2            Achieved Atmosphere Concentrations**

**Sighting Exposure – Group 0.1**

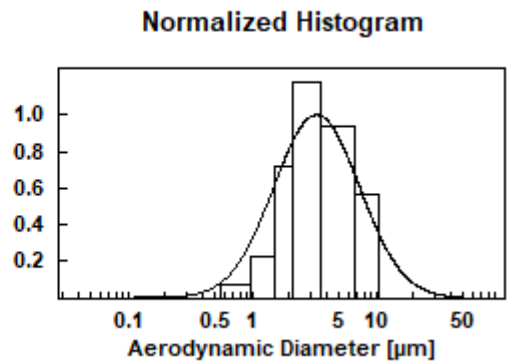
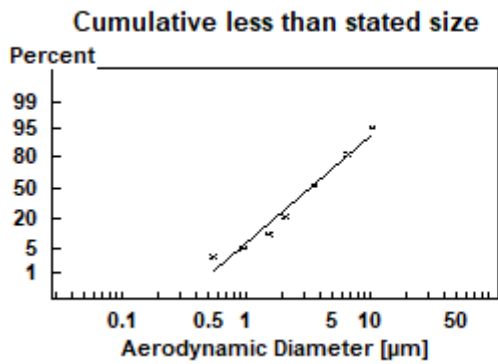
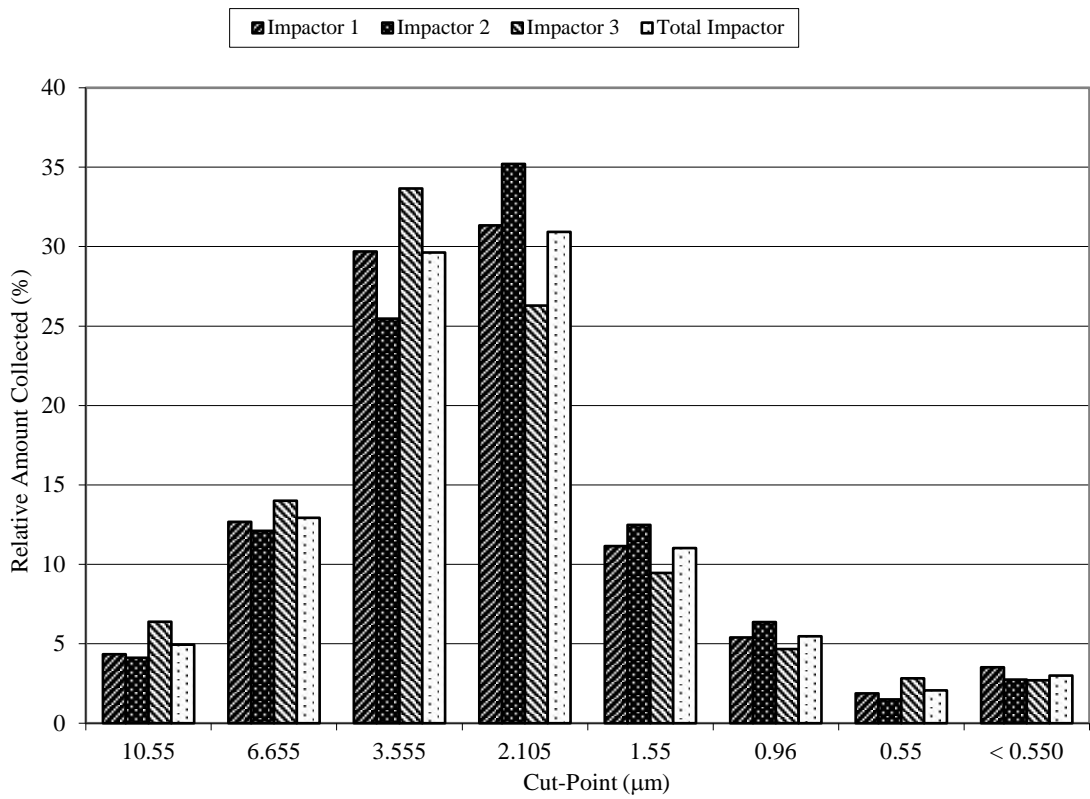


## Main Study – Group 1

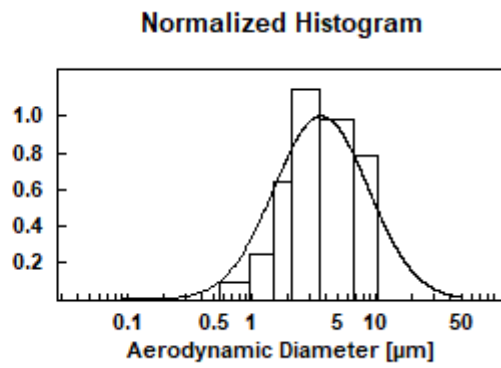
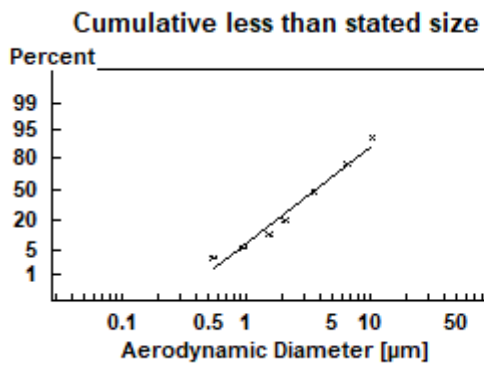
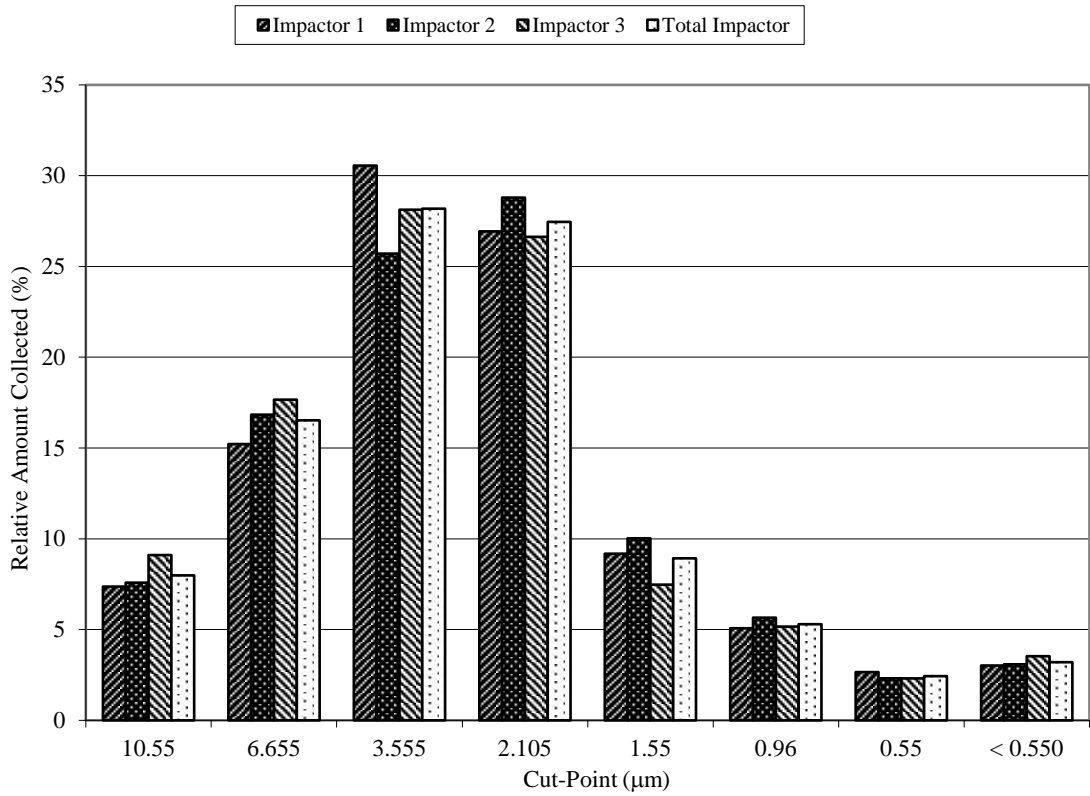


### FIGURE 3 Particle Size Distribution

#### Sighting Exposure – Group 0.1



# Main Study – Group 1



## TABLES SECTION

**TABLE 1                      Test Atmosphere Concentrations**

**Sighting Exposure – Group 0.1**

| <b>Exposure Duration (minutes)</b> | <b>Sample Volume (L)</b> | <b>Amount of Non Volatiles Collected* (mg)</b> | <b>Equivalent Test Item Amount (mg)</b> | <b>Atmospheric Concentration of the Test Item (mg/L)</b> |
|------------------------------------|--------------------------|--|---|--|
| 0                                  | 2.0                      | 2.17   | 5.44                                    | 2.72   |
| 12                                 | 2.0                      | 1.80   | 4.51                                    | 2.26   |
| 24                                 | 2.0                      | 1.93   | 4.84                                    | 2.42   |
| 37                                 | 2.0                      | 1.94   | 4.86                                    | 2.43   |
| 51                                 | 2.0                      | 2.20   | 5.51                                    | 2.76   |
| 63                                 | 2.0                      | 2.38   | 5.96                                    | 2.98   |
| 78                                 | 2.0                      | 2.38   | 5.96                                    | 2.98   |
| 93                                 | 2.0                      | 2.15   | 5.39                                    | 2.69   |
| 109                                | 2.0                      | 2.22   | 5.56                                    | 2.78   |
| 123                                | 2.0                      | 2.40   | 6.02                                    | 3.01   |
| 138                                | 2.0                      | 2.34   | 5.86                                    | 2.93   |
| 153                                | 2.0                      | 2.32   | 5.81                                    | 2.91   |
| 168                                | 2.0                      | 2.22   | 5.56                                    | 2.78   |
| 183                                | 2.0                      | 2.32   | 5.81                                    | 2.91   |
| 199                                | 2.0                      | 2.27   | 5.69                                    | 2.84   |
| 213                                | 2.0                      | 2.19   | 5.49                                    | 2.74   |
| 224                                | 2.0                      | 2.05   | 5.14                                    | 2.57   |

Maximum achievable mean concentration = 2.75 mg/L

Standard Deviation = 0.22

Amount of Test Item Used (g): 625.39

Total Volume of Air Used (L): 10000

Nominal Concentration (mg/L): 62.54

---

\* = non-volatile content of Isocycloseram/emamectin benzoate SC (A23220A) was 39.90%

## 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               | 2.21                                    | 5.54                             | 2.77  |
| 12                          | 2.0               | 2.20                                    | 5.51                             | 2.76  |
| 24                          | 2.0               | 2.01                                    | 5.04                             | 2.52  |
| 37                          | 2.0               | 1.99                                    | 4.99                             | 2.49  |
| 47                          | 2.0               | 2.01                                    | 5.04                             | 2.52  |
| 63                          | 2.0               | 1.98                                    | 4.96                             | 2.48  |
| 78                          | 2.0               | 2.23                                    | 5.59                             | 2.79  |
| 93                          | 2.0               | 2.18                                    | 5.46                             | 2.73  |
| 108                         | 2.0               | 1.72                                    | 4.31                             | 2.16  |
| 123                         | 2.0               | 2.34                                    | 5.86                             | 2.93  |
| 138                         | 2.0               | 1.65                                    | 4.14                             | 2.07  |
| 153                         | 2.0               | 1.99                                    | 4.99                             | 2.49  |
| 169                         | 2.0               | 2.20                                    | 5.51                             | 2.76  |
| 183                         | 2.0               | 2.29                                    | 5.74                             | 2.87  |
| 199                         | 2.0               | 2.16                                    | 5.41                             | 2.71  |
| 213                         | 2.0               | 2.12                                    | 5.31                             | 2.66  |
| 226                         | 2.0               | 1.77                                    | 4.44                             | 2.22  |

Maximum achievable concentration = 2.58 mg/L

Standard Deviation = 0.25

Amount of Test Item Used (g): 622.06

Total Volume of Air Used (L): 10040

Nominal Concentration (mg/L): 61.96

---

\* = non-volatile content of Isocycloseram/emamectin benzoate SC (A23220A) was 39.90%

**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         | 0.37                  | 0.33     | 0.52                | 1.22                           |
| 2                                  | 6.655          | 1.08                  | 0.97     | 1.14                | 3.19                           |
| 3                                  | 3.555          | 2.53                  | 2.04     | 2.74                | 7.31                           |
| 4                                  | 2.105          | 2.67                  | 2.82     | 2.14                | 7.63                           |
| 5                                  | 1.550          | 0.95                  | 1.00     | 0.77                | 2.72                           |
| 6                                  | 0.960          | 0.46                  | 0.51     | 0.38                | 1.35                           |
| 7                                  | 0.550          | 0.16                  | 0.13     | 0.23                | 0.52                           |
| Filter                             | < 0.550        | 0.30                  | 0.22     | 0.22                | 0.74                           |
| <b>Total Amount Collected (mg)</b> |                |                       |          |                     | 24.68                          |
| Size Range (µm)                    |                | Total Mass/stage (mg) |          | Cumulative Mass (%) |                                |
| < 0.550                            |                | 0.74                  |          | 3.00                |                                |
| 0.550 - 0.960                      |                | 0.52                  |          | 5.11                |                                |
| 0.960 - 1.550                      |                | 1.35                  |          | 10.58               |                                |
| 1.550 - 2.105                      |                | 2.72                  |          | 21.60               |                                |
| 2.105 – 3.555                      |                | 7.63                  |          | 52.51               |                                |
| 3.555 - 6.655                      |                | 7.31                  |          | 82.13               |                                |
| 6.655 – 10.550                     |                | 3.19                  |          | 95.06               |                                |
| > 10.550                           |                | 1.22                  |          | 100.00              |                                |

Maximum achievable mean concentration = 2.75 mg/L

Mean Mass Median Aerodynamic Diameter (MMAD) = 3.31 µm

Geometric Standard Deviation (GSD) = 2.22

Respirable Fraction (% < 4µm) = 59.3%

## 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         | 0.61                  | 0.59     | 0.67                | 1.87                           |
| 2                                  | 6.655          | 1.26                  | 1.31     | 1.30                | 3.87                           |
| 3                                  | 3.555          | 2.53                  | 2.00     | 2.07                | 6.60                           |
| 4                                  | 2.105          | 2.23                  | 2.24     | 1.96                | 6.43                           |
| 5                                  | 1.550          | 0.76                  | 0.78     | 0.55                | 2.09                           |
| 6                                  | 0.960          | 0.42                  | 0.44     | 0.38                | 1.24                           |
| 7                                  | 0.550          | 0.22                  | 0.18     | 0.17                | 0.57                           |
| Filter                             | < 0.550        | 0.25                  | 0.24     | 0.26                | 0.75                           |
| <b>Total Amount Collected (mg)</b> |                |                       |          |                     | 23.42                          |
| Size Range (µm)                    |                | Total Mass/stage (mg) |          | Cumulative Mass (%) |                                |
| < 0.550                            |                | 0.75                  |          | 3.20                |                                |
| 0.550 - 0.960                      |                | 0.57                  |          | 5.64                |                                |
| 0.960 - 1.550                      |                | 1.24                  |          | 10.93               |                                |
| 1.550 - 2.105                      |                | 2.09                  |          | 19.85               |                                |
| 2.105 – 3.555                      |                | 6.43                  |          | 47.31               |                                |
| 3.555 - 6.655                      |                | 6.60                  |          | 75.49               |                                |
| 6.655 – 10.550                     |                | 3.87                  |          | 92.02               |                                |
| > 10.550                           |                | 1.87                  |          | 100.00              |                                |

Maximum achievable concentration = 2.58 mg/L

Mean Mass Median Aerodynamic Diameter (MMAD) = 3.67 µm

Geometric Standard Deviation (GSD) = 2.40

Respirable Fraction (% < 4µm) = 53.8%

**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)    | 39.9       | 37.6    | 45.5    |
| Air Flow Out (Outer Cylinder) (L/min) | 40.4       | 40.1    | 40.7    |
| Temperature (°C)                      | 20.9       | 20.6    | 22.1    |
| Relative Humidity* (%)                | -          | -       | -       |
| Oxygen Concentration** (%)            | -          | -       | -       |
| Carbon Dioxide** (%)                  | -          | -       | -       |

Theoretical Chamber Equilibration Time (T<sub>99</sub>):

$$T_{99} = (4.605 \times (\text{Chamber Volume} / \text{Chamber Flow rate})) \text{ (Silver, 1946)}$$

Chamber volume (inner plenum) = 3.85 L (Pauluhn, 1994)

T<sub>99</sub> (Minimum Acceptable Equilibration Time) = 1 minute

Actual equilibration time allowed = 11 minutes

\*The chamber humidity was not evaluated due to the evidently false values caused by the sensor interference.

\*\*Oxygen and Carbon Dioxide concentrations were not measured.

## Main Study – Group 1

| Measurement                                 | Mean Value | Minimum | Maximum |
|---|------------|---------|---------|
| Air Flow In<br>(Inner Plenum)<br>(L/min)    | 39.9       | 34.3    | 41.0    |
| Air Flow Out<br>(Outer Cylinder)<br>(L/min) | 40.4       | 40.2    | 40.7    |
| Temperature*<br>(°C)                        | 24.4       | 24.2    | 24.5    |
| Relative Humidity†<br>(%)                   | 57.6       | 55.9    | 58.3    |
| Oxygen<br>Concentration** (%)               | -          | -       | -       |
| Carbon Dioxide**<br>(%)                     | -          | -       | -       |

Theoretical Chamber Equilibration Time ( $T_{99}$ ):

$$T_{99} = (4.605 \times (\text{Chamber Volume} / \text{Chamber Flow rate})) \text{ (Silver, 1946)}$$

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.

\*\*Oxygen and Carbon Dioxide concentrations were not measured.

† 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<br>(During Exposure) | 0                | 0      | 0       | 0      |
| 0<br>(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       | 1      |
| 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: 2.75 mg/L

SEX: MALE

| Animal number        | Observations                     | Days of study |    |       |    |    |    |    |   |   |   |   |   |   |   | Frequency |    |    |       |        |        |
|----------------------|----------------------------------|---------------|----|-------|----|----|----|----|---|---|---|---|---|---|---|-----------|----|----|-------|--------|--------|
|                      |                                  | 0 (exposure)  |    |       |    |    | 1  | 2  | 3 | 4 | 5 | 6 | 7 | 8 | 9 |           | 10 | 11 | 12    | 13     | 14     |
|                      |                                  | during        |    | after |    |    |    |    |   |   |   |   |   |   |   |           |    |    |       |        |        |
| 1h                   | 2h                               | 3h            | 4h | 5h    |    |    |    |    |   |   |   |   |   |   |   |           |    |    |       |        |        |
| 5603                 | Normal                           | +             | -  | -     | -  | -  | -  | -  | - | - | + | + | + | + | + | +         | +  | +  | +     | +      | 11 /19 |
|                      | Activity decreased               | -             | -  | -     | -  | SI | SI | SI | - | - | - | - | - | - | - | -         | -  | -  | -     | -      | 3 /19  |
|                      | Fur staining by test item - Nose | -             | -  | -     | +  | +  | +  | +  | + | + | - | - | - | - | - | -         | -  | -  | -     | -      | 6 /19  |
|                      | Laboured respiration             | -             | SI | SI    | SI | SI | SI | SI | - | - | - | - | - | - | - | -         | -  | -  | -     | -      | 6 /19  |
|                      | Lack of grooming                 | -             | -  | -     | -  | -  | +  | +  | - | - | - | - | - | - | - | -         | -  | -  | -     | -      | 2 /19  |
|                      | Noisy respiration                | -             | -  | -     | SI | Mo | SI | -  | - | - | - | - | - | - | - | -         | -  | -  | -     | -      | 3 /19  |
|                      | Red-brown staining - Head        | -             | -  | -     | +  | +  | +  | -  | - | - | - | - | - | - | - | -         | -  | -  | -     | -      | 3 /19  |
|                      | Wet fur - Whole body             | -             | -  | -     | +  | +  | -  | -  | - | - | - | - | - | - | - | -         | -  | -  | -     | -      | 2 /19  |
| 5604                 | Normal                           | +             | -  | -     | -  | -  | -  | -  | - | - | + | + | + | + | + | +         | +  | +  | +     | 11 /19 |        |
|                      | Fur staining by test item - Nose | -             | -  | -     | +  | +  | +  | +  | + | + | - | - | - | - | - | -         | -  | -  | -     | 6 /19  |        |
|                      | Laboured respiration             | -             | SI | SI    | SI | SI | -  | -  | - | - | - | - | - | - | - | -         | -  | -  | -     | 4 /19  |        |
|                      | Lack of grooming                 | -             | -  | -     | -  | -  | +  | +  | - | - | - | - | - | - | - | -         | -  | -  | -     | 2 /19  |        |
|                      | Noisy respiration                | -             | -  | -     | SI | SI | -  | -  | - | - | - | - | - | - | - | -         | -  | -  | -     | 2 /19  |        |
|                      | Red-brown staining - Head        | -             | -  | -     | +  | +  | +  | -  | - | - | - | - | - | - | - | -         | -  | -  | -     | 3 /19  |        |
| Wet fur - Whole body | -                                | -             | -  | +     | +  | -  | -  | -  | - | - | - | - | - | - | - | -         | -  | -  | 2 /19 |        |        |

**Standard footnotes:** + = present - = absent  
h = hour (s) ' = minute  
# = Found M = Moribund  
dead

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

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

SIGHTING  
 EXPOSURE  
 DOSE GROUP: 0.1  
 CONCENTRATION: 2.75 mg/L

SEX: FEMALE

| Animal number | Observations                     | 0 (exposure) |    | Days of study |    |    |    |   |   |   |   |   |   |   |    |    |    | Frequency |    |    |        |
|---------------|----------------------------------|--------------|----|---------------|----|----|----|---|---|---|---|---|---|---|----|----|----|-----------|----|----|--------|
|               |                                  | during       |    | after         |    | 1  | 2  | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |           | 13 | 14 |        |
|               |                                  | 1h           | 2h | 3h            | 4h | 5h |    |   |   |   |   |   |   |   |    |    |    |           |    |    |        |
| 5620          | Normal                           | +            | -  | -             | -  | -  | -  | - | - | - | - | + | + | + | +  | +  | +  | +         | +  | +  | 10 /19 |
|               | Fur staining by test item - Nose | -            | -  | -             | +  | +  | +  | + | - | + | + | - | - | - | -  | -  | -  | -         | -  | -  | 6 /19  |
|               | Laboured respiration             | -            | SI | SI            | SI | SI | SI | - | - | - | - | - | - | - | -  | -  | -  | -         | -  | -  | 5 /19  |
|               | Lack of grooming                 | -            | -  | -             | -  | -  | +  | + | + | - | - | - | - | - | -  | -  | -  | -         | -  | -  | 3 /19  |
|               | Noisy respiration                | -            | -  | -             | SI | -  | -  | - | - | - | - | - | - | - | -  | -  | -  | -         | -  | -  | 1 /19  |
|               | Red-brown staining - Head        | -            | -  | -             | +  | +  | +  | - | - | - | - | - | - | - | -  | -  | -  | -         | -  | -  | 3 /19  |
|               | Tremors Continous - Whole body   | -            | -  | -             | -  | -  | +  | + | + | + | - | - | - | - | -  | -  | -  | -         | -  | -  | 4 /19  |
|               | Wet fur - Whole body             | -            | -  | -             | +  | +  | -  | - | - | - | - | - | - | - | -  | -  | -  | -         | -  | -  | 2 /19  |
| 5619          | Normal                           | +            | -  | -             | -  | -  | -  | - | - | - | - | + | + | + | +  | +  | +  | +         | +  | +  | 11 /19 |
|               | Fur staining by test item - Nose | -            | -  | -             | +  | +  | +  | + | + | + | - | - | - | - | -  | -  | -  | -         | -  | -  | 6 /19  |
|               | Heightened startle response      | -            | -  | -             | -  | -  | +  | + | - | - | - | - | - | - | -  | -  | -  | -         | -  | -  | 2 /19  |
|               | Laboured respiration             | -            | SI | SI            | SI | SI | -  | - | - | - | - | - | - | - | -  | -  | -  | -         | -  | -  | 4 /19  |
|               | Lack of grooming                 | -            | -  | -             | -  | -  | +  | + | - | - | - | - | - | - | -  | -  | -  | -         | -  | -  | 2 /19  |
|               | Noisy respiration                | -            | -  | -             | SI | SI | -  | - | - | - | - | - | - | - | -  | -  | -  | -         | -  | -  | 2 /19  |
|               | Red-brown staining - Head        | -            | -  | -             | +  | +  | -  | - | - | - | - | - | - | - | -  | -  | -  | -         | -  | -  | 2 /19  |
|               | Wet fur - Whole body             | -            | -  | -             | +  | +  | -  | - | - | - | - | - | - | - | -  | -  | -  | -         | -  | -  | 2 /19  |

**Standard footnotes:** + = present      - = absent  
 h = hour (s)      ' = minute  
 # = Found dead      M = Moribund  
 Frequency of observation = number of occurrence of observation / total number of observations

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

MAIN STUDY  
DOSE GROUP: 1  
CONCENTRATION: 2.58 mg/L

SEX: MALE

| Animal number        | Observations                     | Days of study |       |    |    |    |    |    |    |   |   |   |   |   |   |    |    |    |    | Frequency |        |
|----------------------|----------------------------------|---------------|-------|----|----|----|----|----|----|---|---|---|---|---|---|----|----|----|----|-----------|--------|
|                      |                                  | 0 (exposure)  |       |    |    |    | 1  | 2  | 3  | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |           | 14     |
|                      |                                  | during        | after |    |    |    |    |    |    |   |   |   |   |   |   |    |    |    |    |           |        |
| 1h                   | 2h                               | 3h            | 4h    | 5h |    |    |    |    |    |   |   |   |   |   |   |    |    |    |    |           |        |
| 5612                 | Normal                           | +             | -     | -  | -  | -  | -  | -  | -  | - | + | + | + | + | + | +  | +  | +  | +  | +         | 11 /19 |
|                      | Fur staining by test item - Nose | -             | -     | -  | +  | +  | +  | +  | +  | + | - | - | - | - | - | -  | -  | -  | -  | -         | 6 /19  |
|                      | Laboured respiration             | -             | SI    | SI | SI | SI | SI | SI | -  | - | - | - | - | - | - | -  | -  | -  | -  | -         | 6 /19  |
|                      | Lack of grooming                 | -             | -     | -  | -  | -  | +  | +  | -  | - | - | - | - | - | - | -  | -  | -  | -  | -         | 2 /19  |
|                      | Noisy respiration                | -             | -     | -  | SI | SI | SI | SI | -  | - | - | - | - | - | - | -  | -  | -  | -  | -         | 4 /19  |
|                      | Red-brown staining - Head        | -             | -     | -  | +  | +  | +  | +  | -  | - | - | - | - | - | - | -  | -  | -  | -  | -         | 4 /19  |
|                      | Wet fur - Whole body             | -             | -     | -  | +  | +  | -  | -  | -  | - | - | - | - | - | - | -  | -  | -  | -  | -         | 2 /19  |
| 5610                 | Normal                           | +             | -     | -  | -  | -  | -  | -  | -  | - | + | + | + | + | + | +  | +  | +  | +  | +         | 11 /19 |
|                      | Fur staining by test item - Nose | -             | -     | -  | +  | +  | +  | +  | +  | + | - | - | - | - | - | -  | -  | -  | -  | -         | 6 /19  |
|                      | Laboured respiration             | -             | SI    | SI | SI | SI | SI | SI | -  | - | - | - | - | - | - | -  | -  | -  | -  | -         | 6 /19  |
|                      | Lack of grooming                 | -             | -     | -  | -  | -  | +  | +  | -  | - | - | - | - | - | - | -  | -  | -  | -  | -         | 2 /19  |
|                      | Noisy respiration                | -             | -     | -  | SI | SI | Mo | Mo | -  | - | - | - | - | - | - | -  | -  | -  | -  | -         | 4 /19  |
|                      | Red-brown staining - Head        | -             | -     | -  | +  | +  | +  | +  | -  | - | - | - | - | - | - | -  | -  | -  | -  | -         | 4 /19  |
|                      | Wet fur - Whole body             | -             | -     | -  | +  | +  | -  | -  | -  | - | - | - | - | - | - | -  | -  | -  | -  | -         | 2 /19  |
| 5615                 | Normal                           | +             | -     | -  | -  | -  | -  | -  | -  | - | + | + | + | + | + | +  | +  | +  | +  | +         | 11 /19 |
|                      | Fur staining by test item - Nose | -             | -     | -  | +  | +  | +  | +  | +  | + | - | - | - | - | - | -  | -  | -  | -  | -         | 6 /19  |
|                      | Laboured respiration             | -             | SI    | SI | SI | SI | SI | SI | -  | - | - | - | - | - | - | -  | -  | -  | -  | -         | 6 /19  |
|                      | Lack of grooming                 | -             | -     | -  | -  | -  | +  | +  | +  | + | - | - | - | - | - | -  | -  | -  | -  | -         | 4 /19  |
|                      | Noisy respiration                | -             | -     | -  | SI | SI | SI | SI | SI | - | - | - | - | - | - | -  | -  | -  | -  | -         | 5 /19  |
|                      | Red-brown staining - Head        | -             | -     | -  | +  | +  | +  | +  | -  | - | - | - | - | - | - | -  | -  | -  | -  | -         | 4 /19  |
|                      | Wet fur - Whole body             | -             | -     | -  | +  | +  | -  | -  | -  | - | - | - | - | - | - | -  | -  | -  | -  | -         | 2 /19  |
| 5606                 | Normal                           | +             | -     | -  | -  | -  | -  | -  | -  | - | + | + | + | + | + | +  | +  | +  | +  | +         | 11 /19 |
|                      | Fur staining by test item - Nose | -             | -     | -  | +  | +  | +  | +  | +  | + | - | - | - | - | - | -  | -  | -  | -  | -         | 6 /19  |
|                      | Laboured respiration             | -             | SI    | SI | SI | SI | SI | SI | -  | - | - | - | - | - | - | -  | -  | -  | -  | -         | 6 /19  |
|                      | Lack of grooming                 | -             | -     | -  | -  | -  | +  | +  | -  | - | - | - | - | - | - | -  | -  | -  | -  | -         | 2 /19  |
|                      | Noisy respiration                | -             | -     | -  | SI | SI | SI | SI | -  | - | - | - | - | - | - | -  | -  | -  | -  | -         | 4 /19  |
|                      | Red-brown staining - Head        | -             | -     | -  | +  | +  | -  | -  | -  | - | - | - | - | - | - | -  | -  | -  | -  | -         | 2 /19  |
|                      | Wet fur - Whole body             | -             | -     | -  | +  | +  | -  | -  | -  | - | - | - | - | - | - | -  | -  | -  | -  | -         | 2 /19  |
| 5613                 | Normal                           | +             | -     | -  | -  | -  | -  | -  | -  | - | + | + | + | + | + | +  | +  | +  | +  | +         | 11 /19 |
|                      | Activity decreased               | -             | -     | -  | SI | SI | -  | -  | -  | - | - | - | - | - | - | -  | -  | -  | -  | -         | 2 /19  |
|                      | Fur staining by test item - Nose | -             | -     | -  | +  | +  | +  | +  | +  | + | - | - | - | - | - | -  | -  | -  | -  | -         | 6 /19  |
|                      | Laboured respiration             | -             | SI    | SI | SI | SI | SI | SI | -  | - | - | - | - | - | - | -  | -  | -  | -  | -         | 6 /19  |
|                      | Lack of grooming                 | -             | -     | -  | -  | -  | +  | +  | -  | - | - | - | - | - | - | -  | -  | -  | -  | -         | 2 /19  |
|                      | Noisy respiration                | -             | -     | -  | SI | SI | SI | SI | -  | - | - | - | - | - | - | -  | -  | -  | -  | -         | 4 /19  |
|                      | Red-brown staining - Head        | -             | -     | -  | +  | +  | +  | +  | -  | - | - | - | - | - | - | -  | -  | -  | -  | -         | 4 /19  |
| Wet fur - Whole body | -                                | -             | -     | +  | +  | -  | -  | -  | -  | - | - | - | - | - | - | -  | -  | -  | -  | 2 /19     |        |

**Standard footnotes:** + = present      - = absent  
h = hour (s)      ' = minute  
# = Found dead      M = Moribund  
Frequency of observation = number of occurrence of observation / total number of observations

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

MAIN STUDY

DOSE GROUP: 1

CONCENTRATION: 2.58 mg/L

SEX: FEMALE

| Animal number             | Observations                     | Days of study |    |    |    |    |    |    |    |    |   |   |   |   |   | Frequency |    |    |       |        |        |
|---------------------------|----------------------------------|---------------|----|----|----|----|----|----|----|----|---|---|---|---|---|-----------|----|----|-------|--------|--------|
|                           |                                  | 0 (exposure)  |    |    |    |    | 1  | 2  | 3  | 4  | 5 | 6 | 7 | 8 | 9 |           | 10 | 11 | 12    | 13     | 14     |
|                           |                                  | 1h            | 2h | 3h | 4h | 5h |    |    |    |    |   |   |   |   |   |           |    |    |       |        |        |
| 5624M                     | Normal                           | +             | -  | -  | -  | -  | -  | -  | -  | -  | - | - | - | - | - | -         | -  | -  | -     | -      | 1 /9   |
|                           | Activity decreased               | -             | -  | -  | -  | -  | -  | -  | Mo | Mo | - | - | - | - | - | -         | -  | -  | -     | -      | 2 /9   |
|                           | Fur staining by test item - Nose | -             | -  | -  | +  | +  | +  | +  | +  | +  | - | - | - | - | - | -         | -  | -  | -     | -      | 6 /9   |
|                           | Hunched back                     | -             | -  | -  | -  | -  | -  | -  | +  | +  | - | - | - | - | - | -         | -  | -  | -     | -      | 2 /9   |
|                           | Laboured respiration             | -             | Sl | Sl | Sl | Sl | Sl | Sl | Sl | Sl | - | - | - | - | - | -         | -  | -  | -     | -      | 8 /9   |
|                           | Lack of grooming                 | -             | -  | -  | -  | -  | +  | +  | +  | +  | - | - | - | - | - | -         | -  | -  | -     | -      | 4 /9   |
|                           | Noisy respiration                | -             | -  | -  | Sl | Sl | Sl | Sl | Sl | Sl | - | - | - | - | - | -         | -  | -  | -     | -      | 6 /9   |
|                           | Red-brown staining - Head        | -             | -  | -  | +  | +  | +  | +  | +  | +  | - | - | - | - | - | -         | -  | -  | -     | -      | 6 /9   |
|                           | Tremors Continous - Whole body   | -             | -  | -  | -  | -  | -  | -  | +  | +  | - | - | - | - | - | -         | -  | -  | -     | -      | 2 /9   |
|                           | Wet fur - Whole body             | -             | -  | -  | +  | +  | -  | -  | -  | -  | - | - | - | - | - | -         | -  | -  | -     | -      | 2 /9   |
| Moribund                  | -                                | -             | -  | -  | -  | -  | -  | -  | -  | +  | - | - | - | - | - | -         | -  | -  | -     | 1 /9   |        |
| 5617                      | Normal                           | +             | -  | -  | -  | -  | -  | -  | -  | -  | + | + | + | + | + | +         | +  | +  | +     | +      | 11 /19 |
|                           | Fur staining by test item - Nose | -             | -  | -  | +  | +  | +  | +  | +  | +  | - | - | - | - | - | -         | -  | -  | -     | -      | 6 /19  |
|                           | Laboured respiration             | -             | Sl | Sl | Sl | Sl | Sl | Sl | -  | -  | - | - | - | - | - | -         | -  | -  | -     | -      | 6 /19  |
|                           | Lack of grooming                 | -             | -  | -  | -  | -  | +  | +  | -  | -  | - | - | - | - | - | -         | -  | -  | -     | -      | 2 /19  |
|                           | Noisy respiration                | -             | -  | -  | Sl | Sl | Sl | Sl | -  | -  | - | - | - | - | - | -         | -  | -  | -     | -      | 4 /19  |
|                           | Red-brown staining - Head        | -             | -  | -  | +  | +  | +  | +  | -  | -  | - | - | - | - | - | -         | -  | -  | -     | -      | 4 /19  |
| Wet fur - Whole body      | -                                | -             | -  | +  | +  | -  | -  | -  | -  | -  | - | - | - | - | - | -         | -  | -  | -     | 2 /19  |        |
| 5630                      | Normal                           | +             | -  | -  | -  | -  | -  | -  | -  | -  | + | + | + | + | + | +         | +  | +  | +     | 9 /19  |        |
|                           | Fur staining by test item - Nose | -             | -  | -  | +  | +  | +  | +  | +  | +  | + | - | - | - | - | -         | -  | -  | -     | 8 /19  |        |
|                           | Laboured respiration             | -             | Sl | Sl | Sl | Sl | Sl | Sl | -  | -  | - | - | - | - | - | -         | -  | -  | -     | 6 /19  |        |
|                           | Lack of grooming                 | -             | -  | -  | -  | -  | +  | +  | -  | -  | - | - | - | - | - | -         | -  | -  | -     | 2 /19  |        |
|                           | Noisy respiration                | -             | -  | -  | Sl | Mo | Sl | Sl | -  | -  | - | - | - | - | - | -         | -  | -  | -     | 4 /19  |        |
|                           | Red-brown staining - Head        | -             | -  | -  | +  | +  | +  | +  | -  | -  | - | - | - | - | - | -         | -  | -  | -     | 4 /19  |        |
| Wet fur - Whole body      | -                                | -             | -  | +  | +  | -  | -  | -  | -  | -  | - | - | - | - | - | -         | -  | -  | 2 /19 |        |        |
| 5622                      | Normal                           | +             | -  | -  | -  | -  | -  | -  | -  | -  | + | + | + | + | + | +         | +  | +  | +     | 11 /19 |        |
|                           | Activity decreased               | -             | -  | -  | -  | Sl | -  | -  | -  | -  | - | - | - | - | - | -         | -  | -  | -     | 1 /19  |        |
|                           | Fur staining by test item - Nose | -             | -  | -  | +  | +  | +  | +  | +  | +  | - | - | - | - | - | -         | -  | -  | -     | 6 /19  |        |
|                           | Laboured respiration             | -             | Sl | Sl | Sl | Sl | Sl | Sl | -  | -  | - | - | - | - | - | -         | -  | -  | -     | 6 /19  |        |
|                           | Lack of grooming                 | -             | -  | -  | -  | -  | +  | +  | -  | -  | - | - | - | - | - | -         | -  | -  | -     | 2 /19  |        |
|                           | Noisy respiration                | -             | -  | -  | Sl | Sl | Sl | Sl | -  | -  | - | - | - | - | - | -         | -  | -  | -     | 4 /19  |        |
| Red-brown staining - Head | -                                | -             | -  | +  | +  | +  | +  | -  | -  | -  | - | - | - | - | - | -         | -  | -  | 4 /19 |        |        |
| Wet fur - Whole body      | -                                | -             | -  | +  | +  | -  | -  | -  | -  | -  | - | - | - | - | - | -         | -  | -  | 2 /19 |        |        |
| 5618                      | Normal                           | +             | -  | -  | -  | -  | -  | -  | -  | -  | + | + | + | + | + | +         | +  | +  | +     | 11 /19 |        |
|                           | Fur staining by test item - Nose | -             | -  | -  | +  | +  | +  | +  | +  | +  | - | - | - | - | - | -         | -  | -  | -     | 6 /19  |        |
|                           | Laboured respiration             | -             | Sl | Sl | Sl | Sl | Sl | Sl | -  | -  | - | - | - | - | - | -         | -  | -  | -     | 6 /19  |        |
|                           | Lack of grooming                 | -             | -  | -  | -  | -  | +  | +  | -  | -  | - | - | - | - | - | -         | -  | -  | -     | 2 /19  |        |
|                           | Noisy respiration                | -             | -  | -  | Sl | Sl | Sl | Sl | -  | -  | - | - | - | - | - | -         | -  | -  | -     | 4 /19  |        |
|                           | Red-brown staining - Head        | -             | -  | -  | +  | +  | +  | +  | -  | -  | - | - | - | - | - | -         | -  | -  | -     | 4 /19  |        |
| Wet fur - Whole body      | -                                | -             | -  | +  | +  | -  | -  | -  | -  | -  | - | - | - | - | - | -         | -  | -  | 2 /19 |        |        |

**Standard footnotes:** + = present      - = absent  
h = hour (s)      ' = minute  
# = Found dead      M = Moribund  
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:

2.75 mg/L

SEX: MALE

| Animal Number | Body weight (g) on days |     |     |     |     | Body weight gain (g) between days |     |     |      |      |
|---------------|-------------------------|-----|-----|-----|-----|-----------------------------------|-----|-----|------|------|
|               | 0                       | 1   | 3   | 7   | 14  | 0-1                               | 1-3 | 3-7 | 7-14 | 0-14 |
| 5603          | 368                     | 339 | 342 | 372 | 406 | -29                               | 3   | 30  | 34   | 38   |
| 5604          | 376                     | 353 | 353 | 373 | 413 | -23                               | 0   | 20  | 40   | 37   |

SIGHTING EXPOSURE

DOSE GROUP:

0.1

CONCENTRATION:

2.75 mg/L

SEX: FEMALE

| Animal Number | Body weight (g) on days |     |     |     |     | Body weight gain (g) between days |     |     |      |      |
|---------------|-------------------------|-----|-----|-----|-----|-----------------------------------|-----|-----|------|------|
|               | 0                       | 1   | 3   | 7   | 14  | 0-1                               | 1-3 | 3-7 | 7-14 | 0-14 |
| 5620          | 229                     | 211 | 205 | 213 | 234 | -18                               | -6  | 8   | 21   | 5    |
| 5619          | 231                     | 234 | 239 | 263 | 274 | 3                                 | 5   | 24  | 11   | 43   |

MAIN STUDY

DOSE GROUP:

1

CONCENTRATION:

2.58 mg/L

SEX: MALE

| Animal Number              | Body weight (g) on days |       |       |       |       | Body weight gain (g) between days |      |      |      |      |
|----------------------------|-------------------------|-------|-------|-------|-------|-----------------------------------|------|------|------|------|
|                            | 0                       | 1     | 3     | 7     | 14    | 0-1                               | 1-3  | 3-7  | 7-14 | 0-14 |
| 5612                       | 397                     | 371   | 376   | 400   | 432   | -26                               | 5    | 24   | 32   | 35   |
| 5610                       | 387                     | 365   | 371   | 395   | 425   | -22                               | 6    | 24   | 30   | 38   |
| 5615                       | 396                     | 387   | 365   | 362   | 390   | -9                                | -22  | -3   | 28   | -6   |
| 5606                       | 385                     | 352   | 360   | 379   | 418   | -33                               | 8    | 19   | 39   | 33   |
| 5613                       | 385                     | 365   | 374   | 394   | 412   | -20                               | 9    | 20   | 18   | 27   |
| <b>Mean:</b>               | 390.0                   | 368.0 | 369.2 | 386.0 | 415.4 | -22.0                             | 1.2  | 16.8 | 29.4 | 25.4 |
| <b>Standard deviation:</b> | 6.0                     | 12.7  | 6.6   | 15.5  | 16.1  | 8.8                               | 13.1 | 11.3 | 7.6  | 18.0 |

DOSE GROUP:

1

CONCENTRATION:

2.58 mg/L

SEX: FEMALE

| Animal Number              | Body weight (g) on days |       |       |       |       | Body weight gain (g) between days |       |      |      |      |
|----------------------------|-------------------------|-------|-------|-------|-------|-----------------------------------|-------|------|------|------|
|                            | 0                       | 1     | 3     | 7     | 14    | 0-1                               | 1-3   | 3-7  | 7-14 | 0-14 |
| 5624M                      | 250                     | 229   | 185   | -     | -     | -21                               | -44   | -    | -    | -    |
| 5617                       | 252                     | 239   | 234   | 248   | 266   | -13                               | -5    | 14   | 18   | 14   |
| 5630                       | 246                     | 239   | 240   | 257   | 271   | -7                                | 1     | 17   | 14   | 25   |
| 5622                       | 259                     | 247   | 240   | 245   | 263   | -12                               | -7    | 5    | 18   | 4    |
| 5618                       | 264                     | 254   | 257   | 276   | 295   | -10                               | 3     | 19   | 19   | 31   |
| <b>Mean:</b>               | 254.2                   | 241.6 | 231.2 | 256.5 | 273.8 | -12.6                             | -10.4 | 13.8 | 17.3 | 18.5 |
| <b>Standard deviation:</b> | 7.2                     | 9.4   | 27.2  | 14.0  | 14.5  | 5.2                               | 19.2  | 6.2  | 2.2  | 12.0 |

Standard footnotes:

# = Found dead

M = Moribund

- = No data

## APPENDIX 3 Individual Necropsy Findings

### SIGHTING EXPOSURE

CONCENTRATION: 2.75 mg/L

SEX: MALE

| Dose group | Animal Number | Necropsy Day | External Observations             | Internal Observations             | Organ/Tissue   |
|------------|---------------|--------------|-----------------------------------|-----------------------------------|----------------|
| 0.1        | 5603♂         | Day 14       | No external observations recorded | No internal observations recorded | Not applicable |
|            | 5604♂         | Day 14       | No external observations recorded | No internal observations recorded | Not applicable |

CONCENTRATION: 2.75 mg/L

SEX: FEMALE

| Dose group | Animal Number | Necropsy Day | External Observations             | Internal Observations             | Organ/Tissue   |
|------------|---------------|--------------|-----------------------------------|-----------------------------------|----------------|
| 0.1        | 5620♀         | Day 14       | No external observations recorded | No internal observations recorded | Not applicable |
|            | 5619♀         | Day 14       | No external observations recorded | No internal observations recorded | Not applicable |

### MAIN STUDY

CONCENTRATION: 2.58 mg/L

SEX: MALE

| Dose group | Animal Number | Necropsy Day | External Observations             | Internal Observations             | Organ/Tissue   |
|------------|---------------|--------------|-----------------------------------|-----------------------------------|----------------|
| 1          | 5612          | Day 14       | No external observations recorded | No internal observations recorded | Not applicable |
|            | 5610          | Day 14       | No external observations recorded | No internal observations recorded | Not applicable |
|            | 5615          | Day 14       | No external observations recorded | No internal observations recorded | Not applicable |
|            | 5606          | Day 14       | No external observations recorded | No internal observations recorded | Not applicable |
|            | 5613          | Day 14       | No external observations recorded | No internal observations recorded | Not applicable |

CONCENTRATION: 2.58 mg/L

SEX: FEMALE

| Dose group | Animal Number | Necropsy Day                      | External Observations             | Internal Observations                        | Organ/Tissue   |
|------------|---------------|-----------------------------------|-----------------------------------|--|----------------|
| 1          | 5624M         | Day 4                             | No external observations recorded | Discoloration, pale, few, left, lateral lobe | Liver          |
|            |               |                                   |                                   | Discoloration, pale, few, right, medial lobe |                |
|            |               |                                   |                                   | Small  | Spleen         |
|            |               |                                   |                                   | Small  | Thymus         |
|            | 5617          | Day 14                            | No external observations recorded | No internal observations recorded            | Not applicable |
|            | 5630          | Day 14                            | No external observations recorded | No internal observations recorded            | Not applicable |
|            | 5622          | Day 14                            | No external observations recorded | Discoloration, dark red, focal, left lobe    | Lungs          |
| 5618       | Day 14        | No external observations recorded | No internal observations recorded | Not applicable                               |                |

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

## APPENDIX 4 Pathology Report

### PATHOLOGY REPORT

#### INTRODUCTION

The objective of the study was to assess the acute inhalation toxicity of the test item, isocycloseram/emamectin benzoate SC (A23220A) in Wistar CrI:WI rats following a single four-hour nose only exposure.

#### METHODS

Necropsy was performed on one pre terminal main study female (No.5624) and all the surviving animals from the sighting exposure and main study. The surviving animals were euthanized upon completion of the observation period on Day 14. These rats were anesthetized with intra peritoneal injection of pentobarbital, followed by exsanguination. Gross pathology consisted of complete examination of the abdominal and thoracic cavities with special attention to respiratory tract for macroscopic signs of irritancy or local toxicity. Histopathological examination was not performed.

#### PRE TERMINAL

One female (No.5624) from the main study was pre terminally euthanised on Day 4 of the treatment.

The clinical observations shown by this animal prior to death included decreased activity, fur staining around the nose by the test item, lack of grooming, hunched back, laboured and noisy respiration, red brown staining of the head, whole body wet fur and whole-body continuous tremors. Necropsy revealed few pale discolouration in the left lateral and right medial lobe of liver, small spleen and small thymus. Histopathological examination was not carried out and probable cause of death was not determined.

#### TERMINAL (DAY 14)

##### Macroscopic Findings

##### *Sighting exposure (2.75 mg/L)*

The two males and two females from the sighting exposure survived the 14-day observation period and necropsy revealed no visible macroscopic lesions.

##### *Main study (2.58 mg/L)*

The remaining animals (5males and 4 females) survived the 14-day observation period.

One of the females (No. 5622) had dark red focal discolouration of the left lobe of the lungs.

All remaining terminal animals had no visible lesions at necropsy.

## CONCLUSION

A single four hours' nose-only exposure of isocycloseram/emamectin benzoate SC (A23220A) in Wistar Cri:WI rats dosed at 2.58 mg/L in the main study resulted in the pre terminal euthanasia of one female on Day 4 of test item exposure. Necropsy revealed few pale discoloration in the left lateral and right medial lobe of liver, small spleen and small thymus.

Two males and two females from the sighting exposure and the remaining animals in the main study (5 males and 4 females) survived the 14-day observation period. One of the main study females had dark red focal discoloration of the left lobe of the lungs and the remaining main study animals had no macroscopic findings at necropsy.



Babu Gangadharan, BVSc&AH, MVSc, Ph. D, DipRCPath.  
Pathologist

14 Dec 2020

Date:

# APPENDIX 5 Copy of the Certificate of Analysis



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

## Certificate of Analysis

**A23220A**  
**isocycloseram/emamectin benzoate**  
**SC (200/050)**  
**TSC002-041-001**

|                             |   |
|-----------------------------|---|
| <b>Batch Identification</b> | TSC002-041-001                                |
| Other Batch ID              | 1122866                                       |
| <b>Product Code</b>         | <b>A23220A</b>                                |
| Other Product Code(s)       | isocycloseram/emamectin benzoate SC (200/050) |

**Chemical Analysis**  
(Active Ingredient content)

- Identity of the Active Ingredient(s)\* confirmed
- Content of isocycloseram\* 17.5 % w/w corresponding to 201 g/l
- Content of emamectin benzoate\* 4.18 % w/w corresponding to 48.1 g/l

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

|   |  |
|---|--|
| Methodology used for Characterization / Recertification | LC, chiral LC, oscillating density meter |
|---|--|

**Physical Analysis**

- Appearance brown liquid
- Density\* 1150 kg/m<sup>3</sup>

**Stability:**

- Storage Temperature < 30°C
- Recertification Date End of January 2023

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

This Certificate of Analysis summarizes data which originates either from a single study or from several individual studies. Tests marked with an asterisk (\*) have been conducted in compliance with GLP. Raw data, documentation, study plans, any amendments to study plans and reports pertaining to this/these study/studies are stored under the study number(s) referenced below within the archives of the GLP Testing Facility WMU at Syngenta Crop Protection AG, Switzerland.

Study number of batch characterization: CHMU200180  
Study number(s) of batch recertification:

Authorization: 19-Feb-2020

Dr. Karine Heintz  
Analytical Development & Product Chemistry

**APPENDIX 6      Attempts to Achieve the Maximum Attainable Concentration**

| <b>Technical Trial</b> | <b>Test Item Concentration (% w/w)</b> | <b>Test Item Flow (mL/hr)</b> | <b>Air Flow In (set) (L/min)</b> | <b>Achieved Test Atmosphere Concentration (mg/L)</b> | <b>MMAD (µm)</b> | <b>GSD</b>  |
|------------------------|--|-------------------------------|----------------------------------|--|------------------|-------------|
| 1                      | 100                                    | 150-200                       | 30                               | 0.11-0.38  | -                | -           |
| 1                      | 80                                     | 200                           | 30                               | 0.50-1.87  | 6.49             | 2.81        |
| 1                      | 60                                     | 200                           | 30                               | 1.88-2.08  | 3.74-3.95        | 2.37        |
| 2                      | 70                                     | 200                           | 30                               | 2.26-2.39  | 4.41             | 2.22        |
| 2                      | 70                                     | 200                           | 40                               | 2.26-2.62  | 3.47             | 2.21        |
| 2                      | 65                                     | 200                           | 40                               | 2.28-2.53  | 3.40             | 2.18        |
| 2                      | 65                                     | 200                           | 30                               | 2.03-2.36  | 3.93             | 2.19        |
| 3                      | 70                                     | 200                           | 30                               | 1.69   | -                | -           |
| <b>3*</b>              | <b>70</b>                              | <b>200</b>                    | <b>40</b>                        | <b>1.99-2.34</b>                                     | <b>3.19</b>      | <b>2.39</b> |
| 3                      | 65                                     | 200                           | 40                               | 1.78-2.23  | 2.96             | 2.24        |

\*Note: This setting was used for animal exposures.

## APPENDIX 7 Good Laboratory Practice (GLP) Certificate



**OGYÉI**  
National Institute of  
Pharmacy and Nutrition

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E-mail: ogyei@ogyei.gov.hu, Web: www.ogyei.gov.hu

**Ref. no: OGYÉI/22762-5/2018**

**Admin.:** Dr. Juhász Uzonka

**Date:** 03 August 2018

### GOOD LABORATORY PRACTICE (GLP) CERTIFICATE

It is hereby certified that the test facility

**CiToxLAB Hungary Ltd.**

**H-8200 Veszprém, Szabadságpuszta**

is able to carry out

*physico-chemical testing, toxicity studies, mutagenicity studies, environmental toxicity studies on aquatic or terrestrial organisms, studies on behaviour in water, soil and air; bio-accumulation, analytical and clinical chemistry, pathology studies, preparation of microscopic tissue sections, reproduction toxicology, in vitro studies, inhalation toxicology, and contract archiving*

in compliance with the Principles of GLP (Good Laboratory Practice) and also complies with the corresponding OECD/European Community requirements.

Date of the inspection: **07-11 May 2018.**



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