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.
H-8200 Veszprém, Szabadságpuszta, hrsz. 028/1.
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

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410 Swing Road
Post Office Box 18300
Greensboro, NC 27419-8300 USA

Submitter: _____ Date: _____

Syngenta is the owner of this information and data. Syngenta has submitted this material to the United States Environmental Protection Agency specifically under the provisions contained in FIFRA as amended and, hereby, consents to use and disclosure of this material by EPA according to FIFRA. In submitting this material to EPA according to method and format requirements contained in PR Notice 2011-3, we do not waive any protection or right involving this material that would have been claimed by the company if this material had not been submitted to the EPA, nor do we waive any protection or right provided under FIFRA Section 3 (concerning data exclusivity and data compensation) or FIFRA Section 10(g) (prohibiting disclosure to foreign and multinational pesticide companies or their agents).

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study has been performed in accordance with the Principles of Good Laboratory Practice (Hungarian GLP Regulations: 42/2014. (VIII. 19.) EMMI decree of the Ministry of Human Capacities which corresponds to the OECD GLP, ENV/MC/CHEM (98) 17.)

This study was conducted in accordance with a written Study Plan and 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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To be completed for USA EPA submission only:
Representative of Submitter/Sponsor:

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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

Ágnes 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:

Name	Function
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Carolina Vaccari	Syngenta Study Manager

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) Crl: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 Crl: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^3	
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 μm 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 4μm (considered to be the respirable portion) was determined.

3.4.3 Chamber environmental conditions

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

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

Summaries of the data are presented in Table 3.

3.5 Observations

3.5.1 Clinical observations

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

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_{50} was not calculated.

4.0 RESULTS AND DISCUSSION

4.1 Test Atmosphere Concentration

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

Group	Maximum achievable mean concentration (mg/L)	Standard Deviation	Nominal Concentration (mg/L)
0.1 (Sighting exposure)	2.75	0.22	62.54
1 (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) (μm)	Geometric Standard Deviation (GSD)	Respirable Fraction (% < 4μm)
0.1 (Sighting exposure)	2.75	3.31	2.22	59.3
1 (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

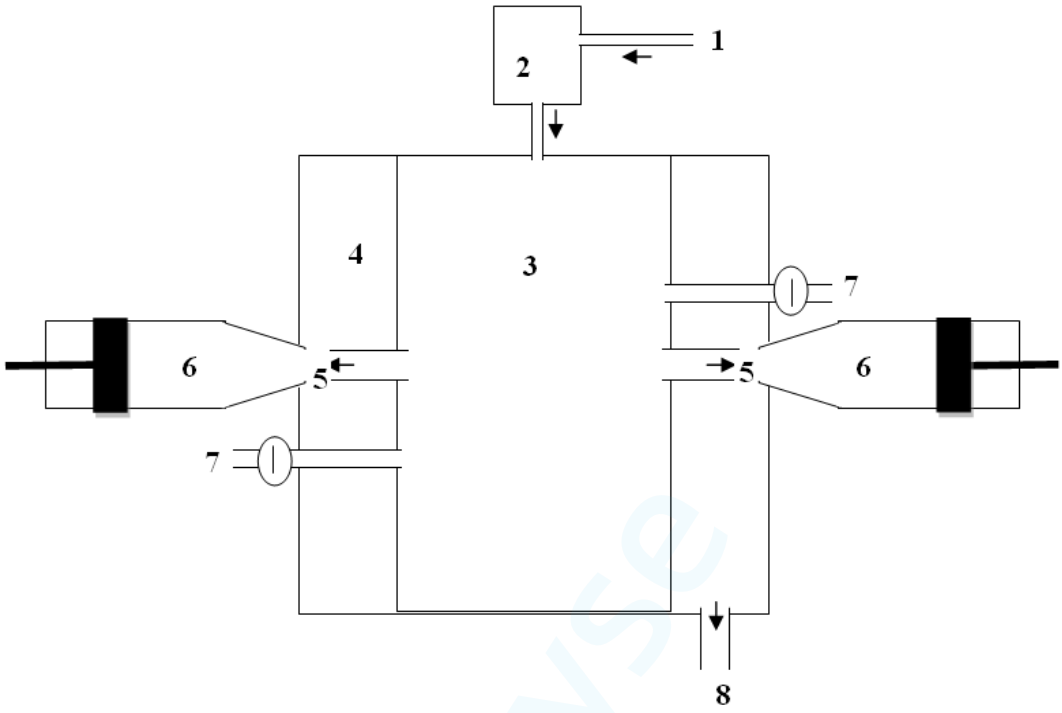
Under the experimental conditions of this study, mortality of one female animal occurred in a group of 10 rats when exposed to 2.58 mg/L of isocycloseram/emamectin benzoate SC (A23220A) for 4 hours. The acute inhalation median lethal concentration of isocycloseram/emamectin benzoate SC (A23220A) in Crl:WI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

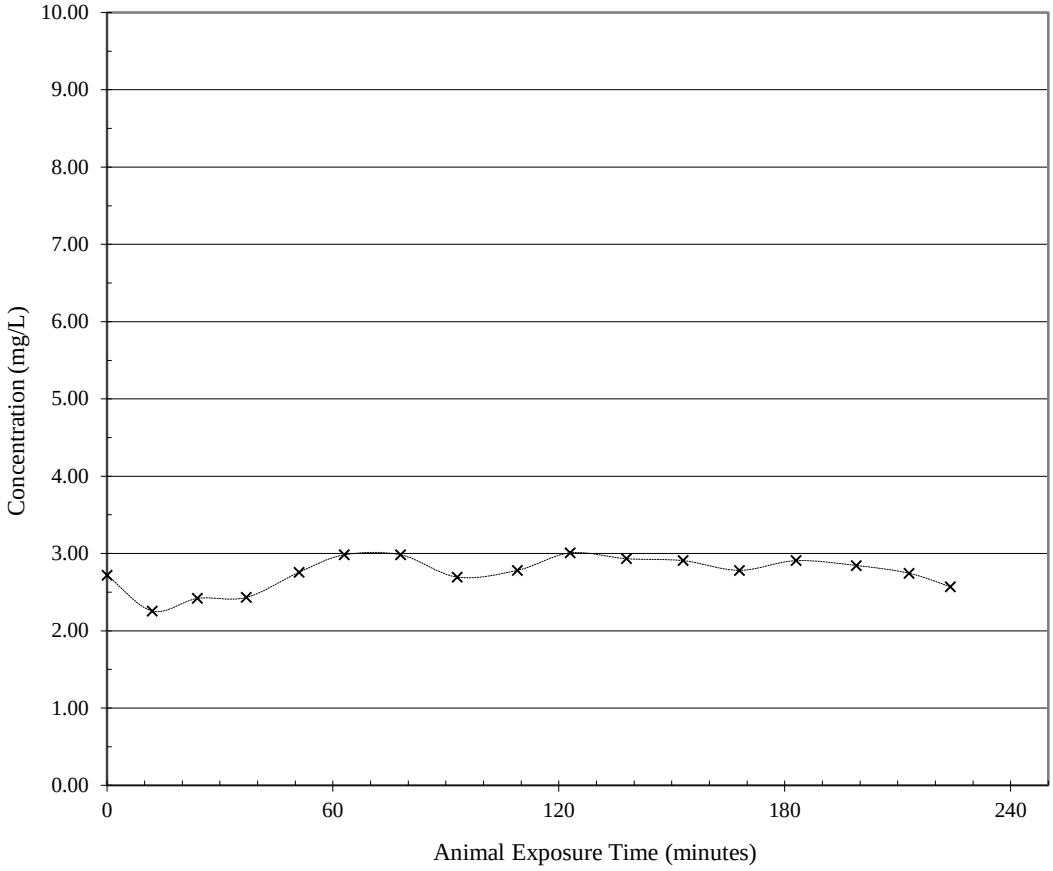


KEY:

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

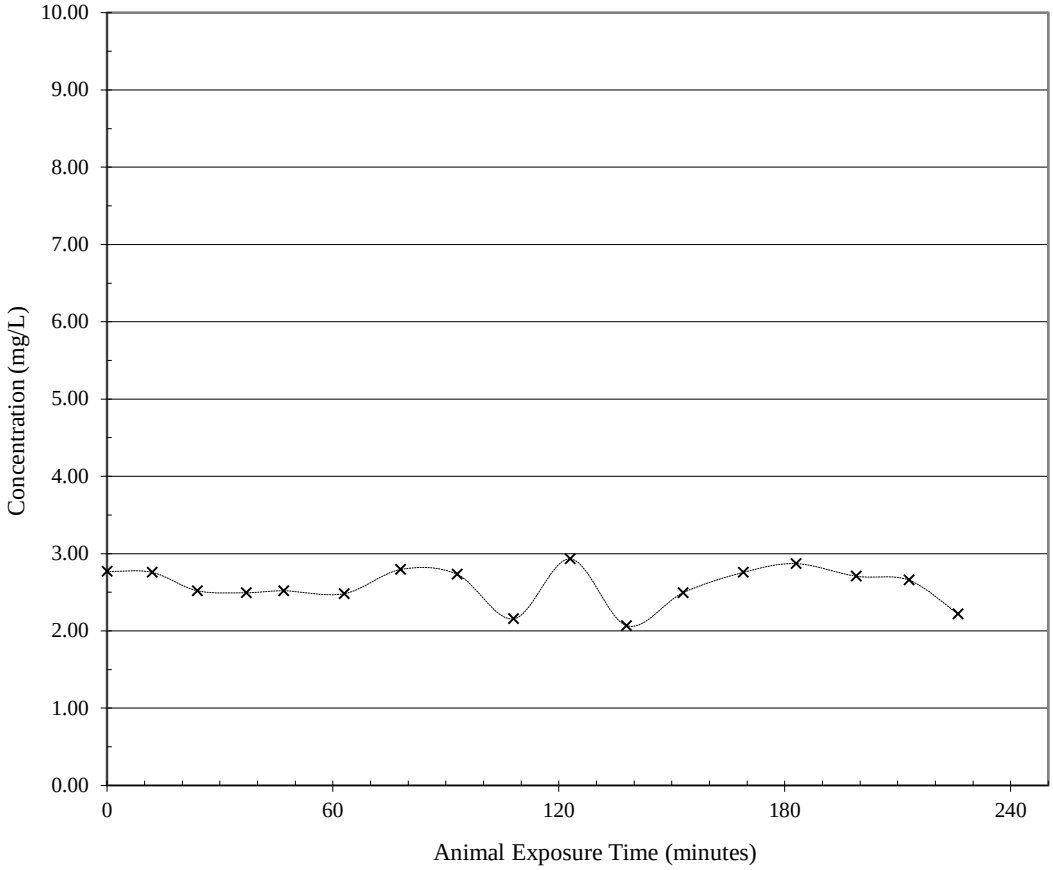
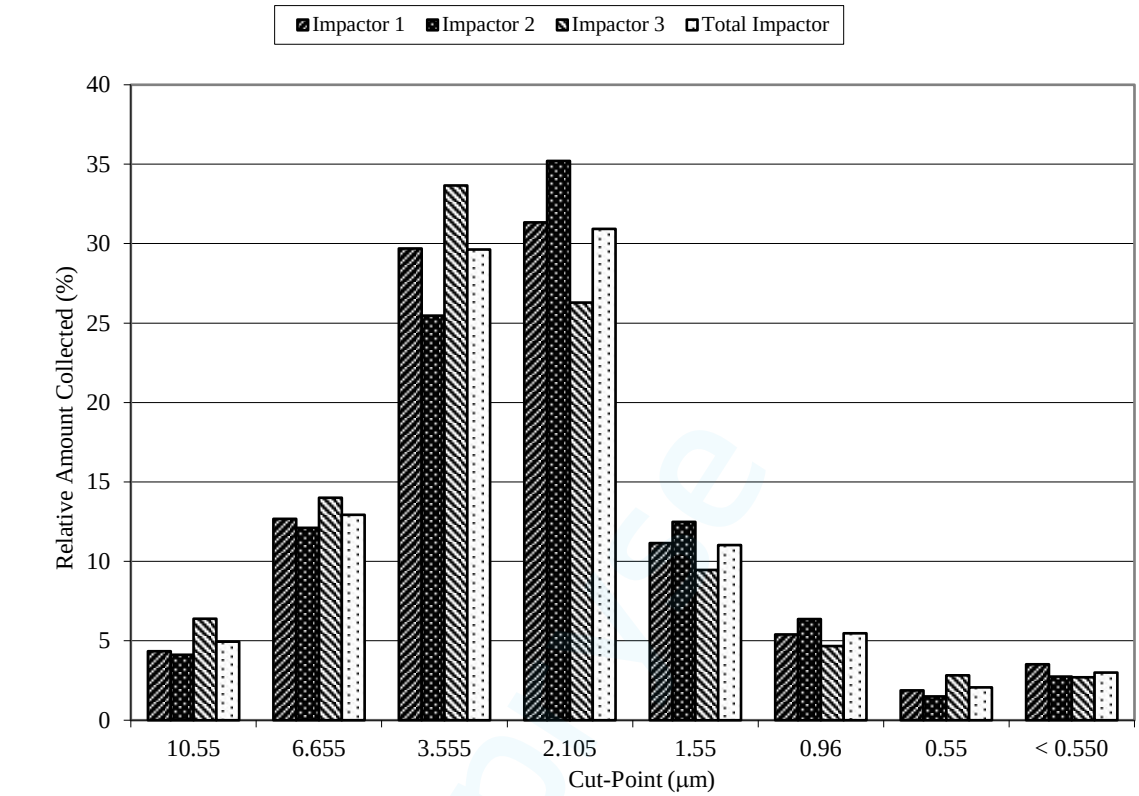
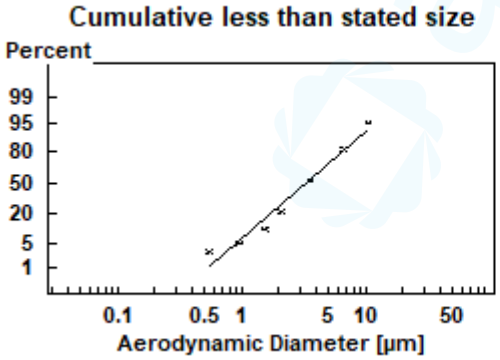
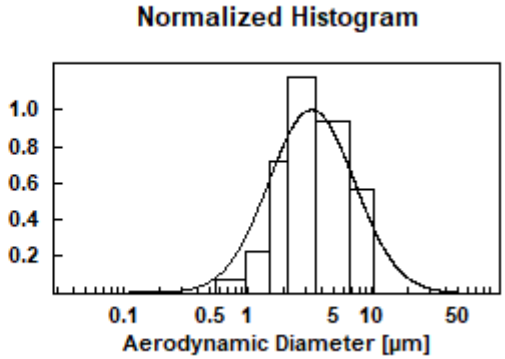


FIGURE 3 Particle Size Distribution

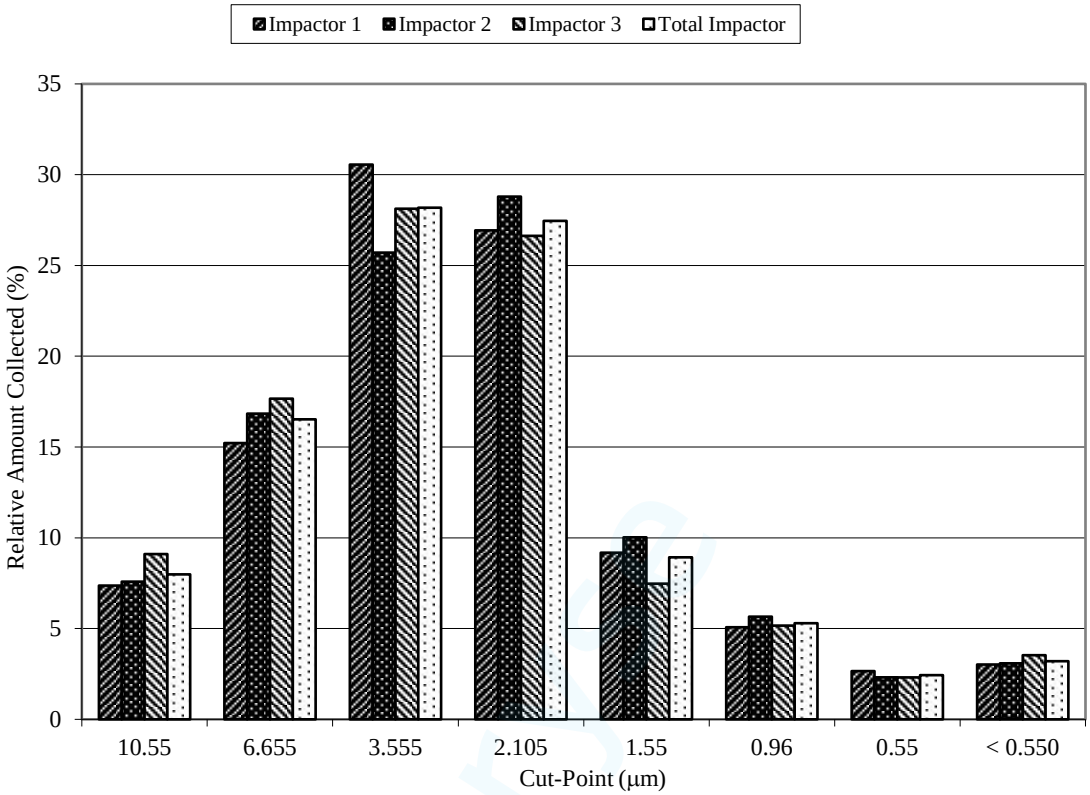
Sighting Exposure – Group 0.1

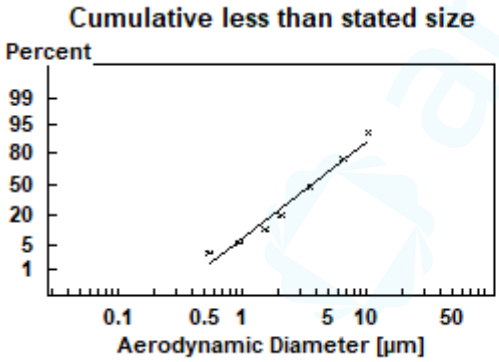


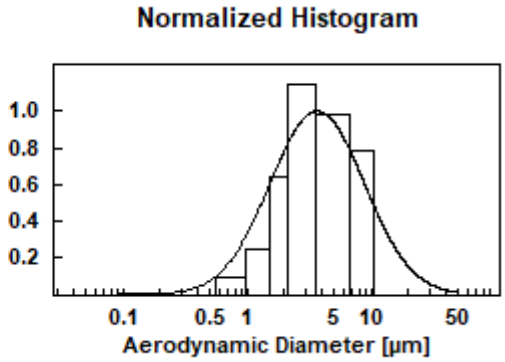




Main Study – Group 1







TABLES SECTION

TABLE 1 Test Atmosphere Concentrations

Sighting Exposure – Group 0.1

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	2.17	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
				Total Amount Collected (mg)	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
				Total Amount Collected (mg)	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_{99}):

$T_{99} = (4.605 \times (^{\text{Chamber Volume}}/_{\text{Chamber Flow rate}})$ (Silver, 1946)
Chamber volume (inner plenum) = 3.85 L (Pauluhn, 1994)
T_{99} (Minimum Acceptable Equilibration Time) = 1 minute
Actual equilibration time allowed = 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 (Inner Plenum) (L/min)	39.9	34.3	41.0
Air Flow Out (Outer Cylinder) (L/min)	40.4	40.2	40.7
Temperature* (°C)	24.4	24.2	24.5
Relative Humidity† (%)	57.6	55.9	58.3
Oxygen Concentration (%)**	-	-	-
Carbon Dioxide (%)**	-	-	-

Theoretical Chamber Equilibration Time (T_{99}):

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

* Temperature was measured by handheld thermometer.
**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 (During Exposure)	0	0	0	0
0 (After Exposure)	0	0	0	0
1	0	0	0	0
2	0	0	0	0
3	0	0	0	0
4	0	0	0	0
5	0	0	0	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	0 (exposure) during 1h	2h	3h	after 4h	5h	Days of study 1	2	3	4	5	6	7	8	9	10	11	12	13	14	Frequency
5603	Normal	+	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	11 /19
	Activity decreased	-	-	-	-	Sl	Sl	Sl	-	-	-	-	-	-	-	-	-	-	-	-	3 /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	Mo	Sl	-	-	-	-	-	-	-	-	-	-	-	-	-	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	-	Sl	Sl	Sl	Sl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4 /19
	Lack of grooming	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	2 /19
	Noisy respiration	-	-	-	Sl	Sl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2 /19
	Red-brown staining - Head	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	3 /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

SIGHTING
EXPOSURE
DOSE GROUP: **0.1**
CONCENTRATION: **2.75 mg/L** SEX: FEMALE

Animal number	Observations	0 (exposure) during 1h	2h	3h	after 4h	5h	Days of study 1	2	3	4	5	6	7	8	9	10	11	12	13	14	Frequency
5620	Normal	+	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	10 /19
	Fur staining by test item - Nose	-	-	-	+	+	+	+	-	+	+	-	-	-	-	-	-	-	-	-	6 /19
	Laboured respiration	-	Sl	Sl	Sl	Sl	Sl	-	-	-	-	-	-	-	-	-	-	-	-	-	5 /19
	Lack of grooming	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	3 /19
	Noisy respiration	-	-	-	Sl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	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	-	Sl	Sl	Sl	Sl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4 /19
	Lack of grooming	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	2 /19
	Noisy respiration	-	-	-	Sl	Sl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	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: Sl = 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	0 (exposure) during 1h	2h	3h	after 4h	5h	Days of study 1	2	3	4	5	6	7	8	9	10	11	12	13	14	Frequency
5612	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
5610	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	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	-	Sl	Sl	Sl	Sl	Sl	Sl	-	-	-	-	-	-	-	-	-	-	-	-	6 /19
	Lack of grooming	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	4 /19
	Noisy respiration	-	-	-	Sl	Sl	Sl	Sl	Sl	-	-	-	-	-	-	-	-	-	-	-	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	-	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	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2 /19
	Wet fur - Whole body	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2 /19
5613	Normal	+	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	11 /19
	Activity decreased	-	-	-	Sl	Sl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2 /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

MAIN STUDY
DOSE GROUP: **1**
CONCENTRATION: **2.58 mg/L** SEX: FEMALE

Animal number	Observations	0 (exposure) during 1h	2h	3h	after 4h	5h	Days of study 1	2	3	4	5	6	7	8	9	10	11	12	13	14	Frequency
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
Mean:	390.0	368.0	369.2	386.0	415.4	-22.0	1.2	16.8	29.4	25.4
Standard deviation:	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
Mean:	254.2	241.6	231.2	256.5	273.8	-12.6	-10.4	13.8	17.3	18.5
Standard deviation:	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 Crl: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 Crl: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 discolouration 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 discolouration 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

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

Certificate of Analysis

A23220A

isocycloseram/emamectin benzoate SC (200/050)

TSC002-041-001

Batch Identification	**TSC002-041-001**
Other Batch ID	1122866
Product Code	**A23220A**
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/m³**
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

Page 1 of 1

APPENDIX 6 Attempts to Achieve the Maximum Attainable Concentration

Technical Trial	Test Item Concentration (% w/w)	Test Item Flow (mL/hr)	Air Flow In (set) (L/min)	Achieved Test Atmosphere Concentration (mg/L)	MMAD (µm)	GSD
1	100	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	-	-
3*	**70**	**200**	**40**	**1.99-2.34**	**3.19**	**2.39**
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

H-1051 Budapest, Zrínyi u. 3.
1372 P.O. Box:450.
Tel: +36 1 88 69-300, Fax: +36 1 88 69 460
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.**

Tarjáni Ibolya
Head of Inspectorate

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."