FINAL REPORT

Acute Inhalation Toxicity Study (Nose-only) in the Rat

Study code:

Study Director:

STATEMENT OF THE STUDY DIRECTOR

Study Code:

Test Item:

Study Title: Acute Inhalation Toxicity Study (Nose-Only) in the Rat

This study has been performed in accordance with the Principles of Good Laboratory Practice (Hungarian GLP Regulations: 42/2014. (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 Amendments, authorized by the sponsor and 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.

Conclusion

Under the experimental conditions of this study, no deaths occurred in a group of ten rats exposed to the target concentration of 5.07 mg/L for four hours. The acute inhalation median lethal concentration (4hr LC_{50}) in CRL: (WI) Wistar strain rats, was therefore considered to be greater than 5.07 mg/L.

Signature: ___ _____ Date: _

Study Director

MANAGEMENT STATEMENT

Study Code:

Test Item:

Study Title: Acute Inhalation Toxicity Study (Nose-Only) in the Rat

According to the conditions of the research and development agreement between (as Sponsor) and (as Test Facility), this study, an "Acute Inhalation Toxicity Study (Nose-Only) in the Rat", has been performed in compliance with the Principles of Good Laboratory Practice.

Signature: _ _____ Date: ____

Managing Director

QUALITY ASSURANCE STATEMENT

Study Code:

Test Item:

Study Title: Acute Inhalation Toxicity Study (Nose-Only) in the Rat

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
	Study Plan		
	Amendment 1 to Study Plan		
	Amendment 2 to Study Plan		
	treatment		
	Amendment 3 to Study Plan		
	Draft Report		
	Final Report		

Signature: [redacted] Date:

STUDY DETAILS

TEST ITEM :

STUDY TITLE : Acute Inhalation Toxicity Study (Nose-Only) in the Rat

SPONSOR :

TEST FACILITY :

START OF EXPERIMENT :

END OF EXPERIMENT :

STUDY DIRECTOR :

RESPONSIBLE PERSONS :

CONTENTS

ACUTE INHALATION TOXICITY STUDY (NOSE-ONLY) IN THE RAT

SUMMARY

Introduction

This study was performed to assess the acute inhalation toxicity The method was designed to meet OECD guideline 403 (07 September 2009), Council Regulation (EC) no. 440/2008, Annex Part B, B.2: "Acute Toxicity (Inhalation)", Official Journal of the European Union No. L 142, dated May 31st, 2008, in line with the Sponsor requirements.

Methods

This study was performed to assess the acute inhalation toxicity following a 4 hours exposure at the target concentration of 5 mg/L to 5 male and 5 female rats.

The study was performed in two phases.

A sighting exposure was performed first: 5.05 mg/L was tested on single animals of both sexes (Group 0.1).

Based on the lack of lethality at this concentration, the main study group, 10 (5 male and 5 female) CRL: (WI) Wistar strain rats, was exposed to the target concentration of 5.07 mg/L 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 euthanized and subjected to a gross examination *post mortem*.

No control group was exposed in this study.

Results

The atmosphere concentration was as follows:

Group Number	Target Concentration (mg/L)	Mean Achieved Concentration (mg/L)	Standard Deviation of Achieved Concentration (mg/L)
0.1	5.00	5.05	0.24
1	5.00	5.07	0.28

The characteristics of the test atmosphere were as follows:

Group	Concentration (mg/L)	Mean Mass Median Aerodynamic Diameter (MMAD) (µm)	Geometric Standard Deviation (GSD)	Inhalable Fraction (% < 4µm)
0.1 Sighting	5.05	3.15	2.94	58.8
1 (Main Study)	5.07	2.92	2.97	61.4

The mortality data were summarised as follows:

Group	Concentration (mg/L)	Male Deaths	Female Deaths	Total Deaths
0.1 Sighting	5.05	0/1	0/1	0/2
1 (Main Study)	5.07	0/5	0/5	0/10

Clinical Observations: Wet fur and fur staining were commonly recorded, mostly on the day of exposure. These observations were considered to be related to the restraint and exposure procedures and, in isolation, were considered not to be biologically significant.

Sighting group (Group 0.1): Slightly laboured respiration was observed during exposure. No abnormalities were detected in either animal from the day following exposure until the end of the observation period.

Main Group (Group 1): Slightly laboured respiration was detected during the exposure. No abnormalities were detected in any animal from the day following exposure until the end of the observation period.

Bodyweights: The exposure procedure caused slight bodyweight loss in 9/10 animals. By the second part of the observation period the bodyweight was normalized in most of the animals.

Necropsy: A single four hour nose-only exposure to Crl:WI rats at a concentration of 5.05 mg/L during Sighting exposure or exposed to a concentration of 5.07 mg/L in the Main study, was not associated with any gross changes.

Conclusion

Under the experimental conditions of this study, no deaths occurred in a group of ten rats exposed to the concentration of 5.07 mg/L for four hours. The acute inhalation median lethal concentration (4hr LC_{50}) in CRL: (WI) Wistar strain rats, was therefore considered to be greater than 5.07 mg/L.

ACUTE INHALATION TOXICITY STUDY (NOSE-ONLY) IN THE RAT

1. INTRODUCTION

This study was performed to assess the acute inhalation toxicity The method was designed to meet OECD guideline 403 (07 September 2009), Council Regulation (EC) No 440/2008, Annex Part B, B.2: "Acute Toxicity (Inhalation)", Official Journal of the European Union No. L 142, dated May 31st, 2008, in line with the Sponsor requirements.

2. MATERIALS AND METHODS

2.1. TEST ITEM

Substance name:

Chemical name:

Batch/Lot No.:

CAS No.:

Purity:

Description:

Manufacture date:

Expiry date:

Storage conditions:

Safety precautions:

Manufacturer:

2.1.1. 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 was made on the basis of these data. The Certificate of Analysis for the test item and its composition is presented in Appendix 9 and Appendix 10, respectively.

2.1.2. Preparation

The test item was used as supplied and no preparation was required.

2.2. EXPERIMENTAL ANIMALS

2.2.1. Specification

Species and strain:	CRL:(WI) rats
Source:	Charles River Laboratories, Research Models and Services, Germany GmbH, Sandhofer Weg 7, D-97633 Sulzfeld
Hygienic level at arrival:	SPF
Hygienic level during the study:	Standard housing conditions
Justification of strain:	Recognized by international guidelines as a recommended test system.
Number of animals:	12
Sex:	6 male and 6 female rats, the females were nulliparous and non-pregnant.
Age and body weight (at dosing):	Sighting exposures: 8 weeks old, 334 g (male) and 220 g (female) Main study: 11 weeks old, 374-398 g (males) and 226-246 g (females).
Randomization:	Selected based on bodyweight prior to the exposure.
Acclimatization period:	10 days (Sighting group), 28 days (Main group)

2.2.2. Husbandry

Animal health:	Only healthy animals were used for the test. The health status was certified by the veterinarian.
Animal room:	245/7
Housing:	Group of 5 (by sex) for main study; individually for sighting
Cage type:	Type III solid floor cages with stainless steel mesh lids
Bedding:	Lignocel Bedding for Laboratory Animals was available to animals during the study.
Light:	12 hours daily, from 6.00 a.m. to 6.00 p.m.
Temperature:	19.4 - 25.5°C
Relative humidity:	41 – 73%
Ventilation:	15-20 air exchanges/hour
Enrichment:	Rodents were housed with deep wood sawdust bedding to allow digging and other normal rodent activities.

2.2.3. Diet and Water

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; batch: 814 3108; expiry: Aug 2015)* and tap water fit for human consumption, *ad libitum*.

A Contents of Diet and the Test Report of the diet are presented in Appendices 11 and 12.

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

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

2.2.4. Identification

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

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

2.3. INHALATION EXPOSURE

2.3.1. 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 varied to achieve the required atmospheric characteristics.

2.3.2. Atmosphere Generation

The test item was aerosolised using two Wright's Dust Feed Systems (TSE Systems GmbH, Bad Homburg, Germany; Serial Number: 040804-59) located at the top of the exposure chamber. Compressed air was supplied by means of an oil-free compressor and passed through a suitable filter system prior to introduction to the dust generator.

2.3.3. 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 two, 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 (ref. 1).

2.3.4. Sighting Exposure

Sighting exposures were performed 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.

2.3.5. 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 (T99) (ref. 2), a group of ten rats (five male and five female) was exposed to an atmosphere of the test material for a period of four hours. Five mg/L as the target concentration was used for the main group. As no death occurred at 5.07 mg/L concentration in the main group, no further data were required.

2.4. EXPOSURE MONITORING

2.4.1. Test Atmosphere Concentrations

The test atmosphere was sampled at regular intervals during each 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 (Whatman GmbH, Hahnestraße 3 – D-37586 Dassel, 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.

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.

2.4.2. 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 (GSD) were calculated. In addition, the proportion (%) of aerosol less than 4μm (considered to be the inhalable portion) was determined.

2.4.3. 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*
Test atmosphere carbon dioxide concentration
Test atmosphere oxygen concentration

Summaries of the data are presented in Appendix 3.

2.5. OBSERVATIONS

2.5.1. Morbidity/Mortality

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

2.5.2. 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, one hour after exposure and subsequently once daily for fourteen days.

2.5.3. Bodyweight

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

2.5.4. Necropsy

At the end of the fourteen day observation period, the animals were euthanised by exsanguination under anaesthesia (intra-peritoneal injection of pentobarbital solution – Euthanimal 40%; Lot No.: 1409236-06; Expiry: 09-2017; Produced by Alfasan Nederland BV, Kulpersweg 9, Woerden, Netherlands) and gross macroscopic examination was 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.

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* = *The humidity value was under the limit during the animal exposure, but based on the guideline it is not qualifying as deviation due to the characteristics of the test item. (OECD 403, ref. 3)*

2.6. 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.

2.7. ARCHIVES

The study plan, amendments, all raw data, sample of the test item, final report and correspondence will be stored for 15 years in the archives
in accordance with the Hungarian GLP requirements and current SOPs.. After the retention time has elapsed, all the archived materials listed above will be offered to the Sponsor or retained for a further period if agreed by a contract. Otherwise the materials will be discarded.

2.8. DEVIATIONS TO THE STUDY PLAN

In the animal room, temperature and humidity deviations from the target range were recorded.

During sighting exposure, temperature deviation was recorded.

The draft report was issued later than stated in the study plan.

For sighting exposure, the animal receipt is 02 June 2015, instead of 04 June 2015.

These deviations had no effect on the purpose, integrity or outcome of the study.

3. RESULTS

3.1. TEST ATMOSPHERE CONCENTRATION

The test atmosphere concentration was sampled 17 times during the exposure at approximately equal intervals and the actual concentration of the test item was calculated. The mean values obtained were:

Group	Concentration (mg/L)	Standard Deviation	Nominal (mg/L)
0.1 (Sighting)	5.05	0.24	13.01
1 (Main Study)	5.07	0.28	12.09

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

3.2. PARTICLE SIZE ANALYSIS

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

Group	Concentration (mg/L)	Mean Mass Median Aerodynamic Diameter (MMAD) (µm)	Geometric Standard Deviation (GSD)	Inhalable Fraction (% < 4µm)
0.1 (Sighting)	5.05	3.15	2.94	58.8
1 (Main Study)	5.07	2.92	2.97	61.4

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

3.3. MORTALITY RATES

The mortality data are detailed in Appendix 4 and summarised as follows:

Group	Concentration (mg/L)	Male Deaths	Female Deaths	Total Deaths
0.1 (Sighting)	5.05	0/1	0/1	0/2
1 (Main Study)	5.07	0/5	0/5	0/10

3.4. CLINICAL OBSERVATIONS

Individual clinical observations are presented in Appendix 5.

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

Sighting group (Group 0.1): Slightly laboured respiration was observed during exposure. No abnormalities were detected in any animal from the day following exposure until the end of the observation period.

Main Group (Group 1): Slightly laboured respiration was detected during the exposure. No abnormalities were detected in any animal from the day following exposure until the end of the observation period.

3.5. BODYWEIGHT

Individual data, together with weekly bodyweight changes, are presented in Appendix 6.

The exposure procedure caused slight bodyweight loss in 9/10 animals. By the second part of the observation period the bodyweight was normalized in most of the animals.

3.6. NECROPSY

Individual data are presented in Appendix 7.

A single four hour nose-only exposure of SH-0850 to Crl:WI rats at a concentration of 5.05 mg/L during Sighting exposure or exposed to a concentration of 5.07 mg/L in Main study, was not associated with any gross changes.

The Pathology Report is presented in Appendix 8.

4. CONCLUSION

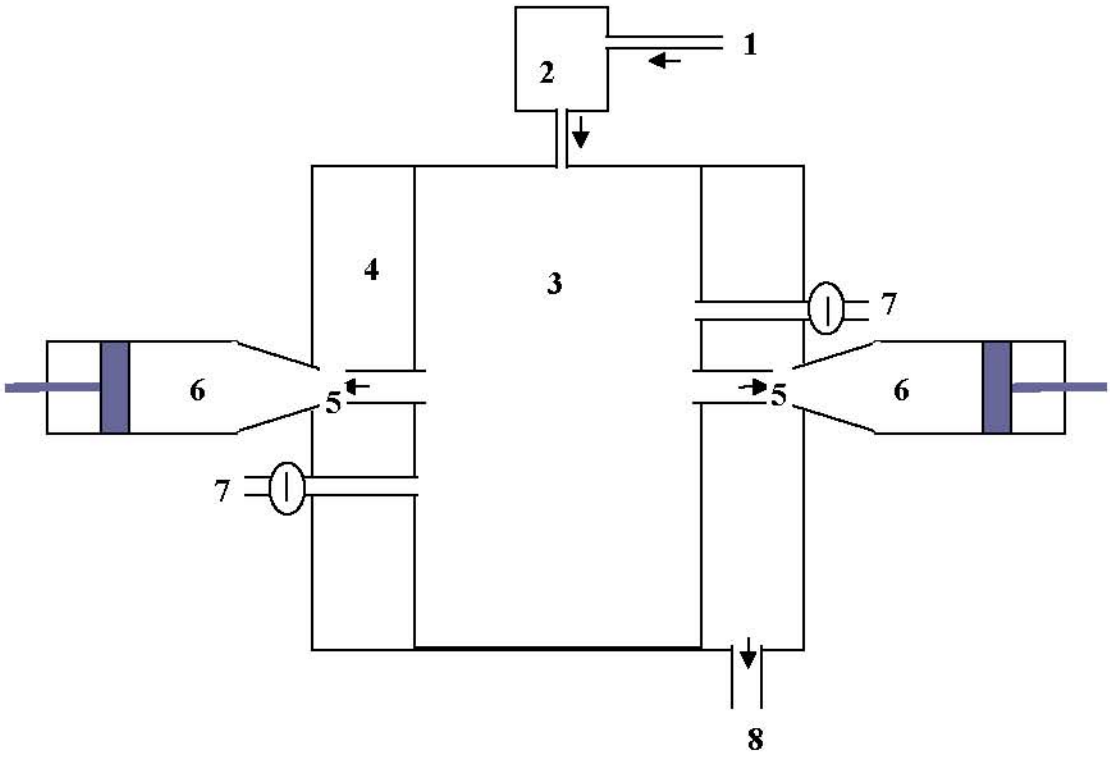
Under the experimental conditions of this study, no deaths occurred in a group of ten rats exposed to a concentration of 5.07 mg/L for four hours. The acute inhalation median lethal concentration (4hr LC_{50}) in CRL: (WI) Wistar strain rats, was therefore considered to be greater than 5.07 mg/L.

5. 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. Pauluhn J. and Mohr U. Repeated 4-week inhalation exposure of rats: effect of low-, intermediate, and high-humidity chamber atmospheres. *Exp Toxic Patho11999; 51: 178-187.*

FIGURES

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: Graph Showing Achieved Atmosphere Concentration – Group 0.1

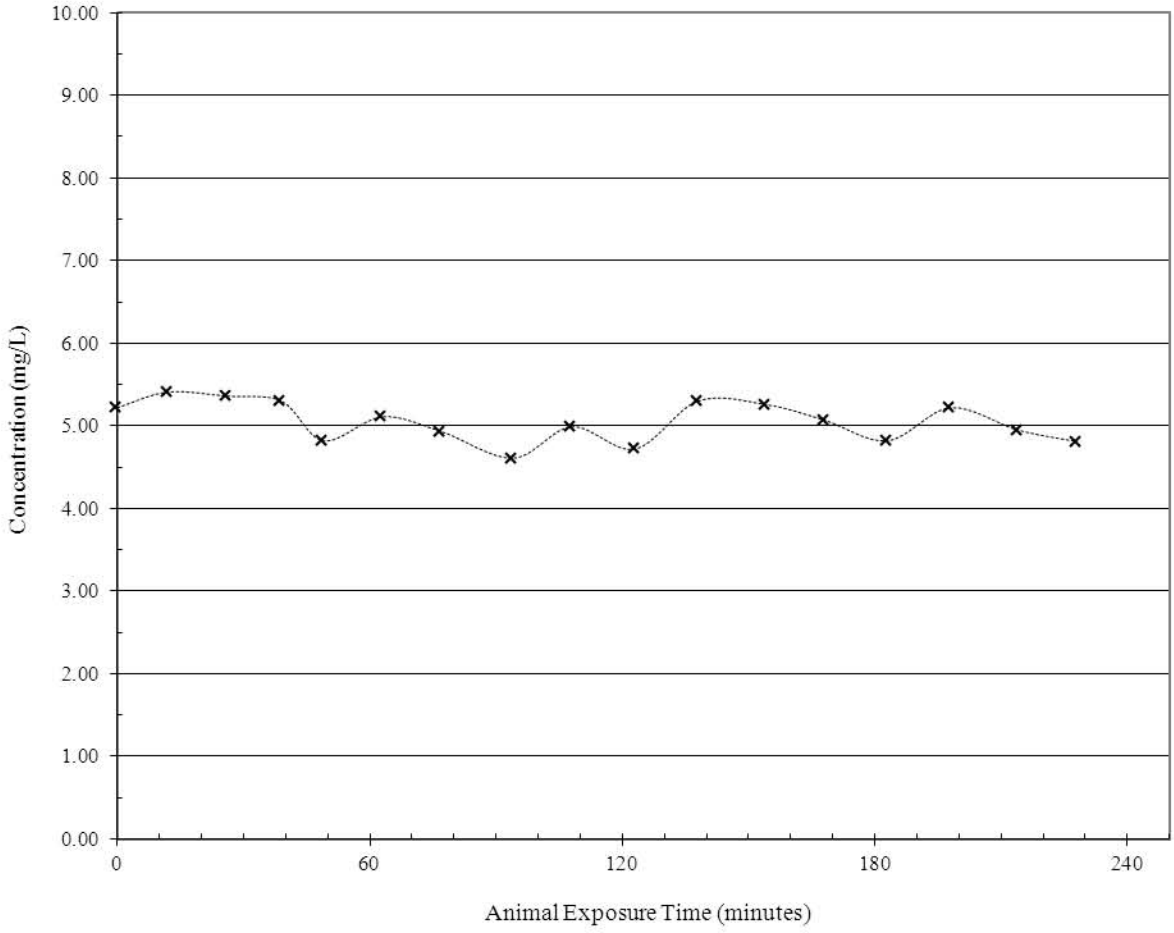


Figure 2 (Continued): Graph Showing Achieved Atmosphere Concentration – Group 1

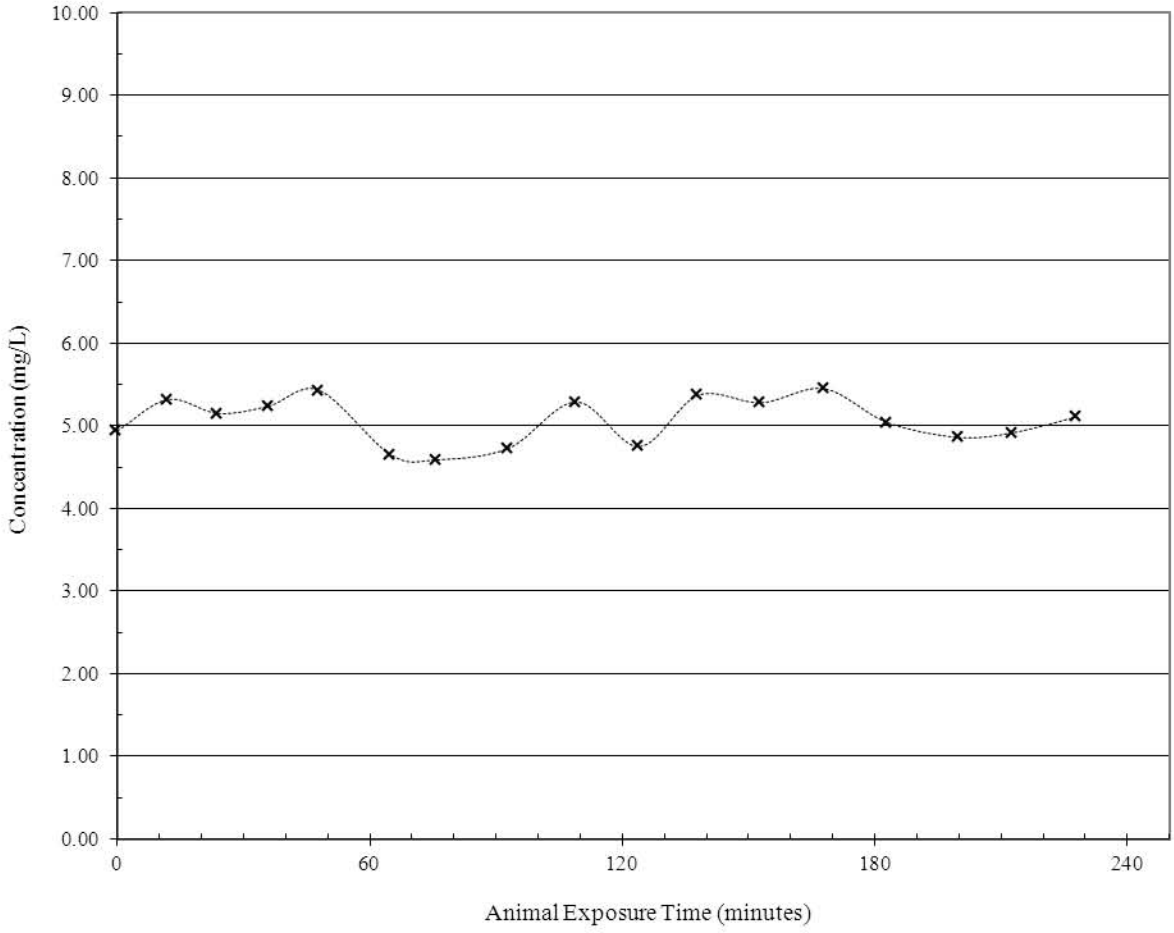
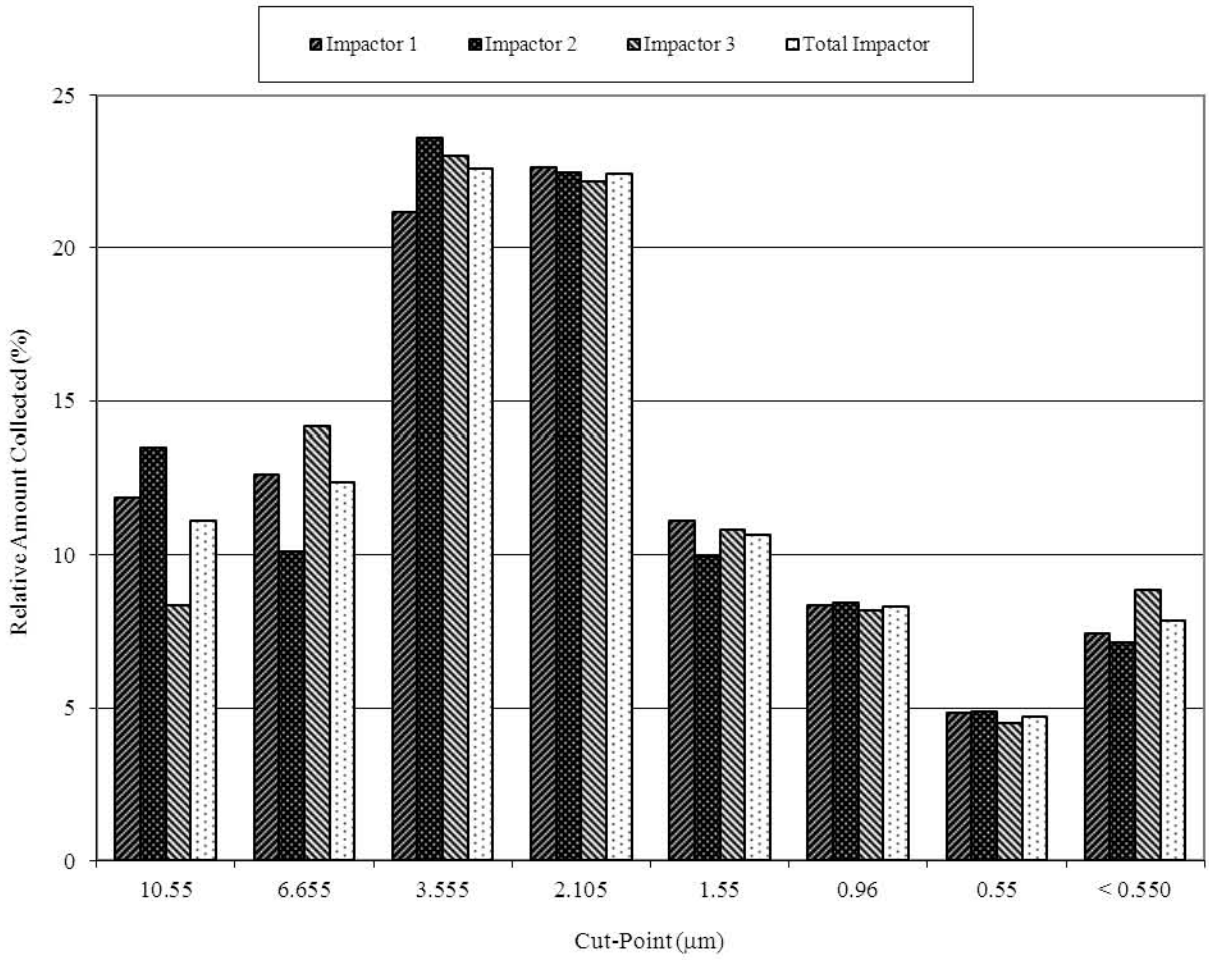
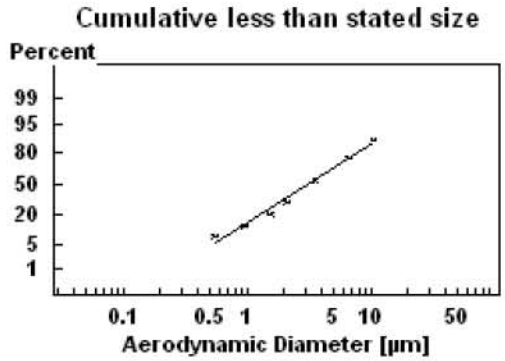


Figure 3: Graphs Showing Particle Size Distribution – Group 0.1





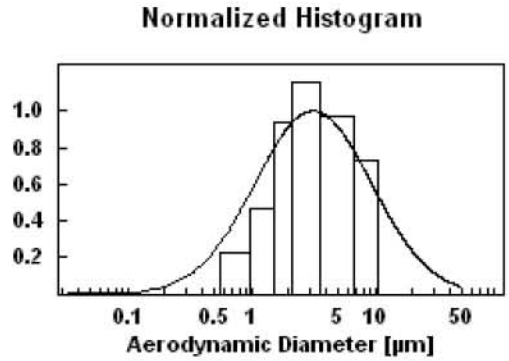
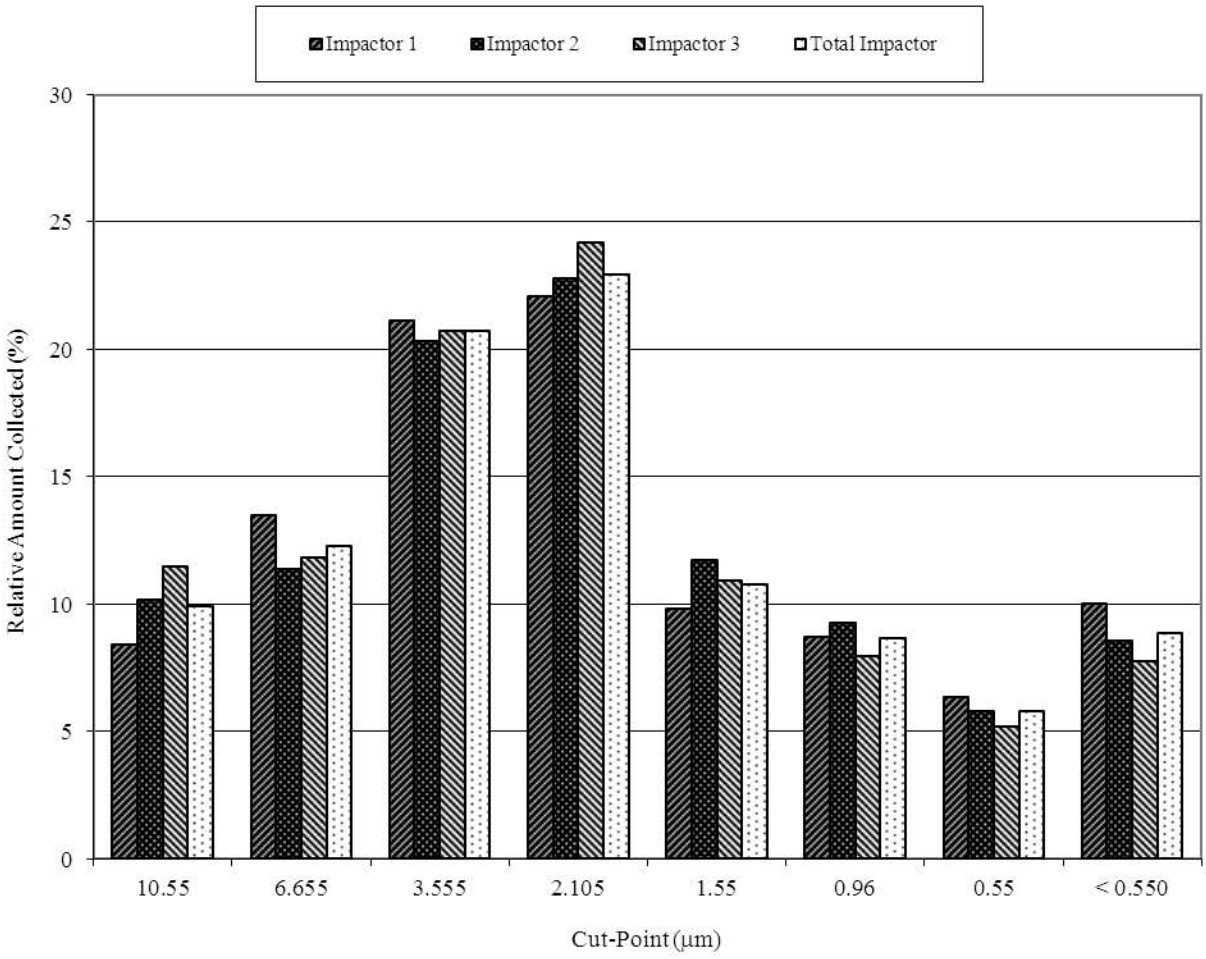
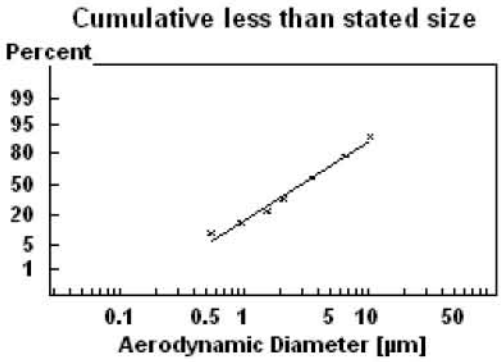
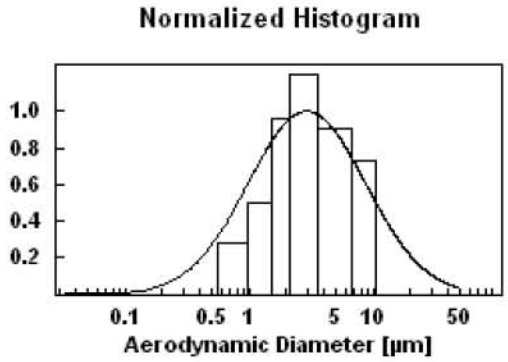


Figure 3 (Continued): Graphs Showing Particle Size Distribution – Group 1







APPENDICES

Appendix 1: Test Atmosphere Concentrations – Group 0.1

Exposure Duration (minutes)	Sample Volume (L)	Atmospheric Concentration of SH-0850 (mg/L)
0	2.0	5.22
12	2.0	5.41
26	2.0	5.36
39	2.0	5.30
49	2.0	4.82
63	2.0	5.11
77	2.0	4.93
94	2.0	4.61
108	2.0	4.99
123	2.0	4.72
138	2.0	5.30
154	2.0	5.26
168	2.0	5.07
183	2.0	4.82
198	2.0	5.22
214	2.0	4.95
228	2.0	4.81

Maximum attainable Atmosphere Concentration = 5.05 mg/L

Standard Deviation = 0.24

Nominal Concentration:

Amount of Test Item Used (g): 97.58

Total Volume of Air Used (L): 7500

Nominal Concentration = 13.01 mg/L

Appendix 1 (Continued): Test Atmosphere Concentrations – Group 1

Exposure Duration (minutes)	Sample Volume (L)	Atmospheric Concentration of SH-0850 (mg/L)
0	2.0	4.94
12	2.0	5.32
24	2.0	5.15
36	2.0	5.24
48	2.0	5.43
65	2.0	4.65
76	2.0	4.59
93	2.0	4.73
109	2.0	5.29
124	2.0	4.75
138	2.0	5.37
153	2.0	5.28
168	2.0	5.45
183	2.0	5.04
200	2.0	4.86
213	2.0	4.92
228	2.0	5.11

Maximum attainable Atmosphere Concentration = 5.07 mg/L

Standard Deviation = 0.28

Nominal Concentration:

Amount of Test Item Used (g): 91.42

Total Volume of Air Used (L): 7560

Nominal Concentration = 12.09 mg/L

Appendix 2: Test Atmosphere Particle Size Distribution Data – 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	0.64	0.72	0.50	1.86
2	6.66	0.68	0.54	0.85	2.07
3	3.56	1.14	1.26	1.38	3.78
4	2.11	1.22	1.20	1.33	3.75
5	1.55	0.60	0.53	0.65	1.78
6	0.96	0.45	0.45	0.49	1.39
7	0.55	0.26	0.26	0.27	0.79
Filter	< 0.55	0.40	0.38	0.53	1.31
				Total Amount Collected (mg)	16.73

Size Range (µm)	Total Mass/stage (mg)	Cumulative Mass (%)
< 0.55	1.31	7.83
0.55 -0.96	0.79	15.55
0.96 - 1.55	1.39	20.86
1.55 - 2.11	1.78	31.50
2.11 – 3.56	3.75	53.92
3.56 – 6.66	3.78	76.51
6.66 – 10.55	2.07	88.88
> 10.55	1.86	100.00

Maximum attainable Atmosphere Concentration = 5.05 mg/L

Mean Mass Median Aerodynamic Diameter (MMAD) = 3.15 µm
Geometric Standard Deviation (GSD) = 2.94
Inhalable Fraction (% < 4µm) = 58.8%

Appendix 2 (Continued): Test Atmosphere Particle Size Distribution Data – Group 1

Stage Number	Cut Point (µm)	Amount Collected (mg)			Total Collected per Stage (mg)
		Sample 1	Sample 2	Sample 3	
1	10.55	0.53	0.58	0.62	1.73
2	6.66	0.85	0.65	0.64	2.14
3	3.56	1.33	1.16	1.12	3.61
4	2.11	1.39	1.30	1.31	4.00
5	1.55	0.62	0.67	0.59	1.88
6	0.96	0.55	0.53	0.43	1.51
7	0.55	0.40	0.33	0.28	1.01
Filter	< 0.55	0.63	0.49	0.42	1.54
				Total Amount Collected (mg)	17.42

Size Range (µm)	Total Mass/stage (mg)	Cumulative Mass (%)
< 0.55	1.54	8.84
0.55 -0.96	1.01	14.64
0.96 - 1.55	1.51	23.31
1.55 - 2.11	1.88	34.10
2.11 – 3.56	4.00	57.06
3.56 – 6.66	3.61	77.78
6.66 – 10.55	2.14	90.07
> 10.55	1.73	100.00

Maximum attainable Atmosphere Concentration = 5.07 mg/L

Mean Mass Median Aerodynamic Diameter (MMAD) = 2.92 µm
Geometric Standard Deviation (GSD) = 2.97
Inhalable Fraction (% < 4µm) = 61.4%

Appendix 3: Test Chamber Environmental and Equilibration Data – Group 0.1

Measurement	Mean Value	Minimum	Maximum
Air Flow In (Inner Plenum) (L/min)	30.4	29.6	31.4
Air Flow Out (Outer Cylinder) (L/min)	24.0	23.7	24.2
Temperature (°C)	25.1	24.8	25.2
Relative Humidity (%)	30.4	23.0	39.0
Oxygen Concentration (%)	20.9	20.8	21.1
Carbon Dioxide (%)	0.0	0.0	0.1

Theoretical Chamber Equilibration Time (T_{99}):

$T_{99} = (4.605 \times (^{\text{Chamber Volume}}/_{\text{Chamber Flow rate}})$ (ref. 2)

Chamber volume (inner plenum) = 3.85L (ref. 1)

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

Actual equilibration time allowed = 11 minutes.

Appendix 3 (Continued): Test Chamber Environmental and Equilibration Data – Group 1

Measurement	Mean Value	Minimum	Maximum
Air Flow In (Inner Plenum) (L/min)	30.5	29.4	31.3
Air Flow Out (Outer Cylinder) (L/min)	24.0	23.7	24.3
Temperature (°C)	24.0	23.0	24.5
Relative Humidity (%)	14.4	6.0	35.0
Oxygen Concentration (%)	21.2	21.1	21.2
Carbon Dioxide (%)	0.0	0.0	0.1

Theoretical Chamber Equilibration Time (T_{99}):

$T_{99} = (4.605 \times (^{\text{Chamber Volume}}/_{\text{Chamber Flow rate}})$ (ref. 2)

Chamber volume (inner plenum) = 3.85L (ref. 1)

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

Actual equilibration time allowed = 13 minutes.

Appendix 4: Mortality Data

Day Number	Number of Deaths			
	Group 0.1 (5.05 mg/L)		Group 1 (5.07 mg/L)	
	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	0
6	0	0	0	0
7	0	0	0	0
8 – 14	0	0	0	0
Total Deaths	0/1	0/1	0/5	0/5
Grand Total Deaths	0/2		0/10	

Appendix 5: Individual Clinical Observations – Group 0.1

SIGHTING ESPOSURE
DOSE GROUP: **0.1**
CONCENTRATION: **5.05 mg/L** SEX: MALE/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
3696	Normal	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15 /19
	Laboured Respiration - Slight	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4 /19
	Wet fur - On/In restraining apparatus	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1 /19
	Wet fur - Whole body	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1 /19
	Fur staining by test item - First third of animal	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1 /19
3711	Normal	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15 /19
	Laboured Respiration - Slight	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4 /19
	Wet fur - On/In restraining apparatus	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1 /19
	Wet fur - Whole body	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1 /19
	Red-brown staining - Nose	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1 /19
	Fur staining by test item - First third of animal	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1 /19

COMMENT:
+ = Present
- = Absent

Appendix 5 (continued): Individual Clinical Observations – Group 1

MAIN STUDY
DOSE GROUP: 1
CONCENTRATION: 5.07 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
3496	Normal	+	+	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	16 /19
	Laboured Respiration - Slight	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2 /19
	Red-brown staining - Nose	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1 /19
	Fur staining by test item - First third of animal	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1 /19
3499	Normal	+	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15 /19
	Laboured Respiration - Slight	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3 /19
	Wet fur - On/In restraining apparatus	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2 /19
	Wet fur - Whole body	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2 /19
	Fur staining by test item - First third of animal	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1 /19
3500	Normal	+	+	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	16 /19
	Laboured Respiration - Slight	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2 /19
	Red-brown staining - Nose	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2 /19
	Fur staining by test item - First third of animal	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1 /19
3501	Normal	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	17 /19
	Laboured Respiration - Slight	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2 /19
	Fur staining by test item - First third of animal	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1 /19
3504	Normal	+	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	16 /19
	Laboured Respiration - Slight	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3 /19
	Fur staining by test item - First third of animal	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1 /19

COMMENT:
+ = Present
- = Absent

MAIN STUDY
DOSE GROUP: 1
CONCENTRATION: 5.07 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
3512	Normal	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	17 /19
	Laboured Respiration - Slight	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2 /19
	Fur staining by test item - First third of animal	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1 /19
3517	Normal	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	17 /19
	Laboured Respiration - Slight	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2 /19
	Fur staining by test item - First third of animal	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1 /19
3519	Normal	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	17 /19
	Laboured Respiration - Slight	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2 /19
	Red-brown staining - Nose	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1 /19
	Fur staining by test item - First third of animal	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1 /19
3523	Normal	+	+	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	16 /19
	Laboured Respiration - Slight	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2 /19
	Red-brown staining - Nose	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2 /19
	Fur staining by test item - First third of animal	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1 /19
3524	Normal	+	+	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	16 /19
	Laboured Respiration - Slight	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2 /19
	Red-brown staining - Nose	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2 /19
	Fur staining by test item - First third of animal	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1 /19

COMMENT: -
+ = Present
- = Absent

Appendix 6: Individual Bodyweight Data – Groups 0.1 and 1

SIGHTING EXPOSURE
DOSE GROUP: 0.1
CONCENTRATION: 5.05 mg/L SEX: MALE/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
3696	334	325	344	362	405	-9	19	18	43	71
3711	220	214	220	229	241	-6	6	9	12	21

MAIN STUDY
DOSE GROUP: 1
CONCENTRATION: 5.07 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
3496	374	366	357	391	409	-8	-9	34	18	35
3499	388	382	368	397	417	-6	-14	29	20	29
3500	398	392	383	404	423	-6	-9	21	19	25
3501	386	384	375	405	426	-2	-9	30	21	40
3504	393	395	386	420	441	2	-9	34	21	48

DOSE GROUP: 1
CONCENTRATION: 5.07 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
3512	246	240	246	250	246	-6	6	4	-4	0
3517	244	239	244	250	243	-5	5	6	-7	-1
3519	230	241	244	255	258	11	3	11	3	28
3523	243	235	238	242	242	-8	3	4	0	-1
3524	226	225	222	229	234	-1	-3	7	5	8

Appendix 7: Individual Necropsy Findings – Group 0.1 and 1

SIGHTING EXPOSURE

DOSE GROUP: 0.1
CONCENTRATION: 5.05 mg/L SEX: MALE/FEMALE

NECROPSY FINDINGS	Animal numbers	
	3696	3711
NO INTERNAL OBSERVATION RECORDED	+	+
NO EXTERNAL OBSERVATION RECORDED	+	+
STUDY DAYS	14	14
DATE OF NECROPSY	26 June 2015	

MAIN STUDY

DOSE GROUP: 1
CONCENTRATION: 5.07 mg/L SEX: MALE

NECROPSY FINDINGS	Animal numbers				
	3496	3499	3500	3501	3504
NO INTERNAL OBSERVATION RECORDED	+	+	+	+	+
NO EXTERNAL OBSERVATION RECORDED	+	+	+	+	+
STUDY DAYS	14	14	14	14	14
DATE OF NECROPSY	02 July 2015				

DOSE GROUP 1
CONCENTRATION: 5.07 mg/L SEX: FEMALE

NECROPSY FINDINGS	Animal numbers				
	3512	3517	3519	3523	3524
NO INTERNAL OBSERVATION RECORDED	+	+	+	+	+
NO EXTERNAL OBSERVATION RECORDED	+	+	+	+	+
STUDY DAYS	14	14	14	14	14
DATE OF NECROPSY	02 July 2015				

COMMENT: NECROPSY FINDINGS PRESENT =+

Appendix 8: Pathology Report

PATHOLOGY REPORT

INTRODUCTION

The objective of the study was to assess the acute inhalation toxicity The results of the study will serve as a basis for hazard assessment and classification and labelling.

RESULTS AND DISCUSSION

All rats survived until the scheduled termination of the study.

All 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.

TERMINAL (DAY 14)

Macroscopic Findings

Sighting exposure

There was no evidence of any changes in the rats dosed at 5.05 mg/L.

Main study

No macroscopic findings were observed at a concentration of 5.07 mg/L.

CONCLUSION

A single four hours nose-only exposure to Crl:WI rats exposed to the concentration of 5.05 mg/L during Sighting exposure or exposed to the concentration of 5.07 mg/L in Main study, was not associated with any gross changes.

_____

Date

Director of Pathology

Appendix 9: Certificate of Analysis

Appendix 10: Composition, Structure and Content

Appendix 11: Contents of the Diet

SSNIFF® SM R/M, AUTOCLAVABLE
Complete feed for rats and mice – breeding and maintenance"

Batch number:	*814 3108*
Expiry date:	*August 2015*

Crude protein	19.00%
Crude oils and fats	3.50%
Crude fibre	3.60%
Crude Ash	6.50%
Lysine	1.10%
Met+Cys	0.89%
Calcium	1.00%
Phosphorus	0.70%
Vitamin A	25000 IU/kg
Vitamin D_3	1500 IU/kg
Vitamin E	125 mg/kg

These data are standard and guaranteed values provided by the supplier.

Appendix 12: The Test Reports of the Diet

LUFA-ITL GmbH
Dr.-Hell-Str. 6, 24107 Kiel, Germany
Fax: +49(0431)1228-496
eMail: zentrale@lufa-itl.de www.agrolab.de

AGROLAB GROUP
Your labs. Your service.

LUFA - ITL Dr.-Hell-Str. 6, 24107 Kiel

SSNIFF SPEZIALDIÄTEN GMBH
FERDINAND-GABRIEL-WEG 16
59494 SOEST

ssniff
Spezialdiäten GmbH
Freigabe / Release
Nach GV-Solas

Date
Customer no.

REPORT

Order
Sample no.
Project
Sample acceptance
Date of sampling — **no information**
Sample code — **ssniff R/M-Zucht + H ungarn autocl, 15 mm**
S8106-S011
Chargen-No.: 814 3108
Packaging — **plastic bag**

	Unit	Result	Declaration		Substance	Method
Nutrition values						
Moisture (4h, 103°C)	%	12,0			OM	REG(EC) 152/2009, III, A
Crude ash	%	5,8	6,50		OM	REG(EC) 152/2009, III, M
Crude protein (Nx6,25)	%	17,5	19,00		OM	REG(EC) 152/2009, III, C
Total fat	%	3,4	3,50		OM	REG(EC) 152/2009, III, H, method B
Crude fibre	%	4,2	3,60		OM	REG(EC) 152/2009, III, I
Calculated values (nutrition/ingredients)						
N-free substances	%	57,1			OM	calculated

Explsnation: OM = on original matter; DM = on dry matter base

LUFA - ITL Frau Dr. Verena Gonzalez Lopez, Tel. 0431/1228-316
Customer Relations Management feed
Start of testing: 23.02.2015
End of testing: 26.02.2015

The analytical results are only valid for the delivered sample material.A plausibility check is hardly possible for samples of unknown origin.Duplication of this document or of parts of it requires the authorization from laboratory.

Appendix 13: Good Laboratory Practice Certificate

GYEMSZI
National Institute for Quality- and Organizational Development in Healthcare and Medicines

National Institute of Pharmacy

Zrínyi utca 3., H-1051 Budapest
H-1372 Budapest, PO Box 450.
Tel.: +36 1 888 9300
Fax: +36 1 888 9480
E-mail: ogyi@ogyi.hu
Web: www.ogyi.hu

GOOD LABORATORY PRACTICE (GLP) CERTIFICATE

It is hereby certified that the test facility

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, 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: