Cyproconazole/Isopyrazam

Cyproconazole/Isopyrazam SC (A19022A) - Acute Inhalation Toxicity Study (Nose-Only) in the Rat

Final Report

DATA REQUIREMENT(S): OECD 403 (2009)
EPA OPPTS 870.1300 (1998)
EC 440/2008, B.2 (2008)

AUTHOR(S): Krisztina Nagy, M.Sc.

STUDY COMPLETION DATE: 04 September 2012

PERFORMING LABORATORY: CiToxLAB Hungary Ltd.
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LABORATORY PROJECT ID: Report Number: 12/055-004P
Study Number: 12/055-004P
Task Number: TK0006571

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

STATEMENT OF DATA CONFIDENTIALITY CLAIMS

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GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study has been performed in accordance with the Principles of Good Laboratory Practice (Hungarian GLP Regulations: 9/2001. (III. 30.) EüM-FVM joint decree of the Minister of Health and the Minister of Agriculture and Regional Development which corresponds to the OECD GLP, ENV/MC/CHEM (98) 17.).

This study was conducted in accordance with a written Study Plan and any Amendments, authorised by the Sponsor and CiToxLAB Hungary Ltd. 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.

Signature: _____ Date: 04 Sept. 2012.

Krisztina Nagy, M.Sc.
Study Director

Performing Laboratory: CiToxLAB Hungary Ltd.
H-8200 Veszprém, Szabadságpuszta
Hungary

FLAGGING STATEMENT

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QUALITY ASSURANCE STATEMENT

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
28 March 2012	Study Plan	28 March 2012	28 March 2012
14 June 2012	Clinical Observation	14 June 2012	14 June 2012
16 July 2012	Draft Report	16 July 2012	16 July 2012
04 September 2012	Final Report	04 September 2012	04 September 2012

Signature: Gyimesi Vanda Date: 04 September 2012

Vanda Gyimesi, M.Sc.
Deputy Head of QAU

MANAGEMENT STATEMENT

According to the conditions of the research and development agreement between Syngenta Ltd. (as Sponsor) and CiToxLAB Hungary Ltd. (as Test Facility), the study titled "Cyproconazole/Isopyrazam SC (A19022A) - Acute Inhalation Toxicity Study (Nose-Only) in the Rat", has been performed in compliance with current SOPs.

Signature: _____ Date: 04 Sept. 2012

Christopher Banks, DABT
Managing Director

GENERAL INFORMATION

Contributors

The following contributed to this report in the capacities indicated:

Name	Function
Krisztina Nagy, M.Sc.	Study Director
András Mátyás, M.Sc.	Assistant Scientist
Szabolcs Gáty, M.Sc.	Head of Quality Assurance
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STUDY DATES

Sighting Exposure – Group 0.1

Receipt of Animals	03 May 2012
Experimental Starting Date	31 May 2012
Experimental Completion Date	01 June 2012
Inhalation Exposure (Day 0)	31 May 2012
Observation	31 May 2012 – 01 June 2012
Necropsy	01 June 2012

Sighting Exposure – Group 0.2

Receipt of Animals	31 May 2012
Experimental Starting Date	06 June 2012
Experimental Completion Date	20 June 2012
Inhalation Exposure (Day 0)	06 June 2012
Observation	06 June 2012 – 20 June 2012
Necropsy	20 June 2012

Group 1

Reception of Animals	05 June 2012
Experimental Starting Date	12 June 2012
Experimental Completion Date	26 June 2012
Inhalation Exposure (Day 0)	12 June 2012
Observation	12 June 2012 – 26 June 2012
Necropsy	26 June 2012

Performing laboratory test substance reference number
12003B

Other
The study documents:
- 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 and applicable SOP's in the archives of CiToxLAB Hungary Ltd. 8200 Veszprém, Szabadságpuszta, 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 Sponsor will be contacted as to the fate of the archived materials.

Deviations to the Study Plan
Due to extended technical phase the date of draft report was changed from 30 June 2012 to 16 July 2012.
Due to failure of the TSE temperature-humidity sensor in the exposure system during the animal exposures, the humidity was not measured and the temperature was recorded using a calibrated mini-max thermometer at hourly intervals.

In the opinion of the Study Director, these deviations had no effect on the purpose, integrity or outcome of the study.

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1.0 EXECUTIVE SUMMARY

1.1 Study Design

The aim of this study was to assess the inhalation toxicity of Cyproconazole/Isopyrazam SC (A19022A). Two sighting exposures using 2 male and 2 female rats each were performed prior to the main study at target aerosol concentrations of 5 and 1 mg/L due to insufficient information about the test item's acute inhalation toxicity.

The main study group, consisting of 10 CRL: (WI) Wistar strain rats (5 males and 5 females) was exposed to a target aerosol concentration of 1 mg/L Cyproconazole/Isopyrazam SC (A19022A). 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 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 sacrificed and subjected to a gross examination *post mortem*.

1.2 Results

Four dead animals were observed in the study following a 4 hour exposure to Cyproconazole/Isopyrazam SC (A19022A) in animal exposures at a concentration of 5.27 mg/L (Group 0.1). No deaths occurred in the sighting exposure study conducted at 1.15 mg/l nor in the main study.

Exposures conditions:

Sighting Exposures - Group 0.1: The mean achieved atmosphere concentration was 5.27 mg/L. The MMAD (Mean Mass Aerodynamic Diameter) was 3.63 µm ± 2.15 (GSD [Geometric Standard Deviation]).

Sighting Exposures - Group 0.2: The mean achieved atmosphere concentration was 1.15 mg/L. The MMAD (Mean Mass Aerodynamic Diameter) was 2.80 µm ± 1.94 (GSD [Geometric Standard Deviation]).

Main Study - Group 1: The mean achieved atmosphere concentration was 1.04 mg/L. The MMAD (Mean Mass Aerodynamic Diameter) was 2.52 µm ± 1.84 (GSD [Geometric Standard Deviation]).

***Clinical observations*:**

Wet fur and fur staining were commonly recorded on the day of exposure and on the day after exposure. These observations were considered to be related to the restraint and exposure procedures and, in isolation, were considered not to be treatment related.

Sighting exposure – Group 0.1: The following clinical signs were recorded on the day of exposure in the sighting exposures: laboured, gasping and noisy respiration and decreased activity. All four animals were found dead on the following day of exposure.
Sighting exposure – Group 0.2: Laboured, noisy respiration and decreased activity were noted for the exposed animals on day of exposure. In addition, sneezing, hunched posure, weak and wasted condition were noted during first week of recovery period. The exposed animals recovered and all clinical signs ceased from Day 10.

Main study: In the main study similar clinical signs were recorded as in the sighting exposures on day of exposure: laboured and noisy respiration, decreased activity. In addition, sneezing, gasping respiration, hunched posure, weak and wasted condition were recorded during the observation period. The exposed animals recovered and all significant clinical signs ceased from Day 10, however fur loss was noted for two females from Day 12 to 14.

Bodyweights:

In the study, normal bodyweight gain was noted for all surviving animals from Day 1 to the end of the observation period, with the exception of three animals in the main study where slight body weight loss was noted during first three days.

Necropsy:

A single four hour nose-only exposure of Cyproconazole/Isopyrazam SC (A19022A) to CRL (WI) Wistar strain rat led to the death of four animals dosed at 5.27 mg/L during a sighting exposure. Dark/red discoloration of the non-collapsed lungs and beige liquid at the fur of perinasal area were considered to be associated with the administration of the test item.

In surviving animals subjected to the necropsy on Day 14, no test item-related macroscopic changes were seen in the sighting study animals exposed at 1.15 mg/l or in the main study at a concentration of 1.04 mg/l.

1.3 Conclusion

Under the experimental conditions of this study, no death occurred in a group of 10 rats exposed to a mean achieved atmosphere of 1.04 mg/L for 4 hours. The acute inhalation median lethal concentration of Cyproconazole/Isopyrazam SC (A19022A), in CRL: (WI) Wistar strain rats is therefore considered to be greater than 1.04 mg/L.

2.0 INTRODUCTION

2.1 Purpose

This study was performed to assess the acute inhalation toxicity of Cyproconazole/Isopyrazam SC (A19022A) following a 4 hour exposure at a target concentration of 1 mg/L to 5 male and 5 female CRL: (WI) Wistar strain rats. Two sighting exposures were performed prior the main study with 2 males and 2 females each at target concentrations of 1.0 mg/L and the maximum attainable concentration of 5.27 mg/L.

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: 7 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).

3.0 MATERIALS AND METHODS

3.1 Test Item

Name:	Cyproconazole/Isopyrazam SC (A19022A)
Synonyms:	cyproconazole/isopyrazam SC (080/125), SAN619/SYN520453 SC (080/125)
Batch number:	J8657/147
Purity:	cyproconazole – 7.45% w/w
	isopyrazam – 12.0% w/w
	SYN534969 – 10.4% w/w
	SYN534968 – 1.58% w/w
Product code:	A19022A
Appearance:	Beige liquid
Recertification date:	End of October 2013
Storage conditions:	< 30^{o}C

Safety precautions:	Routine safety precautions (lab coat, gloves, goggles, face mask) for unknown materials were applied to assure personnel health and safety.

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 Unit of CiToxLAB Hungary Ltd., was made on the basis of these data. The Certificate of Analysis for the material is presented in Appendix 6.

3.1.2 Preparation

Due to the physical properties of the test item as supplied, suitable atmospheres could not be produced. A range of formulations and homogenisation techniques (*T 25 digital ULTRA-TURRAX*®) were therefore attempted in order to improve the physical characteristics of the test item and all data associated is retained in the raw data but not reported. Animals were exposed to a test atmosphere produced from a dilution with distilled water *(TEVA Zrt., H-2100 Gödöllő, Táncsics Mihály u. 82, Hungary; Batch: 3450611; Expiry: June 2014)*. As the formulation Cyproconazole/Isopyrazam SC (A19022A): distilled water (80:20%, w/w) dilution was prepared shortly before use, determination of the concentration, homogeneity and stability of the formulation were not required and were not performed.

3.2 Experimental Animals

3.2.1 Specification

Nine male and nine female CRL: (WI) Wistar strain rats were obtained from Charles River Laboratories, Research Models and Servicies (Germany GmbH, Sanhofer Weg 7, D-97633 Sulzfeld). After arrival, their health was certified by the resident veterinarian and after an acclimatisation period of at least five days, the animals were assigned to the study. The animals were randomised 1 day prior to exposure. At randomisation, the animals were 9 - 11 weeks old and, on day of exposure in the weight range of 180 to 406g (♂: 288-406g; ♀: 180-251g). The females were nulliparous and non-pregnant.

3.2.2 Justification

Rats are the preferred species, as historically they have been used for this type of study and they are specified by the appropriate regulatory authorities.

3.2.3 Husbandry

The animals were housed in groups of 5 (or 2 in the case of the sighting exposures), by sex, in solid-floor cages (Type III) with stainless steel mesh lids and softwood flake bedding. The environmental controls were set to achieve target values of 22 ± 3 °C and 30-70%. The animal room was ventilated for at least 15 air exchanges per hour and the lighting controlled to give 12 hours of continuous artificial light in each 24 hour period.

3.2.4 Diet and water

The animals were provided with ssniff SM R/M-Z+H "Autoclavable Complete Feed for Rats and Mice – Breeding and Maintenance" *(ssniff Spezialdiäten GmbH, D-59494 Soest Germany, Batch No.: i) 719 6627(expiry 05-2012), ii) 601 7197(expiry 09-2012)* and tap water fit for human consumption, *ad libitum*.

The content of the diet and the test report of the diet analysis are available in the raw data.

The diet and drinking water are routinely analysed and are considered not to contain any contaminants that could reasonably be expected to affect the purpose or integrity of the study. Copies of the relevant Certificates of Analysis are retained in the archive of CiToxLAB Hungary Ltd.

Water quality control analysis is performed once every 3 months and microbiological assessment is performed monthly, by Veszprém County Institute of State Public Health and Medical Officer Service (ÁNTSZ, H-8201 Veszprém, József A.u.36, Hungary). The quality control results are retained in the archive of CiToxLAB Hungary Ltd.

3.2.5 Identification

Each animal was identified by a unique number marked on the tail. The animal number was assigned on the basis of the CiToxLAB Hungary Ltd. master file.

Cages were identified by cage card, giving details of study code, sex, dose-group, cage number and individual animal numbers.

3.3 Inhalation Exposure

3.3.1 Technical trials

Prior to animal exposures, test material atmospheres were generated within the exposure chamber. During these technical trials, air-flow settings and test material input rates were adjusted to achieve the required atmospheric characteristics.

3.3.2 Atmosphere generation

The test item formulation was aerosolised using a stainless steel concentric jet nebuliser (TSE Systems GmbH, Bad Homburg, Germany) located at the top of the exposure chamber. The rate of formulation 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 comprises 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 nares 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 0.7 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 (Pauluhn, 1994).

3.3.4 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}) (Silver, 1946), groups of rats were exposed to target atmospheres of either 1.0 mg/L or 5 mg/L for a period of 4 hours.

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 GF10, Whatman, Germany). The filters were then dried, at atmospheric pressure, in a dessicator at room temperature for approximately 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 33.57% (n = 10) with a standard deviation 0.29%. Data associated with this is kept in the raw data.

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). The difference in the pre and post sampling weights, divided by the volume of atmosphere sampled, was equal to the actual achieved test atmosphere concentration.

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, divided by the volume of atmosphere sampled, was the chamber concentration in terms of non-volatile component.

Based on the results of the preliminary work, these figures were adjusted to obtain a true figure for the test material concentration in the test atmospheres.

The nominal concentration was calculated by dividing the mass of test material disseminated into the chamber by the total volume of air that 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 0.55, 0.96, 1.55, 2.11, 3.56, 6.66 and 10.55 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 were calculated. In addition, the proportion (%) of aerosol less than 4μm (considered to be the inhalable portion) was determined.

3.4.3 Chamber environmental conditions

The following variables were monitored continuously and recorded every minute during each exposure period by the TSE-DACO monitoring system integrated into the exposure system:

- Chamber airflow rates
- Test atmosphere temperature*
- Test atmosphere relative humidity*
- Test atmosphere carbon dioxide concentration
- Test atmosphere oxygen concentration

Summaries of the data are presented in Table 3.

3.5 Observations

3.5.1 Morbidity/mortality

Animals were checked hourly during exposure, 1 hour after exposure and twice daily (early and late in the working day) during the 14 days of the observation period for morbidity and/or mortality.

3.5.2 Clinical signs

All animals were observed for clinical signs at hourly intervals during exposure, as soon as practically possible following removal from restraint at the end of exposure, 1 hour after exposure and once daily for 14 days.

3.5.3 Bodyweight

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

3.6 Necropsy

At the end of the 14 day observation period, the animals were sacrificed by exsanguination under anaesthesia (intra-peritoneal injection of pentobarbital solution - *'Euthasol® 40%'; Lot*

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* = *Due to failure of the TSE temperature-humidity sensor in the exposure system during the animal exposures, the humidity was not measured and the temperature was recorded using a calibrated mini-max thermometer at hourly intervals.*

No.: 11B15 8; Expiry: 07-2014; Produced by AST Beheer B.V. Oudewater Netherlands (Produlab Pharma, Raamsdonksveer) and a gross macroscopic examination performed. All animals were subject to a gross necropsy which included a detailed examination of the abdominal and thoracic cavities. Special attention was given to the respiratory tract for macroscopic signs of irritancy or local toxicity.

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 observations, bodyweight changes, macroscopic abnormalities or any other toxicological effects.

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 Number	Mean Achieved (mg/L)	Standard Deviation	Nominal (mg/L)
Sighting Exposure 0.1	5.27	0.19	87.24
Sighting Exposure 0.2	1.15	0.23	8.84
Main Study 1	1.04	0.09	10.94

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 Number	Mean Achieved (mg/L)	Mean Mass Median Aerodynamic Diameter (MMAD) (μm)	Geometric Standard Deviation	Inhalable Fraction (% < 4μm)
Sighting Exposure 0.1	5.27	3.63	2.15	54.9
Sighting Exposure 0.2	1.15	2.80	1.94	70.4
Main Study 1	1.04	2.52	1.84	77.6

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

4.3 Mortality Rates

Mortality data are detailed in Table 4.

All animals from the Group 0.1 sighting exposure were found dead in the study following a 4 hour exposure to Cyproconazole/Isopyrazam SC (A19022A) at a maximum attainable concentration of 5.27 mg/L. No deaths were recorded in either sighting study Group 0.2 or main study 1.

4.4 Clinical Observations

Individual clinical observations are presented in Appendix 2.

Wet fur and fur staining were commonly recorded on the day of and the day following exposure. These observations were considered to be related to the restraint and exposure procedures and, in isolation, were considered not to be treatment related.

Sighting exposure – Group 0.1: The following clinical signs were recorded on the day of exposure in the sighting exposures: laboured, gasping and noisy respiration and decreased activity. All four animals were found dead on the following day of exposure.

Sighting exposure – Group 0.2: Laboured, noisy respiration and decreased activity were noted for the exposed animals on day of exposure. In addition, sneezing, hunched posture, weak and wasted condition were noted during first week of recovery period. The exposed animals recovered and all clinical signs ceased from Day 10.

Main study: In the main study similar clinical signs were recorded as in the sighting exposures on day of exposure: laboured and noisy respiration, decreased activity. In addition, sneezing, gasping respiration, hunched posure, weak and wasted condition were recorded during the observation period. The exposed animals recovered and all significant clinical signs ceased from Day 10, however fur loss was noted for two females from Day 12 to 14.

4.5 Bodyweight

Individual data, together with bodyweight changes, are presented in Appendix 3.

In the study, normal bodyweight gain was noted for all surviving animals from Day 1 to the end of the observation period, with the exception of three animals (one in the sighting and two in the main study) where slight body weight loss was noted during first three days.

4.6 Necropsy

Individual data are presented in Appendix 4. A single four hour nose-only exposure of Cyproconazole/Isopyrazam SC (A19022A) to CRL (WI) Wistar strain rat led to the death of four animals dosed at 5.27 mg/L during a sighting exposure. Dark/red discoloration of the non-collapsed lungs and beige liquid at the fur of perinasal area were considered to be associated with the administration of the test item.

In surviving animals subjected to the necropsy on Day 14, no test item-related macroscopic changes were seen in the sighting study animals exposed at 1.15 mg/l or in the main study at a concentration of 1.04 mg/l.

5.0 CONCLUSIONS

Under the experimental conditions of this study, no death occurred in a group of 10 rats exposed to a mean achieved atmosphere of 1.04 mg/L for 4 hours. The acute inhalation median lethal concentration of Cyproconazole/Isopyrazam SC (A19022A), in CRL: (WI) Wistar strain rats is therefore considered to be greater than 1.04 mg/L.

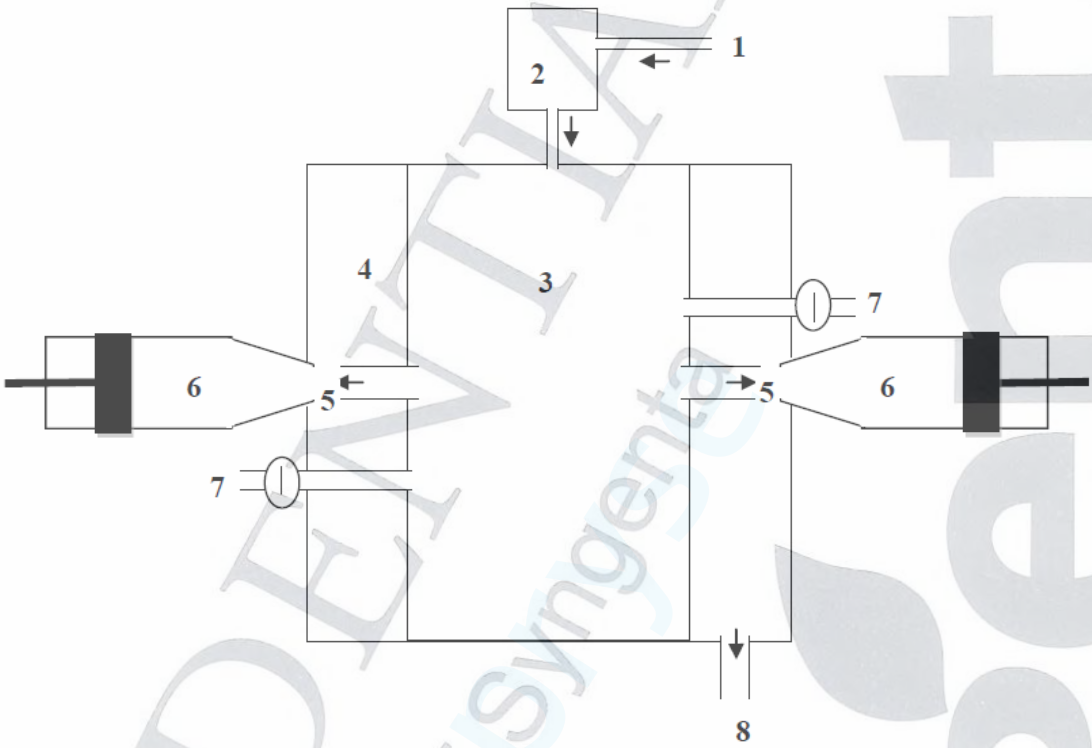
6.0 REFERENCES

Pauluhn J (1994) Validation of an Improved Nose-Only Exposure System for Rodents. *J App Tox* **14** (1), 55-62

Silver S D (1946) Constant flow gassing chambers: Principles influencing design and operation. *J Lab Clin Med* **31**, 1153-1161

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

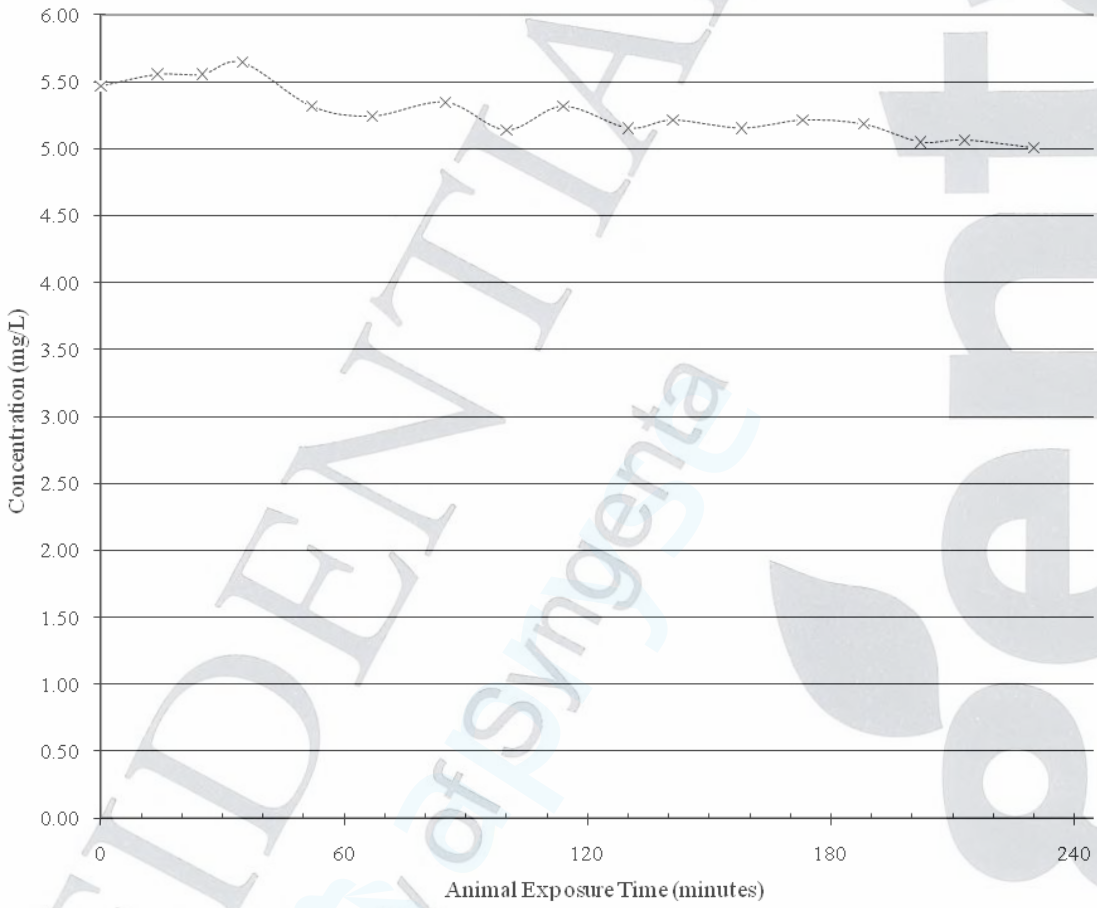


FIGURE 2 Achieved Atmosphere Concentrations (Continued)

Sighting Exposure – Group 0.2

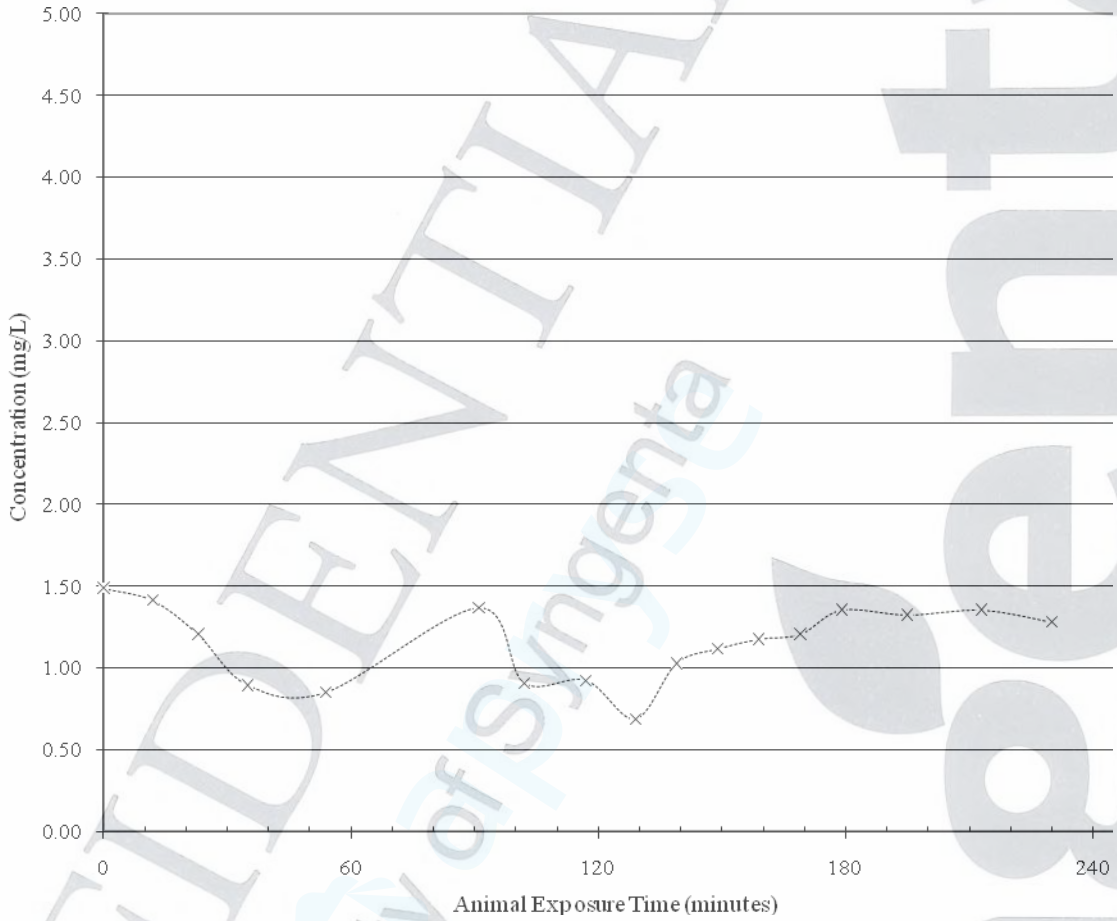


FIGURE 2 Achieved Atmosphere Concentrations (Continued)

Main Study - Group 1

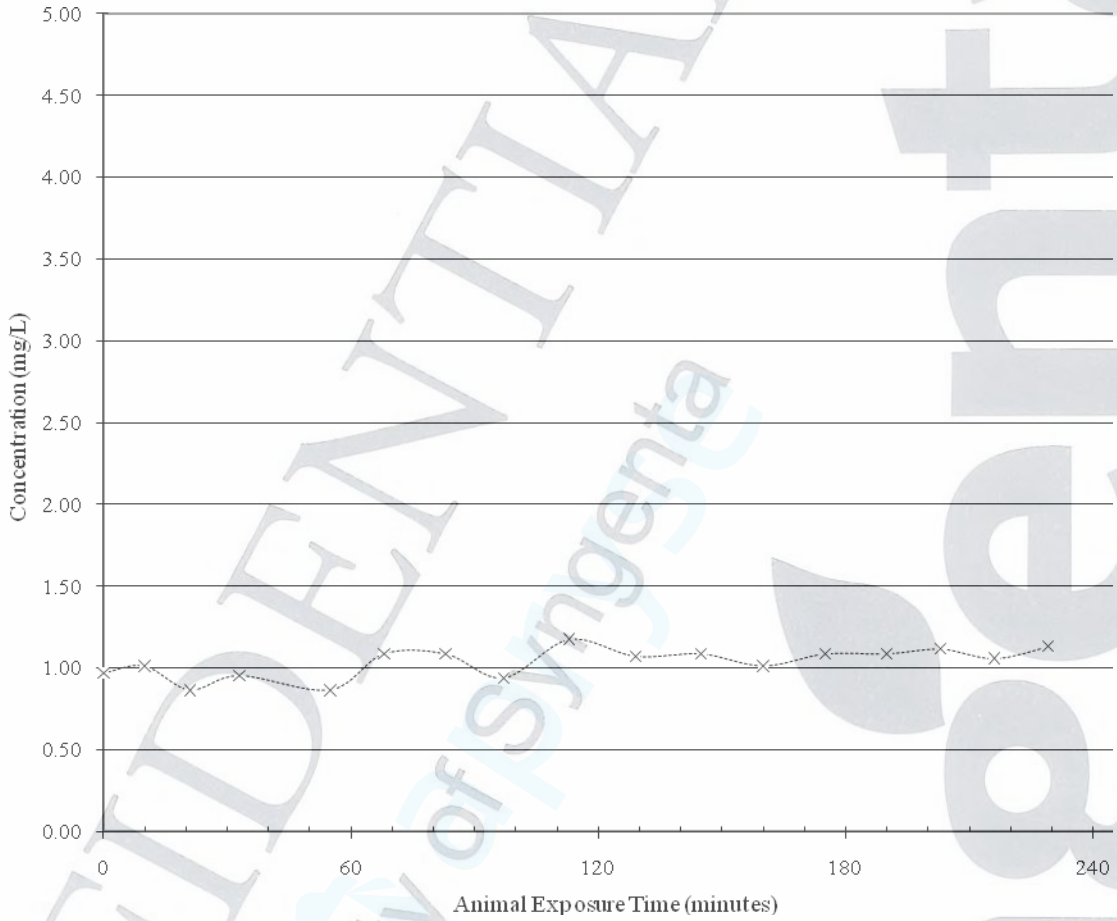
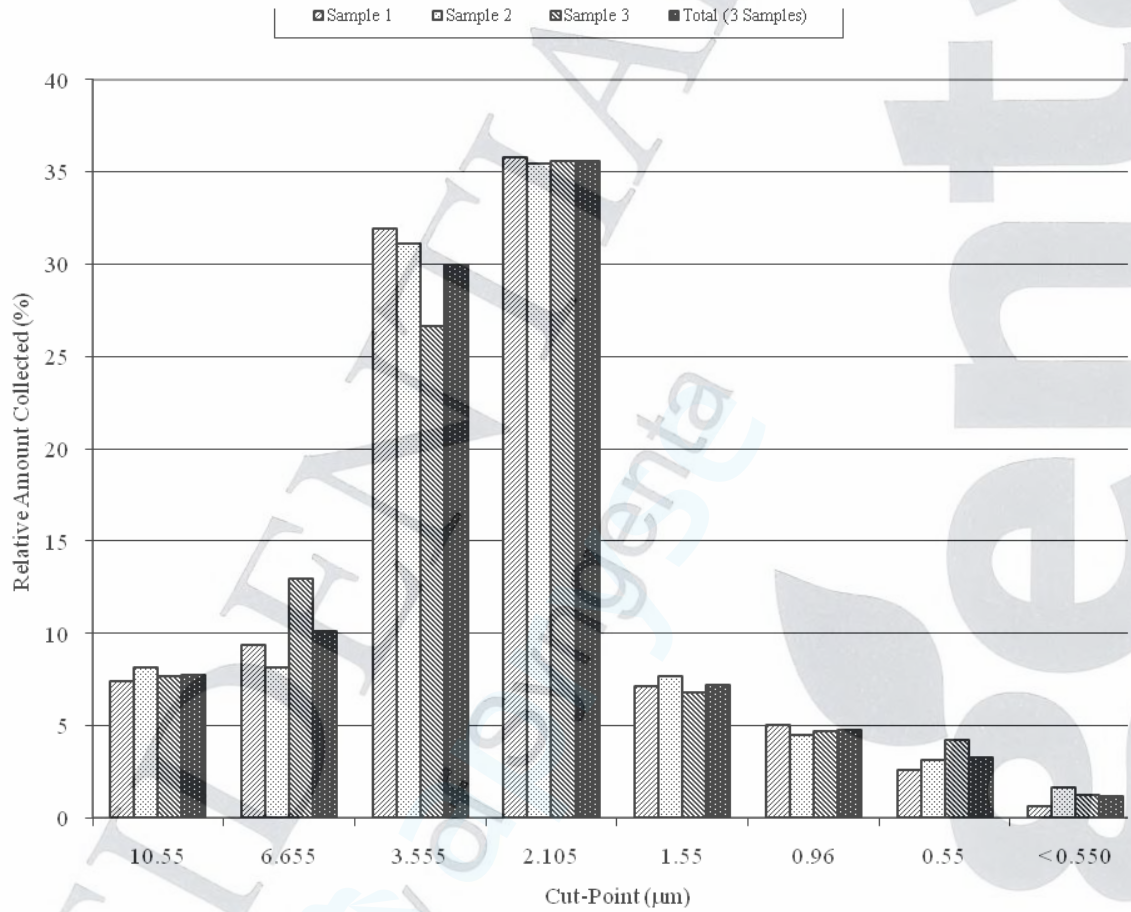
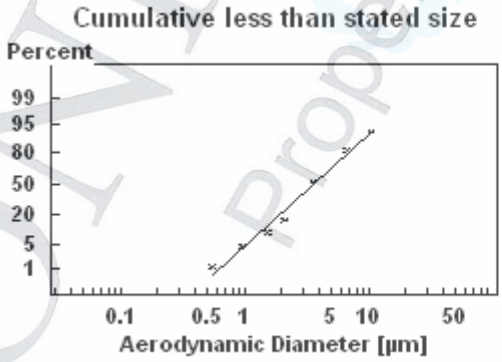


FIGURE 3 Particle Size Distribution

Sighting Exposure – Group 0.1





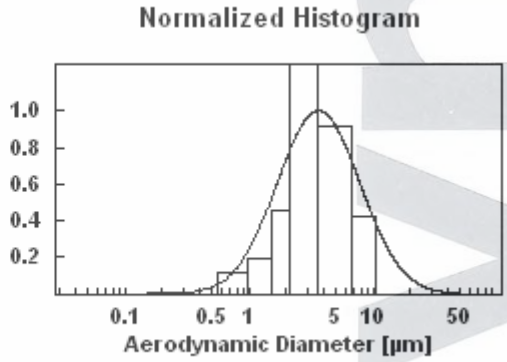
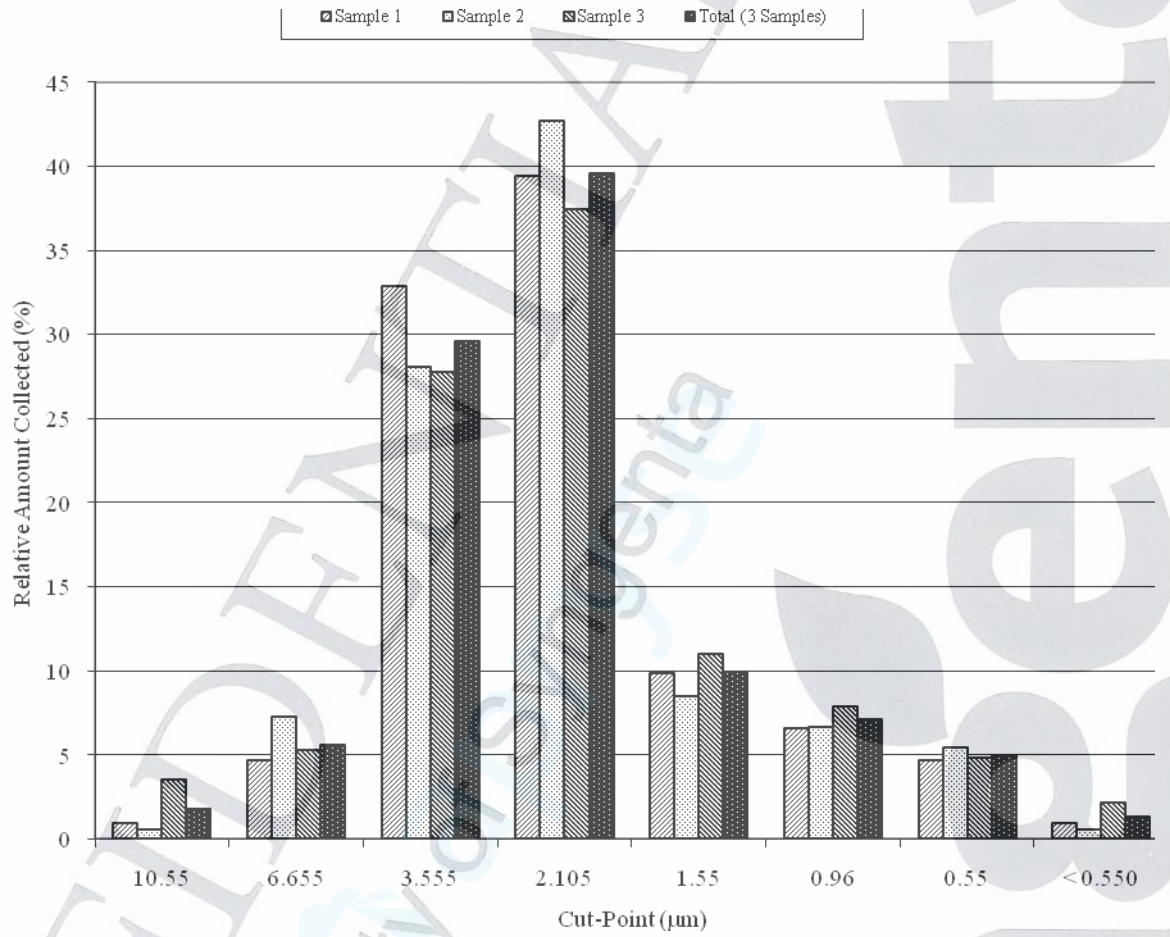
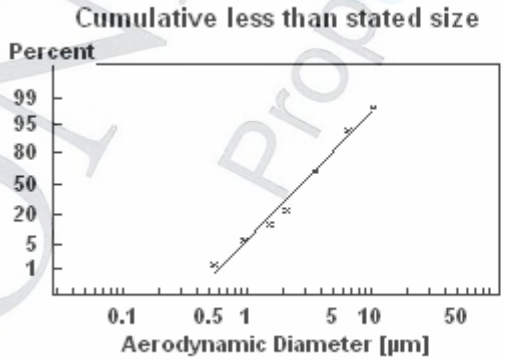


FIGURE 3 Particle Size Distribution (Continued)

Sighting Exposure – Group 0.2





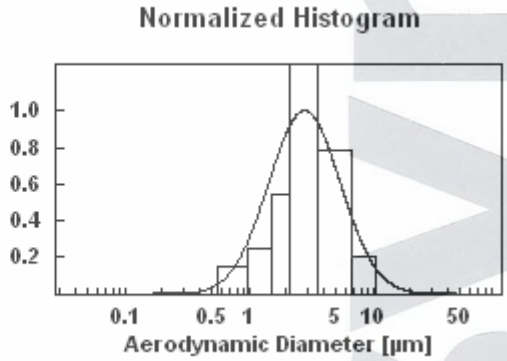
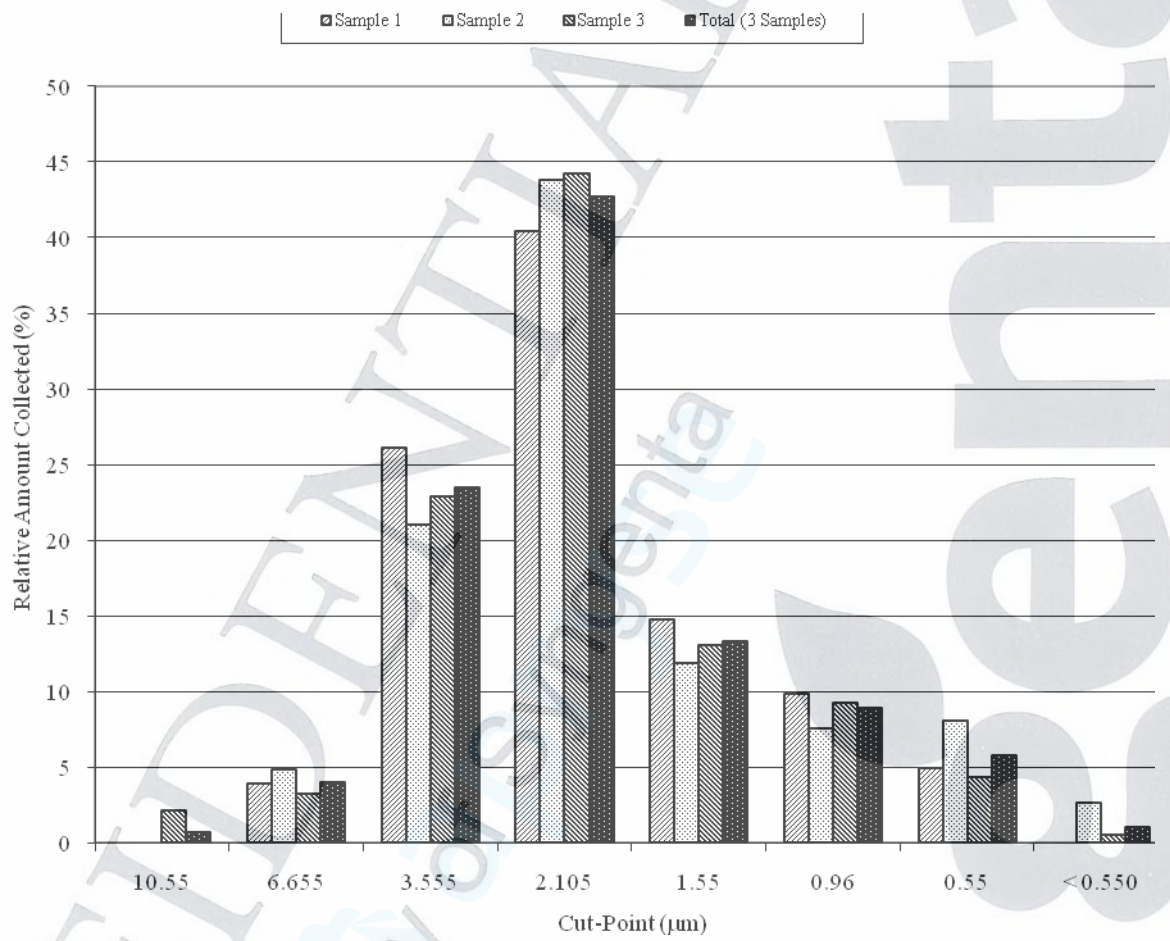
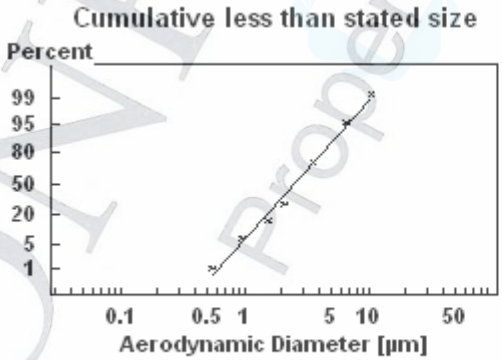
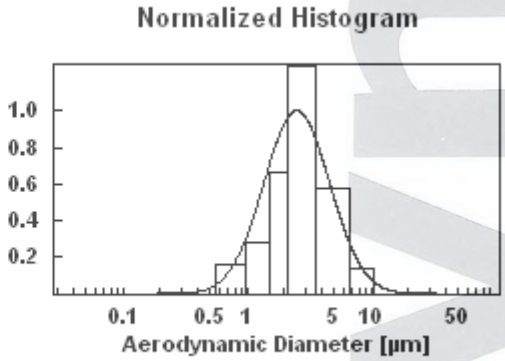


FIGURE 3 Particle Size Distribution (Continued)

Main Study - Group 1







TABLES SECTION

TABLE 1 Test Atmosphere Concentrations

Sighting Exposure – Group 0.1

Exposure Duration (minutes)	Amount of Non Volatiles Collected (mg)	Equivalant Test Item Amount (mg)*	Sample Volume (L)	Atmospheric Concentration of Cyproconazole/Isopyrazam SC (A19022A) (mg/L)
0	3.67	10.93	2.0	5.47
14	3.73	11.11	2.0	5.56
25	3.73	11.11	2.0	5.56
35	3.79	11.29	2.0	5.64
52	3.57	10.63	2.0	5.32
67	3.52	10.49	2.0	5.24
85	3.59	10.69	2.0	5.35
100	3.45	10.28	2.0	5.14
114	3.57	10.63	2.0	5.32
130	3.46	10.31	2.0	5.15
141	3.50	10.43	2.0	5.21
158	3.46	10.31	2.0	5.15
173	3.50	10.43	2.0	5.21
188	3.48	10.37	2.0	5.18
202	3.39	10.10	2.0	5.05
213	3.40	10.13	2.0	5.06
230	3.36	10.01	2.0	5.00

Mean Achieved Atmosphere Concentration = 5.27 mg/L
Standard Deviation = 0.19

Amount of Test Item Used (g): 675.23
Total Volume of Air Used (L): 7740
Nominal Concentration (mg/L): 87.24

* = *non-volatile content of Cyproconazole/Isopyrazam SC (A19022A) was 33.57%.*

TABLE 1 Test Atmosphere Concentrations (Continued)

Sighting Exposure – Group 0.2

Exposure Duration (minutes)	Amount of Non Volatiles Collected (mg)	Equivalant Test Item Amount (mg) *	Sample Volume (L)	Atmospheric Concentration of Cyproconazole/Isopyrazam SC (A19022A) (mg/L)
0	1.00	2.98	2.0	1.49
12	0.95	2.83	2.0	1.41
23	0.81	2.41	2.0	1.21
35	0.60	1.79	2.0	0.89
54	0.57	1.70	2.0	0.85
91	0.92	2.74	2.0	1.37
102	0.61	1.82	2.0	0.91
117	0.62	1.85	2.0	0.92
129	0.46	1.37	2.0	0.69
139	0.69	2.06	2.0	1.03
149	0.75	2.23	2.0	1.12
159	0.79	2.35	2.0	1.18
169	0.81	2.41	2.0	1.21
179	0.91	2.71	2.0	1.36
195	0.89	2.65	2.0	1.33
213	0.91	2.71	2.0	1.36
230	0.86	2.56	2.0	1.28

Mean Achieved Atmosphere Concentration = 1.15 mg/L
Standard Deviation = 0.23

Amount of Test Item Used (g): 67.33
Total Volume of Air Used (L): 7620
Nominal Concentration (mg/L): 8.84

* = *non-volatile content of Cyproconazole/Isopyrazam SC (A19022A) was 33.57%.*

TABLE 1 Test Atmosphere Concentrations (Continued)

Main Study - Group 1

Exposure Duration (minutes)	Amount of Non Volatiles Collected (mg)	Equivalant Test Item Amount (mg) *	Sample Volume (L)	Atmospheric Concentration of Cyproconazole/Isopyrazam SC (A19022A) (mg/L)
0	0.65	1.94	2.0	0.97
10	0.68	2.03	2.0	1.01
21	0.58	1.73	2.0	0.86
33	0.64	1.91	2.0	0.95
55	0.58	1.73	2.0	0.86
68	0.73	2.17	2.0	1.09
83	0.73	2.17	2.0	1.09
97	0.63	1.88	2.0	0.94
113	0.79	2.35	2.0	1.18
129	0.72	2.14	2.0	1.07
145	0.73	2.17	2.0	1.09
160	0.68	2.03	2.0	1.01
175	0.73	2.17	2.0	1.09
190	0.73	2.17	2.0	1.09
203	0.75	2.23	2.0	1.12
216	0.71	2.11	2.0	1.06
229	0.76	2.26	2.0	1.13

Mean Achieved Atmosphere Concentration = 1.04 mg/L
Standard Deviation = 0.09

Amount of Test Item Used (g): 86.65
Total Volume of Air Used (L): 7920
Nominal Concentration (mg/L): 10.94

* = *non-volatile content of Cyproconazole/Isopyrazam SC (A19022A) was 33.57%.*

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	10.55	0.46	0.49	0.44	1.39
2	6.66	0.58	0.49	0.74	1.81
3	3.56	1.97	1.86	1.52	5.35
4	2.11	2.21	2.12	2.03	6.36
5	1.55	0.44	0.46	0.39	1.29
6	0.96	0.31	0.27	0.27	0.85
7	0.55	0.16	0.19	0.24	0.59
Filter	< 0.55	0.04	0.10	0.07	0.21
				Total Amount Collected (mg)	17.85

Size Range (µm)	Total Mass/stage (mg)	Cumulative Mass (%)
< 0.55	0.21	1.18
0.55 - 0.96	0.59	4.48
0.96 - 1.55	0.85	9.24
1.55 - 2.11	1.29	16.47
2.11 – 3.56	6.36	52.10
3.56 - 6.66	5.35	82.07
6.66 – 10.55	1.81	92.21
> 10.55	1.39	100.00

Mean Achieved Atmosphere Concentration = 5.27 mg/L

Mean Mass Median Aerodynamic Diameter (MMAD) = 3.63 µm

Geometric Standard Deviation = 2.15

Inhalable Fraction (% < 4µm) = 54.9%

TABLE 2 Test Atmosphere Particle Size Distribution Data (Continued)

Sighting Exposure – Group 0.2

Stage Number	Cut Point (µm)	Amount Collected (mg)			Total Collected per Stage (mg)
		Sample 1	Sample 2	Sample 3	
1	10.55	0.02	0.01	0.08	0.11
2	6.66	0.10	0.12	0.12	0.34
3	3.56	0.70	0.46	0.63	1.79
4	2.11	0.84	0.70	0.85	2.39
5	1.55	0.21	0.14	0.25	0.60
6	0.96	0.14	0.11	0.18	0.43
7	0.55	0.10	0.09	0.11	0.30
Filter	< 0.55	0.02	0.01	0.05	0.08
				Total Amount Collected (mg)	6.04

Size Range (µm)	Total Mass/stage (mg)	Cumulative Mass (%)
< 0.55	0.08	1.32
0.55 - 0.96	0.30	6.29
0.96 - 1.55	0.43	13.41
1.55 - 2.11	0.60	23.34
2.11 – 3.56	2.39	62.91
3.56 - 6.66	1.79	92.55
6.66 – 10.55	0.34	98.18
> 10.55	0.11	100.00

Mean Achieved Atmosphere Concentration = 1.15 mg/L

Mean Mass Median Aerodynamic Diameter (MMAD) = 2.80 µm

Geometric Standard Deviation = 1.84

Inhalable Fraction (% < 4µm) = 77.6 %

TABLE 2 Test Atmosphere Particle Size Distribution Data (Continued)

Main Study - Group 1

Stage Number	Cut Point (µm)	Amount Collected (mg)			Total Collected per Stage (mg)
		Sample 1	Sample 2	Sample 3	
1	10.55	0.00	0.00	0.04	0.04
2	6.66	0.08	0.09	0.06	0.23
3	3.56	0.53	0.39	0.42	1.34
4	2.11	0.82	0.81	0.81	2.44
5	1.55	0.30	0.22	0.24	0.76
6	0.96	0.20	0.14	0.17	0.51
7	0.55	0.10	0.15	0.08	0.33
Filter	< 0.55	0.00	0.05	0.01	0.06
				Total Amount Collected (mg)	5.71

Size Range (µm)	Total Mass/stage (mg)	Cumulative Mass (%)
< 0.55	0.06	1.05
0.55 - 0.96	0.33	6.83
0.96 - 1.55	0.51	15.76
1.55 - 2.11	0.76	29.07
2.11 – 3.56	2.44	71.80
3.56 - 6.66	1.34	95.27
6.66 – 10.55	0.23	99.30
> 10.55	0.04	100.00

Mean Achieved Atmosphere Concentration = 1.04 mg/L

Mean Mass Median Aerodynamic Diameter (MMAD) = 2.52 µm

Geometric Standard Deviation = 1.84

Inhalable Fraction (% < 4µm) = 77.6%

TABLE 3 Test Chamber Environmental and Equilibration Data

Sighting Exposure – Group 0.1

Measurement	Mean Value	Minimum	Maximum
Air Flow In (Inner Plenum) (L/min)	30.1	29.7	30.5
Air Flow Out (Outer Cylinder) (L/min)	27.1	26.8	27.5
Temperature* (°C)	19.2	19.4	20.9
Relative Humidity* (%)	-	-	-
Oxygen Concentration (%)	20.7	20.6	20.8
Carbon Dioxide (%)	0.0	0.0	0.0

Theoretical Chamber Equilibration Time (T_{99}):

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

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

T_{99} (Minimum Acceptable Equilibration Time) = 1 minute

Actual equilibration time allowed = 19 minutes.

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* = *Due to failure of the TSE temperature-humidity sensor in the exposure system during the animal exposures, the humidity was not measured and the temperature was recorded using a calibrated mini-max thermometer at hourly intervals.*

TABLE 3 Test Chamber Environmental and Equilibration Data (Continued)

Sighting Exposure – Group 0.2

Measurement	Mean Value	Minimum	Maximum
Air Flow In (Inner Plenum) (L/min)	30.1	29.7	30.9
Air Flow Out (Outer Cylinder) (L/min)	27.2	26.8	27.5
Temperature* (°C)	21.9	21.8	22.0
Relative Humidity* (%)	-	-	-
Oxygen Concentration (%)	20.7	20.6	20.8
Carbon Dioxide (%)	0.0	0.0	0.1

Theoretical Chamber Equilibration Time (T_{99}):

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

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

T_{99} (Minimum Acceptable Equilibration Time) = 1 minute

Actual equilibration time allowed = 15 minutes.

* = *Due to failure of the TSE temperature-humidity sensor in the exposure system during the animal exposures, the humidity was not measured and the temperature was recorded using a calibrated mini-max thermometer at hourly intervals.*

TABLE 3 Test Chamber Environmental and Equilibration Data (Continued)

Main Study - Group 1

Measurement	Mean Value	Minimum	Maximum
Air Flow In (Inner Plenum) (L/min)	30.1	29.6	30.4
Air Flow Out (Outer Cylinder) (L/min)	27.1	26.8	27.5
Temperature* (°C)	24.2	24.2	24.3
Relative Humidity* (%)	-	-	-
Oxygen Concentration (%)	20.5	20.4	20.7
Carbon Dioxide (%)	0.1	0.0	0.1

Theoretical Chamber Equilibration Time (T_{99}):

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

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

T_{99} (Minimum Acceptable Equilibration Time) = 1 minute

Actual equilibration time allowed = 25 minutes.

* *= Due to failure of the TSE temperature-humidity sensor in the exposure system during the animal exposures, the humidity was not measured and the temperature was recorded using a calibrated mini-max thermometer at hourly intervals.*

TABLE 4 Mortality Data

Day Number	Number of Deaths					
	Group 0.1 (5.27 mg/L)		Group 0.2 (1.15 mg/L)		Group 1 (1.04 mg/L)	
	Males	Females	Males	Females	Males	Females
0 (During Exposure)	0	0	0	0	0	0
0 (After Exposure)	0	0	0	0	0	0
1	2	2	0	0	0	0
2	0	0	0	0	0	0
3	0	0	0	0	0	0
4	0	0	0	0	0	0
5	0	0	0	0	0	0
6	0	0	0	0	0	0
7	0	0	0	0	0	0
8 – 14	0	0	0	0	0	0
Total Deaths	2/2	2/2	0/2	0/2	0/5	0/5
Total Deaths	4/4		0/4		0/10	

APPENDICES SECTION

APPENDIX 1 Key to Clinical Observations

OBSERVATIONS	
Code	**Description**
ADR	Activity Decreased
FU1	Ruffled Coat
FU3	Wet Fur
FU5	Fur Loss
H	Hunched Back
N	No Abnormalities Detected
RB	Red-Brown Staining
RG	Gasping Respiration
RL	Laboured Respiration
RN	Noisy Respiration
RS	Sneezing
WAD	Wasted
WEK	Weak
X	Found Dead
SEVERITIES	
Code	**Description**
1	Slight *or* Small *or* Few *or* Small amount
2	Moderate *or* Several *or* Moderate amount
3	Severe *or* Large *or* Many *or* Large amount
LOCATIONS	
Code	**Description**
A08	Area Around Eyes
C32	Head, Carnium
L30	Left Cheek
M15	Snout
N14	Nose
R99	On/In Restraining Apparatus
W56	Whole Body

APPENDIX 2 Individual Clinical Observations

Sighting Exposure – Group 0.1

Sex	Animal Number	Hours During Exposure			On Removal From Restraint at End of Exposure (~4 Hours)	One-hour After-Exposure (~5 Hours)
		1	2	3		
Male	2991	N	FU3-R99	FU3-R99, RL-1	FU3-W56, RB-C32, RN-3, RG, RL-3, ADR-1	FU3-W56, RB-C32, RN-3, RG, RL-3, ADR-1
	2992	N	FU3-R99	FU3-R99, RL-1	FU3-W56, RB-C32, RN-1, RL-2	FU3-W56, RB-C32, RN-1, RL-2
Female	3009	N	FU3-R99	FU3-R99, RL-1	FU3-W56, RB-C32, RN-2, RG, RL-3, ADR-1	FU3-W56, RB-C32, RN-2, RG, RL-3, ADR-1
	3010	N	FU3-R99	FU3-R99, RL-1	FU3-W56, RB-C32, RN-2, RG, RL-3, ADR-1	FU3-W56, RB-C32, RN-2, RG, RL-3, ADR-1

Sex	Animal Number	Day Number						
		1	2	3	4	5	6	7-14
Male	2991	X	-	-	-	-	-	-
	2992	X	-	-	-	-	-	-
Female	3009	X	-	-	-	-	-	-
	3010	X	-	-	-	-	-	-

APPENDIX 2 Individual Clinical Observations (Continued)

Sighting Exposure – Group 0.2

Sex	Animal Number	Hours During Exposure			On Removal From Restraint at End of Exposure (~4 Hours)	One-hour After-Exposure (~5 Hours)
		1	2	3		
Male	3630	FU3-R99	FU3-R99	FU3-R99, RL-1	FU3-W56, RN-2, RL-2, RB-N14, RB-A08	FU3-W56, RN-2, RL-2, RB-N14, RB-A08, ADR-1
	3632	FU3-R99	FU3-R99	FU3-R99, RL-1	FU3-W56, RN-2, RL-2, RB-N14	FU3-W56, RN-2, RL-2, RB-N14, ADR-1
Female	3647	FU3-R99	FU3-R99	FU3-R99, RL-1	FU3-W56, RN-2, RL-2, RB-N14	FU3-W56, RN-2, RL-2, RB-N14
	3648	FU3-R99	FU3-R99	FU3-R99, RL-1	FU3-W56, RN-2, RL-2, RB-N14	FU3-W56, RN-2, RL-2, RB-N14

Sex	Animal Number	Day Number						
		1	2	3	4	5	6	7-14
Male	3630	FU1, RB-N14, RL-1, RS-1, ADR-1, WEK-2	RL-1, RS-1, WEK-2, H	RL-1, RS-1	RS-1	RS-1	RS-1	$RS\text{-}1_{(Day7)}$ $N_{(Day8\text{-}14)}$
	3632	FU1, RB-N14, RL-3, RG, RN-2, RS-1, H, ADR-1, WEK-3	RB-N14, RL-1, RN-1, RS-1, H, WEK-3	RL-1, RS-1, H, WAD	RS-1, WAD, H	RS-1, WAD	RS-1, WAD	$RS\text{-}1_{(Day7\text{-}9)}$ $N_{(Day10\text{-}14)}$
Female	3647	FU1, RN-1, RS-1, WEK-3	WEK-3, RS-1	RS-1	RS-1	RS-1	RS-1	$RS\text{-}1_{(Day7)}$ $N_{(Day8\text{-}14)}$
	3648	FU1, RS-1, WEK-2	WEK-2, RS-1	RS-1	RS-1	RS-1	RS-1	$RS\text{-}1_{(Day7)}$ $N_{(Day8\text{-}14)}$

APPENDIX 2 Individual Clinical Observations (Continued)

Main Study - Group 1

Sex	Animal Number	Hours During Exposure			On Removal From Restraint (4 Hours)	One-hour Post-Exposure
		1	2	3		
Male	3746	N	FU3-R99	FU3-R99	FUR-W56, RL-1, RN-1, RB-C32	FUR-W56, RL-2, RN-2, RB-C32
	3747	N	FU3-R99	FU3-R99	FUR-W56, RL-1, RN-1, RB-N14	FUR-W56, RL-2, RN-2, RB-N14, ADR-1
	3752	N	FU3-R99	FU3-R99	FUR-W56, RL-1, RN-1	FUR-W56, RL-2, RN-2, ADR-1
	3756	N	FU3-R99	FU3-R99	FUR-W56, RL-1, RN-1, RB-N14	FUR-W56, RL-1, RN-1, RB-N14
	3757	N	FU3-R99	FU3-R99	FUR-W56, RL-1, RN-1	FUR-W56, RL-1, RN-1
Female	3759	N	FU3-R99	FU3-R99	FU3-W56, RL-1	FUR-W56, RL-1, RN-1
	3760	N	FU3-R99	FU3-R99	FUR-W56, RL-1, RN-1	FU3-W56, RL-1
	3767	N	FU3-R99	FU3-R99	FU3-W56, RL-1	FUR-W56, RL-1, RN-1
	3770	N	FU3-R99	FU3-R99	FU3-W56, RL-1	FUR-W56, RL-1, RN-1
	3771	N	FU3-R99	FU3-R99	FU3-W56, RL-1	FUR-W56, RL-1, RN-1

APPENDIX 2 Individual Clinical Observations (Continued)

Main Study - Group 1

Sex	Animal Number	Day Number						
		1	2	3	4	5	6	7-14
Male	3746	FU1, RB-C32, RL-1, RN-1, RS-1, WEK-2	RL-1, RS-1, WEK-2	RL-1, RS-1, WAD	RL-1, RS-1, WAD	RL-1, RS-1, WAD	WAD	$N_{(Day7-14)}$
	3747	FU1, RB-M15, RL-2, RN-1, WEK-2	FU1, RL-1, RS-1, WEK-2	RS-1, RL-1	RS-1	RS-1	RS-1	$RS\text{-}1_{(Day7-9)}$ $N_{(Day10-14)}$
	3752	FU1, RB-M15, RL-2, RG, RN-2, ADR-1	FU1, RB-M15, RL-1, RN-1, RS-1, ADR-1, H	FU1, RL-1, RN-1, RS-1, ADR-1, H, WAD	RS-1, WAD	RS-1, WAD	N	$N_{(Day7-14)}$
	3756	FU1, RB-N14, RL-2, RN-2, RG, WEK-2, RS-1	RL-1, RS-2, RN-1, WEK-2	RL-1, RS-1, WAD	RS-1, WAD	RS-1, WAD	RS-1, WAD	$RS\text{-}1_{(Day7-9)}$ $N_{(Day10-14)}$
	3757	FU1, RB-M15, RL-2, RN-2, RG, WEK-2	RL-1, RN-1, RS-1, WEK-2, H	RL-1, RN-1, RS-1	RS-1	RS-1	N	$N_{(Day7-14)}$
Female	3759	FU1, RB-M15, RL-1	N	N	N	N	N	$N_{(Day7-14)}$
	3760	FU1, RB-M15, RL-1, RN-1, WEK-1	WEK-1	N	N	N	N	$N_{(Day7-11)}$ $FU5\text{-}L30_{(Day12-14)}$
	3767	FU1, RB-M15, RL-2, RN-2, WEK-2	FU1, RL-2, RN-1, RG, WEK-2	RL-1, WAD	N	N	N	$N_{(Day7-11)}$ $FU5\text{-}L30_{(Day12-14)}$
	3770	FU1, RB-M15, RL-1, RN-1, WEK-2, H	WEK-2	N	N	N	N	$N_{(Day7-14)}$
	3771	FU1, RB-M15, RL-1, RN-2, WEK-2	WEK-2, H	RS-1	N	N	N	$N_{(Day7-14)}$

APPENDIX 3 Individual Bodyweight Data

Sighting Exposure – Group 0.1

Sex	Animal Number	Bodyweight (g)						Bodyweight change (g)			
		Day 0	Day 1	Day 3	Day 7	Day 14	At death	Day 0-1	Day 1-3	Day 3-7	Day 7-14
Male	2991	382	-	-	-	-	344	-	-	-	-
	2992	406	-	-	-	-	359	-	-	-	-
Female	3009	249	-	-	-	-	236	-	-	-	-
	3010	251	-	-	-	-	223	-	-	-	-

Sighting Exposure – Group 0.2

Sex	Animal Number	Bodyweight (g)						Bodyweight change (g)			
		Day 0	Day 1	Day 3	Day 7	Day 14	At death	Day 0-1	Day 1-3	Day 3-7	Day 7-14
Male	3630	333	294	308	338	389	-	-39	14	30	51
	3632	327	279	275	304	370	-	-48	-4	29	66
Female	3647	211	177	202	221	248	-	-34	25	19	27
	3648	204	183	198	217	238	-	-21	15	19	21

APPENDIX 3 Individual Bodyweight Data (Continued)

Main Study - Group 1

Sex	Animal Number	Bodyweight (g)						Bodyweight change (g)			
		Day 0	Day 1	Day 3	Day 7	Day 14	At death	Day 0-1	Day 1-3	Day 3-7	Day 7-14
Male	3746	292	254	255	294	346	-	-38	1	39	52
	3747	290	245	263	290	324	-	-45	18	27	34
	3752	288	245	242	284	335	-	-43	-3	42	51
	3756	293	251	250	282	336	-	-42	-1	32	54
	3757	297	260	269	306	351	-	-37	9	37	45
Female	3759	192	183	196	206	211	-	-9	13	10	5
	3760	186	171	186	194	217	-	-15	15	8	23
	3767	188	163	167	194	218	-	-25	4	27	24
	3770	185	162	184	206	217	-	-23	22	22	11
	3771	180	158	174	191	213	-	-22	16	17	22

APPENDIX 4 Individual Necropsy Findings

Cyproconazole/Isopyrazam SC (A19022A) - Acute Inhalation Toxicity Study (Nose-Only) in the Rat

INDIVIDUAL NECROPSY FINDINGS

SIGHTING EXPOSURE

Male - Group 0.1 - 5.27 mg/L

NECROPSY FINDINGS	Animal ID		NECROPSY FINDINGS	
	2991#	2992#	∑	%
Lungs: Dark discoloration, red, diffuse, all lobes	+	+	**2**	**100**
Lungs: Non collapsed	+	+	**2**	**100**
Fur: Liquid material, beige, perinasal area	-	+	**1**	**50**
NO EXTERNAL OBSERVATIONS RECORDED	+	-	**1**	**50**
STUDY DAYS	1	1		
DATE OF NECROPSY	01 June 2012			

Female - Group 0.1 - 5.27 mg/L

NECROPSY FINDINGS	Animal ID		NECROPSY FINDINGS	
	3009#	3010#	∑	%
Lungs: Dark discoloration, red, diffuse, all lobes	+	+	**2**	**100**
Lungs: Non collapsed	+	+	**2**	**100**
Fur: Liquid material, beige, perinasal area	+	-	**1**	**50**
NO EXTERNAL OBSERVATIONS RECORDED	-	+	**1**	**50**
STUDY DAYS	1	1		
DATE OF NECROPSY	01 June 2012			

COMMENT:

NECROPSY FINDINGS PRESENT = +
NECROPSY FINDINGS PRESENT = -
FOUND DEAD = #

APPENDIX 4 Individual Necropsy Findings (Continued)

Cyproconazole/Isopyrazam SC (A19022A) - Acute Inhalation Toxicity Study (Nose-Only) in the Rat

INDIVIDUAL NECROPSY FINDINGS

SIGHTING EXPOSURE

Male - Group 0.2 - 1.15 mg/L

NECROPSY FINDINGS	Animal ID		NECROPSY FINDINGS	
	3630	3632	∑	%
NO INTERNAL OBSERVATIONS RECORDED	+	+	**2**	**100**
NO EXTERNAL OBSERVATIONS RECORDED	+	+	**2**	**100**
STUDY DAYS	14	14		
DATE OF NECROPSY	20 June 2012			

Female - Group 0.2 - 1.15 mg/L

NECROPSY FINDINGS	Animal ID		NECROPSY FINDINGS	
	3647	3648	∑	%
NO INTERNAL OBSERVATIONS RECORDED	+	+	**2**	**100**
NO EXTERNAL OBSERVATIONS RECORDED	+	+	**2**	**100**
STUDY DAYS	14	14		
DATE OF NECROPSY	20 June 2012			

COMMENT:

NECROPSY FINDINGS PRESENT = +
NECROPSY FINDINGS PRESENT = -

APPENDIX 4 Individual Necropsy Findings (Continued)

Cyproconazole/Isopyrazam SC (A19022A) - Acute Inhalation Toxicity Study (Nose-Only) in the Rat

INDIVIDUAL NECROPSY FINDINGS

MAIN STUDY

Male - Group 1 - 1.04 mg/L

NECROPSY FINDINGS	Animal ID					NECROPSY FINDINGS	
	3746	3747	3752	3756	3757	∑	%
NO INTERNAL OBSERVATIONS RECORDED	+	+	+	+	+	**5**	**100**
NO EXTERNAL OBSERVATIONS RECORDED	+	+	+	+	+	**5**	**100**
STUDY DAYS	14	14	14	14	14		
DATE OF NECROPSY	26 June 2012						

Female - Group 1 - 1.04 mg/L

NECROPSY FINDINGS	Animal ID					NECROPSY FINDINGS	
	3759	3760	3767	3770	3771	∑	%
NO INTERNAL OBSERVATIONS RECORDED	+	+	+	+	+	**5**	**100**
Fur: Thin cranium left	-	+	+	-	-	**2**	**40**
NO EXTERNAL OBSERVATIONS RECORDED	+	-	-	+	+	**3**	**60**
STUDY DAYS	14	14	14	14	14		
DATE OF NECROPSY	26 June 2012						

COMMENT:

NECROPSY FINDINGS PRESENT = +
NECROPSY FINDINGS PRESENT = -

APPENDIX 5 Pathology Report

Study code. 12/055-004P

PATHOLOGY REPORT

INTRODUCTION

The objective of this study was to assess the acute inhalation toxicity of Cyproconazole/Isopyrazam SC (A19022A) in the rat following a single four-hour nose-only exposure. The results of the study will serve as a basis for hazard assessment and classification and labelling.

RESULTS AND DISCUSSION

Surviving animals were euthanized upon completion of the observation period on Day 14. These rats were anesthetized with pentobarbital, followed by exsanguination. Gross pathology consisted of an external examination, including identification of all clinically-recorded lesions, as well as a detailed internal examination. Histopathological examination was not performed.

MORTALITY

A total of four rats were found dead on Day 1. Necropsy was performed on 4/4 animals dosed at 5.27 mg/L during first sighting exposure.

FOUND DEAD

Macroscopic findings

Sighting Exposure – Group 0.1 (5.27 mg/L)

Dark/red diffuse discoloration of the non-collapsed lungs was found in 4/4 found dead rats at necropsy. Beige liquid at the fur of perinasal area was also seen in 2/4 of these rats.

These macroscopic changes were considered to be associated with administration of the test item.

TERMINAL

Macroscopic findings

Sighting Exposure – Group 0.2 (1.15 mg/L)

No macroscopic observations were seen in animals dosed at 1.15 mg/L and subjected to the necropsy on Day 14.

Study code. 12/055-004P

Main study – Group 1 (1.04 mg/L)

There was no evidence of test item-related gross changes in surviving animals at a dose level of 1.04 mg/L. Thin fur at the left cranial area was incidentally noted in 2/10 animals.

CONCLUSION

A single four hours nose-only exposure of Cyproconazole/Isopyrazam SC (A19022A) to CRL (WI) Wistar strain rat led to the death of four animals dosed at 5.27 mg/L during sighting exposure. Dark/red discoloration of the non-collapsed lungs an beige liquid at the fur of perinasal area were considered to be associated with the administration of the test item.

In surviving animals subjected to the necropsy on Day 14, no test item-related macroscopic changes at a target dose level of 1.0 mg/L were seen.

Peter Maslej, D.V.M., Ph.D.
Head, Pathology Department

18 July 2012
Date

APPENDIX 6 Certificate of Analysis

syngenta

GLP Testing Facility WMU
Analytical Development &
Product Chemistry

Syngenta Crop Protection Münchwilen AG
Breitenloh 5
CH-4333 Münchwilen

Certificate of Analysis

A19022A
cyproconazole/isopyrazam SC (080/125)
J8657/147

Batch Identification	**J8657/147**
Product Code	**A19022A**
Other Product Code(s)	SAN619/SYN520453 SC (080/125)

Chemical Analysis
(Active Ingredient Content)

– **Identity of the Active Ingredients***	**confirmed**
– **Content of Cyproconazole***	**79.9 g/l corresponding to 7.45 % w/w**
– **Content of Isopyrazam (sum of epimers)***	**129 g/l corresponding to 12.0 % w/w**
– **Content of SYN534969 (syn-epimer of isopyrazam)***	**112 g/l corresponding to 10.4 % w/w**
– **Content of SYN534968 (anti-epimer of isopyrazam)***	**17.0 g/l corresponding to 1.58 % w/w**

The Active Ingredients content is within the FAO limits.
Methodology used for Characterization: HPLC

Physical Analysis

– **Appearance**	**beige liquid**
– **Density***	**1073 kg/m^3**

Stability:

– **Storage Temperature**	**< 30°C**
– **Recertification Date**	**End of October 2013**

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 Muenchwilen AG.

Study number of batch characterization: 123779
Study number(s) of batch recertification: ---
Authorisation:

14 November 2011

Dr. R. Kettner
Analytical Development & Product Chemistry

APPENDIX 7 Good Laboratory Practice Certificate

ORSZÁGOS GYÓGYSZERÉSZETI INTÉZET
National Institute of Pharmacy

FŐIGAZGATÓ
1051 Budapest, Zrínyi u. 3.
tel: (1) 8869-320
fax: (1) 8869-480
e-mail: szepezdi.zsuzsanna@ogyi.hu

Ref. no: OGYI/8242-11/2010
Admin.: Urbin Magdolna Zita
Date: 16 December, 2010

GOOD LABORATORY PRACTICE (GLP) CERTIFICATE

It is hereby certified that the test facility

LAB Research Kft.

(Base facility: H-8201 Veszprém, Szabadságpuszta, Hungary)

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 , safety pharmacology testing, reproduction toxicology, inhalation toxicology, analytical chemistry 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: **4-8 October, 2010.**

Zsuzsanna Szepezdi, Ph. D.
Director-General

The facility name until 1st September 2011 was LAB Research Ltd. From that date, the registered name has been CiToxLAB Hungary Ltd., this information has been transmitted to the GLP competent authority. The above GLP certificate is valid for this facility (now known as CiToxLAB Hungary Ltd.) until the certificate expires (16 December 2012).

Translation (from Hungarian to English):

Stamp Translation = Országos Gyógyszerészeti Intézet (OGYI) = National Institute of Pharmacy
Főigazgató = Director-General