Study Report

(Original 1/2)

Study Number: U-15006

Study Title

Acute Inhalation Toxicity Study of GFS HG-AM GFS BioProtect TM Hospital Grade Disinfectant - GFS BioProtect TM Anti Mould in Sprague Dawley Rats

OECD Test Guideline: 403

Study Completion Date: 05 January 2016

SPONSOR

Global Future Solution Ltd
41 Magnesium Street
Narangba, QLD, Australia-4504

TEST FACILITY

Syngene International Limited
Biocon Park, Plot No. 2 & 3
Bommasandra IV Phase
Jigani Link Road
Bangalore – 560099, India

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1.0 STUDY DETAILS

1.1 GENERAL

Study Title	Acute Inhalation Toxicity Study of GFS HG-AM GFS BioProtect TM Hospital Grade Disinfectant - GFS BioProtect TM Anti Mould in Sprague Dawley Rats
Study Number	U-15006
Test Item Name	GFS BIOPROTECT-HG-AM
Test Item Code	56-0003
Test Facility	Syngene International Limited Biocon Park, Plot No. 2 & 3, Bommasandra IV Phase Jigani Link Road, Bangalore – 560099, India
Study Sponsor	Global Future Solution Ltd 41 Magnesium Street Narangba, QLD, Australia-4504
Monitoring Scientist	Mike Bralkowski E-mail: m.bralkowski@globalfuturesolutions.com Phone: 1-336-724-2244

1.2 RESPONSIBILITIES

Study Director	Abhijit Suresh Vichare M.V.Sc., DABT Syngene International Limited Biocon Park, Plot No. 2 & 3, Bommasandra IV Phase Jigani Link Road, Bangalore – 560099, India Email: abhijit.vichare@syngeneintl.com Mobile: +91 97410 16888
Study Personnel	Raghu N M.Sc. M Lakshmi Narayana M.Pharm. C Ravi Kiran M.Sc. Prathap Kumar K M.V.Sc. Bibhuti Ranjan M.V.Sc., Ph.D Sohel Rana B.Sc. STG Ranganath MSc Prabhakar Bhoite M.V.Sc., DABT
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1.3 STUDY SCHEDULE

Study Initiation Date	28 October 2015
Experiment Start Date	29 October 2015
Acclimatization Start Date	29 October 2015
Exposure	06 November 2015
Experiment Completion Date	20 November 2015
Draft Report to Sponsor	09 December 2015
Study Completion Date	05 January 2016

2.0 ABBREVIATIONS

%	:	Percent
±	:	Plus or minus
≤	:	Less than and equal to
°C	:	Degree Celsius
CAS	:	Chemical Abstract Service
CoA	:	Certificate of Analysis
cm	:	Centimeters
g/L	:	Gram per liter
GLP	:	Good Laboratory Practices
GSD	:	Geometric Standard Deviation
Hr	:	Hour
IAQ	:	Indoor Air Quality
ISO	:	International Organisation for Standardisation
L	:	Liters
LC_{50}	:	Median Lethal Concentration
LPM	:	Litres per minute
L x B x H	:	Length x Breadth x Height
mg	:	Milligram
mg/L	:	Milligram per liter
mL	:	Milliliter
mm	:	Millimeter
MMAD	:	Mass Median Aerodynamic Diameter
SDS	:	Safety Data Sheet
NaOH	:	Sodium Hydroxide
OECD	:	Organization for Economic Co-operation and Development
ppm	:	Parts per Million
SOP	:	Standard Operating Procedure
TIDS	:	Test Item Data Sheet

3.0 GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

Study Number : U-15006
Test Item : GFS BIOPROTECT-HG-AM
Study Title : Acute Inhalation Toxicity Study of GFS HG-AM GFS BioProtect TM Hospital Grade Disinfectant - GFS BioProtect TM Anti Mould in Sprague Dawley Rats

This study contains confidential information of the sponsor, which is not disclosed to anyone other than sponsor.

This is to state that the above mentioned study was performed as per the study plan and as per the guidelines OECD Guideline for testing of chemicals; No. 403; Acute Inhalation Toxicity; adopted on 07 September, 2009.

The study was conducted in compliance with the OECD Principles of Good Laboratory Practice (as revised in 1997), ENV/MC/CHEM (98) 17, OECD, Paris, 1998. (No. 1 in OECD Series on Good Laboratory Practice and Compliance Monitoring) concerning Mutual Acceptance of Data in the Assessment of Chemicals, [C (81)30(FINAL)] and Council Decision-Recommendation on Compliance with Principles of Good Laboratory Practice [C (95)8(FINAL)] and as per test facility standard operating procedures prevailing during the experiment. The study plan was signed by the study director on 28 October 2015 and by the Study Sponsor on 26 December 2015.

The study director declares that the experiment was performed under his supervision and as per the study plan. All the results given in the report represent the raw data. The study director takes the entire responsibility of conduct of the study, documentation of raw data, interpretation of the results, preparation and finalization of the report.

Study Director:

Name: **ABHIJIT SURESH VICHARE M.V.Sc., DABT**

Signature: 

Date: 05 Jan 2016

4.0 AFFIRMATION STATEMENT

Study Number	: U-15006
Study Title	: Acute Inhalation Toxicity Study of GFS HG-AM GFS BioProtect TM Hospital Grade Disinfectant - GFS BioProtect TM Anti Mould in Sprague Dawley Rats
Study Director	: Abhijit Suresh Vichare M.V.Sc., DABT

It is certified that the study in its entirety was performed in accordance with the Principles of Good Laboratory Practice and the final report stands complete in its originality and accuracy as per the principles of GLP.

Management:

Name: **SATHEESH V.K.**

Signature: 

Date: 05 Jan 2016

5.0 QUALITY ASSURANCE STATEMENT

This is to state that the following study had been inspected in compliance with the OECD principles of Good Laboratory Practice [C (97) 186 / final].

The study was inspected as per the Standard Operating Procedures of the test facility's Quality Assurance Unit and findings were reported to Management and Study Director. The details of the Study inspections are given below:

Study Title : Acute Inhalation Toxicity Study of GFS HG-AM GFS BioProtect TM Hospital Grade Disinfectant - GFS BioProtect TM Anti Mould in Sprague Dawley Rats

Study Number : U-15006

Test Item : GFS BIOPROTECT-HG-AM

Study Director : Abhijit Suresh Vichare M.V.Sc.

Details of Inspection :

S. No.	Inspection Phase	Date of Inspection		Date of Reporting To	
		From	To	Study Director	Test Facility Management
1	Draft Study Plan	26 Oct 2015	26 Oct 2015	26 Oct 2015	26 Oct 2015
2	Inhalation Exposure	06 Nov 2015	06 Nov 2015	06 Nov 2015	06 Nov 2015
3	Draft Study Report	07 Dec 2015	07 Dec 2015	07 Dec 2015	07 Dec 2015
4	Final Study Report	05 Jan 2016	05 Jan 2016	05 Jan 2016	05 Jan 2016

The below mentioned procedures were monitored during the process based inspections (independent of this Study) and the findings were reported to the Head of the Department, Study Directors and Management.

5	Animal body weighing	05 Nov 2015	05 Nov 2015	05 Nov 2015	05 Nov 2015
6	Clinical observations	12 Nov 2015	12 Nov 2015	13 Nov 2015	13 Nov 2015
7	Acclimatization	07 Dec 2015	07 Dec 2015	08 Dec 2015	08 Dec 2015

All inspections were conducted against the approved Study Plan and the Standard Operating Procedures. This statement also confirms that the final report reflects the raw data of the Study.

Quality Assurance Unit:

Name: **ANANTA KUMAR M**

Signature:

Date: 05 Jan 2016

6.0 SUMMARY

The Study titled "Acute Inhalation Toxicity Study of GFS HG-AM GFS BioProtect TM Hospital Grade Disinfectant - GFS BioProtect TM Anti Mould in Sprague Dawley Rats" was conducted with the objective to assess medial lethal concentration LC_{50} value of test item following nose only, flow-past inhalation exposure for a single 4-hour period in rats and to classify the test item according to Globally Harmonized System for Classification of Chemicals and OECD Harmonized Integrated Classification System for Human Health and Environmental Hazards of Chemical Substances and Mixtures. The test item was used as supplied by the Study Sponsor to generate aerosols. No analysis for its characterization was performed at the test facility. The Study was conducted as per the OECD Test Guideline 403 Acute Inhalation Toxicity; adopted on 07 September, 2009.

A limit test was conducted with one group consisting of three male and three female Sprague Dawley rats. Rats were exposed to the maximum attainable mean actual chamber concentration (1.48 mg/L) of GFS BIOPROTECT-HG-AM. Rats were exposed for a cumulative period of four hours followed by an observation period of 14 days.

The oxygen concentration, temperature, relative humidity and carbon-di-oxide concentration at the animal breathing zone measured and recorded during the exposure period were 20.8 to 21.0%, 22.6^{0}C to 23.4^{0}C, 58.2% to 63.4% and 740 to 1663 ppm respectively.

The rats were observed daily throughout the experimental period for mortality/viability and clinical signs. All rats were observed for clinical signs at 30 minutes and at approximately 1, 2, 3 and 4 hours during exposure, twice after exposure and once daily thereafter till day 15. Body weights were recorded on day 1 (prior to exposure), day 2, day 4, day 8 and day 15. All animals were subjected to gross pathological examination at necropsy.

No mortality was noted in any of the exposed rats throughout the experimental period. All the rats were found to be normal during exposure, post exposure on Day 1 and from Day 2 onwards up to sacrifice. Lethargy and abdominal breathing was observed in 2 males at 0.5 and 1 hour immediately post exposure.

Reduction in body weights was observed in all rats up to Day 4 post exposure. All rats gained weight from Day 8 onwards up to sacrifice in comparison to Day 1 body weights.

No abnormalities were detected in any rats at terminal sacrifice when subjected to gross pathological examination.

Based on these results, the median lethal concentration (LC_{50}) of GFS HG-AM GFS BioProtect TM Hospital Grade Disinfectant - GFS BioProtect TM Anti Mould after exposure in male and female Sprague Dawley rats through inhalation route for four hours, in accordance with Globally Harmonized System for Classification and Labeling of Chemicals (GHS) and OECD Harmonized Integrated Classification System for Human Health and Environmental Hazards of Chemical Substances and Mixtures:

LC_{50}: Greater than 1.48 mg/L (highest technically achievable concentration) and categorized as "Unclassified"

7.0 SAFETY PRECAUTIONS

The test item was handled with all recommended personal protective equipment and safety measures as per the relevant SOP, TIDS and MSDS. Personal protective equipment such as lab coat, mask, cap, gloves, safety goggles, shoes and half face respirators (during exposure) were used, wherever applicable to ensure adequate personal health and safety.

8.0 PRINCIPLE

The study plan was prepared based on the traditional protocol of the OECD test guideline 403 in which groups of rats are exposed through inhalation route to a limit concentration (limit test) or a series of concentrations (main test) for a pre-determined period of usually four hours and observed thereafter for fourteen days. The study was designed to obtain sufficient information on the acute toxicity of the test item to enable its classification and to provide acute lethality data (LC_{50}) for one or both sexes as needed for quantitative risk assessments.

9.0 ANIMAL WELFARE

All rats were handled humanely with due regard for their welfare. Care of rats complied with the Regulations of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India and Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC). The study was designed to use the fewest number of animals possible. The 'Form B' for carrying out animal experimentation was reviewed and approved by the Institutional Animal Ethics Committee (IAEC Protocol Approval No: SYNGENE/IAEC/666/10-2015).

10.0 OBJECTIVE

a) To assess median lethal concentration LC_{50} value of GFS HG-AM GFS BioProtect TM Hospital Grade Disinfectant- GFS BioProtect TM Anti Mould following nose only, flow-past inhalation exposure for a single 4-hour period to rats.

b) To classify the test item according to Globally Harmonized System for Classification of Chemicals and OECD Harmonized Integrated Classification System for Human Health and Environmental Hazards of Chemical Substances and Mixtures.

11.0 MATERIAL & METHOD

11.1 TEST ITEM DETAILS

Test Item Name	:	GFS BIOPROTECT-HG-AM
Test Item Code	:	56-0003
Name to be used in the study plan and report	:	GFS HG-AM GFS BioProtect TM Hospital Grade Disinfectant-GFS BioProtect TM Anti Mould
Chemical Name	:	Proprietary Blend
Type of Test Item	:	Industrial chemical
Batch No.	:	1005
Physical Appearance	:	Clear Liquid
Odour	:	Odourless
Specific gravity g/mL	:	0.996
pH	:	4.9
Photosensitive	:	No
Solubility	:	Totally miscible with water
Manufactured and Supplied by	:	GFS Australasia Pty Ltd. 41 Magnesium Street Narangba, QLD, Australia-4504
Manufactured Date	:	15/09/14
Expiry Date	:	15/09/16
Storage Condition	:	Ambient (20 to 25°C)

The test item details provided here were based on the information provided by Study Sponsor in CoA, TIDS and SDS. Sponsor was responsible for the authenticity of the test item. No further characterization of test item was performed at Syngene International Limited.

11.2 TEST SYSTEM

Test Species	Rat
Strain	Sprague Dawley
Sex	Male and Female (Females used were nulliparous and non-Pregnant)
Source	Vivo BioTech Limited, Hyderabad, India
Age during exposure	11 weeks
Number of animals	Limit Test (3 Male and 3 Females)

Extra animals for selection	Limit Test (1 male and 1 female)
Body Weight at Exposure (Day 1)	Males: 349.85 to 368.19 Females: 228.86 to 254.99
Justification	Rat is the most preferred rodent species by the OECD test guidelines.

11.3 ANIMAL HUSBANDRY

11.3.1 ENVIRONMENTAL CONDITIONS

Rats were maintained in a controlled environment with temperature range of 19.6°C to 23.1 °C (acceptable range 22 ± 3°C), relative humidity in between 47 to 69% (acceptable range between 30 - 70 %), a light/dark cycle of 12 hours each and at least 15 fresh air changes per hour. The air changes measured before start of the study was determined to be 18 per hour which were within the acceptable range. The maximum and minimum temperature and relative humidity in the experimental room was recorded once daily. Copy of room activity data sheets and record of photoperiod check were filed in the raw data.

11.3.2 ROOM SANITATION

Prior to occupancy, the microbial load of the experimental room (C-04) was checked and the results were found to be within acceptance limits. The experimental room floor was cleaned every day.

11.3.3 HOUSING

Rats were housed individually in clean sterilized polycarbonate cages (with sterilized grill tops) of dimensions approximately 41.0 x 26.5 x 20.0 cm (L x B x H). Enrichment was placed in each cage. During exposure, the rats were held in restrainers for a cumulative period of four hours. The rats were acclimatized to the restrainers for four days prior to exposure for a period of one hour each day to lessen the stress caused by introduction to the new environment. Autoclaved corncob was used as bedding and was changed along with the cage immediately after exposure and at least twice a week. Bedding material is analyzed for chemical contaminants annually in an ISO certified laboratory. Each batch of the bedding material is screened in-house for the microbial load. The contaminant and microbial levels were within the maximum permissible limits.

Name of Bedding Material	Manufacturer	Batch No.	Date of manufacturing	Date of expiry
Corn cobb	Sparconn Life Sciences	SPAR 30/2015	August 2015	July 2016

11.3.4 DIET

The rats were fed, *ad libitum*, with Teklad global rodent diet manufactured by Harlan except during exposure, wherein they were restrained in the restrainers and also prior to exposure, wherein the rats were acclimatized in restrainers for a period of one hour for 4 days. On receipt of each batch, a sample is screened in-house for microbial load. Chemical contaminants and proximate analysis are performed annually in an ISO certified laboratory. The microbial levels, proximate and contaminant levels were within the maximum permissible limits.

Name of Diet	Manufacturer	Batch No.	Date of manufacturing	Date of Expiry
Teklad Certified IR Global 18% Protein Rodent Diet	Harlan	2918C-060215MA	2 June 2015	27 Feb 2016
		2918C-061515MA	15 June 2015	11 Mar 2016

11.3.5 WATER

Potable autoclaved water filtered through Reverse Osmosis, was provided *ad libitum* to all rats, *via* autoclaved polycarbonate bottles fitted with stainless steel nozzles except during exposure. Water analysis for microbiological load and chemical contaminants is conducted in-house once every month. Contaminant analysis is performed annually in an ISO certified laboratory. The microbial and contaminant levels were within maximum permissible limits.
Reports of all analyses of bedding, diet and water are maintained with the facility records.

11.4 PREPARATION OF ANIMALS

11.4.1 ACCLIMATIZATION AND SELECTION

The rats were acclimatized in the experiment room (C-04) for a period of 8 days. Rats were selected and grouped manually for limit test. Rats selected for exposure were examined for clinical signs before treatment.

11.4.2 ANIMAL IDENTIFICATION

On receipt, rats were assigned a temporary number (1-4 for males and 5-8 for females) at the tip of tail by using a black indelible marker pen. Immediately after selection, the rats were tattooed with a unique permanent number from Ra9922 to Ra9924 for males and Ra9925 to Ra9927 for females at the base of the tail using a tattoo machine. Cage cards indicating the study number, animal number, group number and other relevant details were displayed on the corresponding cages.

11.5 LIST OF INSTRUMENTS AND MATERIALS

Name of Instrument	Make	Instrument ID
Thermohygrometer	Equinox	SLAR-AHU-32/26
Weighing Balance	Sartorius	SLAR-LI-157
Weighing Balance	Sartorius	SLAR-LI-202
Weighing Balance	Sartorius	SLAR-LI-054
IAQ Probe	Wolfsense	SLAR-LI-221
Vacuum Pump	Rocker	SLAR-LI-219
Vacuum Pump	Rocker	SLAR-LI-233
Collison Nebulizer	BGI Inc.	SLAR-LI-227
Fume Hood	Kewaunee Scientific Corporation	SLAR-LI-229
Oxymeter	Technovation Analytical Instruments	SLAR-LI-223
Cascade Impactor	Intox Products	SLAR-LI-224
Standard Weight	Zwiebel	SLAR-LI-258
Open Phase Sampler	BGI, Inc	SLAR-LI-226
Control Panel	Palas GmbH	SLAR-LI-227/01
Tattoo Machine	AIMS	SLAR-LI-066

Material used	Make	Batch No.	Date of Manufacture/Receipt	Date of Expiry/Best Before
Sodium Hydroxide Pellets	RFCL Limited	P121D08	18 Apr 2012	26 Apr 2017
Acetone	SD Fine Chem Limited	G13A/0813/0807/13	July 2013	June 2019
Thiopentone Sodium	Neon Laboratories	172358	May 2015	April 2017
47 mm Filter paper	PSI	-	-	-

11.6 TEST ITEM PREPARATION

The test item was used as supplied by the study sponsor for aerosol generation.

11.7 INHALATION EXPOSURE SYSTEM

Inhalation exposure was performed using a nose only, flow past dynamic inhalation chamber (CH Technologies make, USA). The rats were confined individually in restraining tubes which were positioned radially around the flow-past, nose-only exposure chamber. It ensured a uniform distribution of test item aerosol, provided a constant stream of "fresh" aerosolized test item to each rat, and precluded re-breathing of exhaled air. The basic design of the dynamic inhalation exposure system included an Inhalation Chamber (Chamber Dimensions: 841.90 cm^3; Chamber volume: 0.841 L), Collison Nebulizer, Control Panel to regulate the air flow to nebulizer and peripheral devices like Cascade Impactor, Oxymeter, Open Phase Sampler and IAQ probe.

The rats were exposed in flow-past inhalation equipment designed to sustain a dynamic airflow that ensures an adequate air exchange of at least 2-3 times the respiratory minute volume of rats exposed (i.e., at least 0.5 L/min per exposure port for rats) and each exposure port (One chamber has 12 ports) had similar exposure conditions with an Oxygen concentration of at least 19% and Carbon dioxide concentration not exceeding 1% (10000 ppm). Positive pressure was maintained to prevent the dilution of aerosolized test item at breathing zone and the inhalation chamber was operated in a fume hood with negative pressure to prevent leakage of test item aerosol to surrounding areas.

The outlet of inhalation chamber was connected to an impinger with 1% NaOH in two measuring cylinders connected serially immediately after inhalation chamber followed by two measuring cylinders with dry cotton for absorption of the moisture in the extraction air. Thus extracted air was fed via fume hood to the scrubber and then released to the environment after filtering.

11.8 TECHNICAL TRIALS

Before commencement of the animal exposure, technical trial was conducted (without rats) using the inhalation system to achieve the following objectives:

- To establish the exposure conditions necessary to achieve the maximal attainable concentration of test item aerosol (≤ 5 mg/L) for limit test.
- To measure the test item aerosol concentration achieved.
- To measure the particle size distribution of the test item aerosol.

The maximum attainable concentration for the limit test was decided with the calculated MMAD value of 2.41 µm and GSD value of 2.96 attained during the technical trial. The details were documented in the raw data. The actual aerosol gravimetric concentration was found to be in the range of 1.33 to 1.64 mg/L. (Refer Table 10).

11.9 NOSE-ONLY INHALATION EXPOSURE

The exposure was for single four hours after the equilibration of the inhalation chamber. Brief interruption of the exposure was made once to replace the test item reservoir supplying test item. The rats were exposed for a cumulative period of 4 hours, to account for such short interruptions.

A limit test was conducted by exposing 3 male and 3 female rats to maximum attainable mean actual chamber concentration of 1.48 mg/L. Since mortality was not observed in the 14 day observation period, the study was concluded with the limit test and reported.

11.10 TEST AEROSOL GENERATION

Test item aerosol was generated using a 6 Jet Collison Nebulizer (Make: BGI Inc.). Compressed air was supplied to the Nebulizer through the control panel. Brief interruption of the exposure was made once to replace the test item reservoir supplying test item. The aerosol generation air in LPM was set at pre-determined level and the aerosol thus generated was discharged into the inhalation exposure chamber. The quality of air used for aerosol generation is checked annually in an ISO certified laboratory and the photocopy of test results were kept in the raw data.

11.11 EXPOSURE CONDITIONS MONITORING

Test atmosphere concentration and particle size distribution were determined gravimetrically. Relative humidity, temperature, oxygen and carbon-di-oxide concentration were measured at an empty port of the inhalation chamber. Airflow rates for aerosol generation were recorded from the control panel. The monitoring of temperature, relative humidity and carbon-di-oxide concentration was performed using IAQ probe.

11.11.1 NOMINAL CONCENTRATION DETERMINATION

The test item usage was measured by weighing the test item reservoir containing the test item before and after the exposure to determine the quantity of test item consumption in aerosol

generation. This weight (consumed quantity of test item) was divided by the total air-flow volume into the chamber during the exposure period to give the nominal concentration.

11.11.2 GRAVIMETRIC CONCENTRATION DETERMINATION

Gravimetric determinations of aerosol concentration was performed using open phase sampler, a stainless steel sampling device with single filter (47 mm diameter; Make: Performance Systematix Inc). These determinations were performed six times during the exposure.

11.11.3 PARTICLE SIZE DISTRIBUTION: MMAD AND GSD CALCULATION

Particle size distribution was measured gravimetrically twice during the exposure using a 7 stage Cascade Impactor (In-Tox. Products Inc., Moriarty, NM, USA). Mass Median Aerodynamic Diameters (MMAD) and Geometric Standard Deviation (GSD) were calculated on the basis of the results from the Cascade Impactor using a validated statistical software (SigmaPlot 12.3).

11.11.4 OXYGEN CONCENTRATION

Oxygen concentration in % was monitored and recorded four times during the whole exposure period using a calibrated Percentage Oxymeter (Model EC-3 supplied by Technovation Analytical Instruments (P) Ltd., India).

11.11.5 TEMPERATURE AND RELATIVE HUMIDITY

Temperature in °C and Relative Humidity in % was monitored and recorded four times during the whole exposure period using an Indoor Air Quality (IAQ) Probe (Serial No. 11-514, GreyWolf Sensing Solutions, Ireland).

11.11.6 CARBONDIOXIDE CONCENTRATION

Carbon-di-oxide concentration in ppm was monitored and recorded four times during the whole exposure period using an Indoor Air Quality (IAQ) Probe (Serial No. 11-514, GreyWolf Sensing Solutions, Ireland).

11.11.7 EXPOSURE AIR FLOW RATE

The exposure air in-flow rate was monitored during each exposure through the Control Panel of the exposure system and extraction air flow rate was monitored using calibrated rotameter set to the definite flow rate and recorded four times during the whole exposure period.

12.0 OBSERVATIONS

12.1 MORBIDITY & MORTALITY

All the rats were checked twice daily for morbidity and mortality, once daily on weekends, on general holidays and on terminal sacrifice.

12.2 CLINICAL SIGNS

All rats were observed individually for visible clinical signs during acclimatization with special emphasis after acclimatizing the rats to restrainers (before the scheduled exposure) and at 30 minutes, 1, 2, 3 and 4 hours (only gross abnormal signs were recorded as the animals were restrained) during exposure and twice after exposure. Clinical signs were recorded once daily thereafter for 14 days.

12.3 BODY WEIGHT

The body weights were recorded individually for all rats at receipt, prior to the exposure (Day 1), at Day 2, Day 4, Day 8 and at terminal sacrifice (on Day 15). Body weight changes with respect to Day 1 body weights were calculated.

13.0 PATHOLOGY

13.1 NECROPSY

At the end of the observation period, all the rats were sacrificed using Thiopentone Injection administered intra-peritoneally and subjected to gross necropsy. Gross necropsy was performed with particular reference to changes in the respiratory tract.

14.0 RESULTS

14.1 NOMINAL CONCENTRATION DETERMINATION

Refer Table 1

The nominal concentration of test item was determined for the total exposure period of 240 minutes as follows:

40.01 g (total consumption) x 1000 / 12 LPM x 240 min = 13.89 mg/L

14.2 GRAVIMETRIC CONCENTRATION DETERMINATION

Refer Table 2

The actual concentration of test item determined gravimetrically during the exposure period ranged from 1.37 mg/L to 1.57 mg/L. These determinations were performed six times during the exposure. The mean actual chamber concentration was 1.48 mg/L.

14.3 PARTICLE SIZE DISTRIBUTION, MMAD AND GSD CALCULATION

Refer Table 3 and Table 4, Annexure 01

Mass Median Aerodynamic Diameter (MMAD) and Geometric Standard Deviation (GSD) were calculated on the basis of the results from the Cascade Impactor using a validated statistical software (SigmaPlot 12.3). The MMAD calculated was 2.37 µm and GSD was 2.93 and the graph is presented as Annexure 01.

14.4 OXYGEN CONCENTRATION

Refer Table 5

The oxygen concentrations at the animal breathing zone measured and recorded during the exposure period was 20.8 to 21.0% and it was observed to be within the stipulated range.

14.5 TEMPERATURE AND RELATIVE HUMIDITY

Refer Table 5

The temperature at the animal breathing zone, measured and recorded during the exposure period was in the range 22.6°C to 23.4°C. The relative humidity at the animal breathing zone measured and recorded during the exposure period was in the range of 58.2% to 63.4%.

14.6 CARBON DI OXIDE CONCENTRATION

Refer Table 5

The carbon-di-oxide concentration at the animal breathing zone measured and recorded during the exposure period was in the range of 740 to 1663 ppm and the values were found to be within the stipulated range.

14.7 EXPOSURE AIR FLOW RATE

Refer Table 5

The exposure air in-flow rate recorded was 12 LPM and exhaust air flow rate recorded was 10 LPM throughout the exposure period of four hours.

14.8 MORBIDITY AND MORTALITY

Refer Table 6

There was no morbidity and mortality observed in any of the exposed animals (G1).

14.9 CLINICAL SIGNS

Refer Table 6

All the animals were observed to be normal during the exposure period. Clinical signs of lethargy was observed in one male (Ra9923) and lethargy along with abdominal respiration was observed in 2 males (Ra9922 and Ra9924) at 0.5 and 1 hour post exposure. Clinical sign of lethargy persisted till Day 2 in 2 males (Ra9922 and Ra9924). All the males were found to be normal from Day 3 onwards up to sacrifice. All females were found to be normal post exposure up to sacrifice.

14.10 BODY WEIGHT

Refer Table 7 and Table 8

Reduction in body weights was observed in all the rats up to Day 4 post exposure. All rats showed gain in body weight from Day 8 onwards up to sacrifice.

14.11 NECROPSY

Refer Table 9

No abnormalities were detected in any of the animals at terminal sacrifice when subjected to gross pathological examination.

15.0 CONCLUSION

Based on the results, the median lethal concentration (LC_{50}) of GFS HG-AM GFS BioProtect TM Hospital Grade Disinfectant- GFS BioProtect TM Anti Mould after exposure of male and female Sprague Dawley rats through inhalation route for four hours, in accordance with Globally Harmonized System for Classification and Labeling of Chemicals (GHS) and OECD Harmonized Integrated Classification System for Human Health and Environmental Hazards of Chemical Substances and Mixtures:

LC_{50}: Greater than 1.48 mg/L (highest technically achievable concentration) and is categorized as "Unclassified"

16.0 ARCHIVING

On completion of the study, all raw data, the Study Plan, QA audited draft report and an original final report will be stored in the archives of the test facility for a minimum period of 9 years. Also, the test item is archived for a minimum period of 9 years. After the completion of this period, the Sponsor's consent will be sought to either extend the archiving period or to return the archived material to the Sponsor or for discarding the material.

17.0 REPORT DISTRIBUTION

Original 1/2 – Study Sponsor

Original 2/2 – Archives of test facility

18.0 REFERENCES

1. OECD Guidelines for the Testing of Chemicals, No.403, Acute Inhalation Toxicity, Adopted on 7th September, 2009.
2. OECD Series on Testing and Assessment, Number 33 - Harmonised Integrated Classification System for Human Health and Environmental Hazards of Chemical Substances and Mixtures. ENV/JM/MONO (2001)6. OECD, August, 2001.
3. Globally Harmonised System of Classification and Labeling of Chemicals (GHS) Fourth Revised Edition, United Nations (2013). ST/SG/AC.10/30/Rev 5.
4. Guidance Document on Acute Inhalation Toxicity Testing. INV/JM/MONO(2009) 28, OECD Environment, Health and Safety Publications Series on Testing and Assessment No. 39, 2009.

19.0 TABLES

TABLE 1: NOMINAL CONCENTRATION DETERMINATION

$$\text{Nominal Concentration (mg/L)} = \frac{\text{Total Test Item Consumption (g)} \times 1000}{\text{Air Flow Rate (LPM)} \times \text{Exposure Duration (min)}}$$

Test Item Consumption (g)	Inlet Air Flow Rate (LPM)	Exposure Duration (min)	Nominal Concentration (mg/L)
40.01	12	240	13.89

Key: LPM = Liters per minute, min= minutes

TABLE 2: ACTUAL AEROSOL CONCENTRATION OF TEST ITEM

Sample No.	Filter Weight (mg)		Duration (min)	Air Flow Rate (LPM)	Actual Aerosol Concentration (mg/L)
1	Pre-weight	352.57	1	1	1.37
	Post-weight	353.94			
	Wt. Gain	1.37			
2	Pre-weight	356.10	1	1	1.44
	Post-weight	357.54			
	Wt. Gain	1.44			
3	Pre-weight	351.81	1	1	1.55
	Post-weight	353.35			
	Wt. Gain	1.55			
4	Pre-weight	353.28	1	1	1.45
	Post-weight	354.73			
	Wt. Gain	1.45			
5	Pre-weight	354.13	1	1	1.57
	Post-weight	355.70			
	Wt. Gain	1.57			
6	Pre-weight	352.91	1	1	1.50
	Post-weight	354.41			
	Wt. Gain	1.50			
Mean Actual Concentration of Test Item (mg/L)					**1.48**

Key: Wt. = Weight, LPM = Liters per minute, min= minutes,
Actual Aerosol Concentration (mg/L) = Weight gain (mg) / Duration (min) x Airflow (LPM)
Wt. Gain = (Post-weight - Pre-weight)

TABLE 3: MEAN PARTICLE SIZE DISTRIBUTION AT INHALATION CHAMBER

Particle Size Range (µm)	Group G1	
	Mean Percent Particle Size (%)	Cumulative Percent Particle Size (%)
> 5.09	9.8	100.00
3.13 – 5.09	33.9	90.18
1.93 – 3.13	24.1	56.25
1.21 – 1.93	13.4	32.14
0.77 – 1.21	9.8	18.75
0.50 – 0.77	7.1	8.93
0.33 – 0.50	1.8	1.79
0-0.33	0.0	0.00

TABLE 4: PARTICLE SIZE DISTRIBUTION

Particle Size Range (µm)	ECD (µm)	Group G1			
		Sample 1		Sample 2	
		Weight Difference (mg)	Percent Particle size (%)	Weight Difference (mg)	Percent Particle size (%)
> 5.09	5.09	0.06	10.71	0.05	8.93
3.13 – 5.09	3.13	0.19	33.93	0.19	33.93
1.93 – 3.13	1.93	0.13	23.21	0.14	25.00
1.21 – 1.93	1.21	0.08	14.29	0.07	12.50
0.77 – 1.21	0.77	0.05	8.93	0.06	10.71
0.50 – 0.77	0.50	0.04	7.14	0.04	7.14
0.33 – 0.50	0.33	0.01	1.79	0.01	1.79
0-0.33	-	0.00	0.00	0.00	0.00
Sum		**0.56**	**100**	**0.56**	**100**

Key: ECD = Effective cut-off diameter

TABLE 5: EXPOSURE CONDITIONS RECORD

S. No.	Temperature (°C)	Relative Humidity (%)	Oxygen Concentration (%)	CO_2 Level (ppm)	Inlet Air Flow (LPM)	Exhaust Air Flow Rate (LPM)
1	22.6	58.2	20.8	740	12	10
2	22.8	63.4	21.0	1663	12	10
3	23.4	61.8	21.0	1150	12	10
4	23.1	61.2	21.0	1028	12	10

Key: ppm = Parts per million, LPM = Liters per minute

TABLE 6: MORTALITY, MORBIDITY AND CLINICAL SIGNS

Group Number	Animal Number	Mean Actual Chamber Conc. (mg/L)	Sex	Day																				
				1							2	3	4	5	6	7	8	9	10	11	12	13	14	15
				During Exposure					Post Exposure															
				0.5h	1h	2h	3h	4h	*	**														
G1	Ra9922	1.48	M	1	1	1	1	1	37,15	37,15	15	1	1	1	1	1	1	1	1	1	1	1	1	1
	Ra9923		M	1	1	1	1	1	15	15	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	Ra9924		M	1	1	1	1	1	37,15	37,15	15	1	1	1	1	1	1	1	1	1	1	1	1	1
	Ra9925		F	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	Ra9926		F	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	Ra9927		F	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

* About 0.5 hour post exposure
**About 1 hour post exposure
Key: 1 = Normal, 37 - Abdominal respiration, 15 - Lethargy

TABLE 7: INDIVIDUAL ANIMAL BODY WEIGHTS

Group Number	Mean Actual Chamber Conc.	Animal Number	Sex	Day 1 (g)	Day 2 (g)	Day 4 (g)	Day 8 (g)	Day 15 (g)
G1	1.48 mg/L	Ra9922	Male	349.85	330.51	334.93	351.66	362.79
		Ra9923		368.19	345.69	360.65	375.34	387.80
		Ra9924		366.96	350.48	363.87	370.48	384.51
Mean				**361.67**	**342.23**	**353.15**	**365.83**	**378.37**
SD				**10.25**	**10.43**	**15.86**	**12.51**	**13.59**
G1	1.48 mg/L	Ra9925	Female	254.99	244.26	245.21	256.19	264.62
		Ra9926		228.86	215.57	213.15	228.96	242.46
		Ra9927		235.22	223.11	220.28	240.47	249.41
Mean				**239.69**	**227.65**	**226.21**	**241.87**	**252.16**
SD				**13.63**	**14.87**	**16.83**	**13.67**	**11.33**

Key: SD = Standard Deviation

TABLE 8: INDIVIDUAL ANIMAL BODY WEIGHT CHANGES

Group Number	Mean Actual Chamber Conc.	Animal Number	Sex	Day 1-2 (g)	Day 1-4 (g)	Day 1-8 (g)	Day 1-15 (g)
G1	1.48 mg/L	Ra9922	Male	-19.34	-14.92	1.81	12.94
		Ra9923		-22.50	-7.54	7.15	19.61
		Ra9924		-16.48	-3.09	3.52	17.55
Mean				**-19.44**	**-8.52**	**4.16**	**16.70**
SD				**3.01**	**5.98**	**2.73**	**3.42**
G1	1.48 mg/L	Ra9925	Female	-10.73	-9.78	1.20	9.63
		Ra9926		-13.29	-15.71	0.10	13.60
		Ra9927		-12.11	-14.94	5.25	14.19
Mean				**-12.04**	**-13.48**	**2.18**	**12.47**
SD				**1.28**	**3.22**	**2.71**	**2.48**

Key: SD = Standard Deviation

TABLE 9: INDIVIDUAL ANIMAL NECROPSY FINDINGS

Group Number	Mean Actual Chamber Conc.	Animal Number	Sex	Mode of death	Gross Pathology Observations	
G1	1.48 mg/L	Ra9922	Male	TS	NAD	NAD
		Ra9923	Male	TS	NAD	NAD
		Ra9924	Male	TS	NAD	NAD
		Ra9925	Female	TS	NAD	NAD
		Ra9926	Female	TS	NAD	NAD
		Ra9927	Female	TS	NAD	NAD

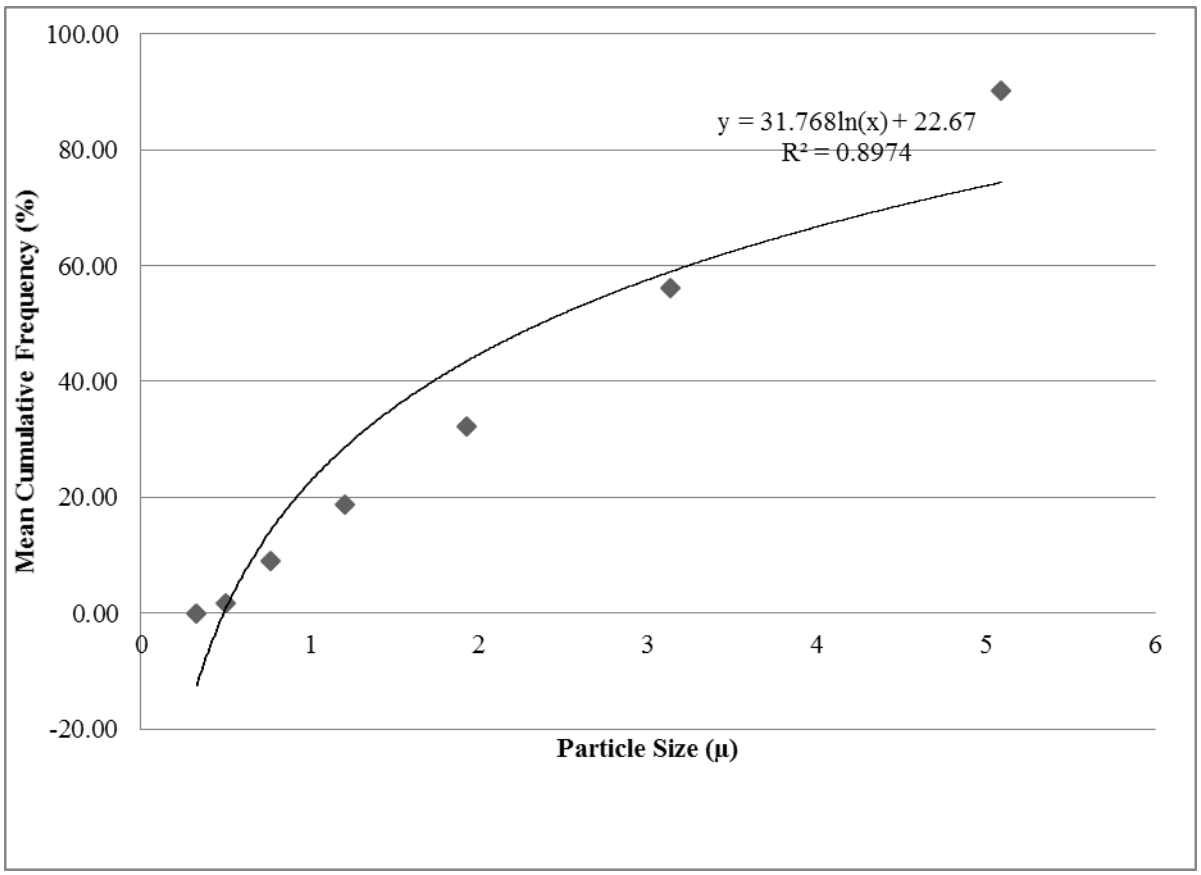
Key: TS = Terminal Sacrifice, NAD = No Abnormalities Detected

TABLE 10: TECHNICAL TRIAL DETAILS

Technical Trial 1
➢ The mean actual chamber concentration achieved during the technical trial 1 was in the range of 0.42 to 0.50 mg/L. ➢ Exposure Settings: Aerosol generation air – 6.5 LPM and Dilution air – 5.5 LPM. ➢ Since, the achieved concentration was very less and foam was getting created in the nebulizer jar, a 2nd technical trial was conducted with modified exposure settings.
Technical Trial 2
➢ The mean actual chamber concentration achieved during the technical trial 2 was in the range of 1.33 to 1.64 mg/L ➢ Exposure Settings: Aerosol generation air – 9.7 LPM and Dilution air – 2.3 LPM. ➢ Since, the concentration achieved was the maximum achievable concentration, particle size was evaluated. ➢ The MMAD was found to be 2.41 microns. Since, the MMAD was found to be in range, the above concentration was selected for animal exposure in the limit test.

20.0 ANNEXURE

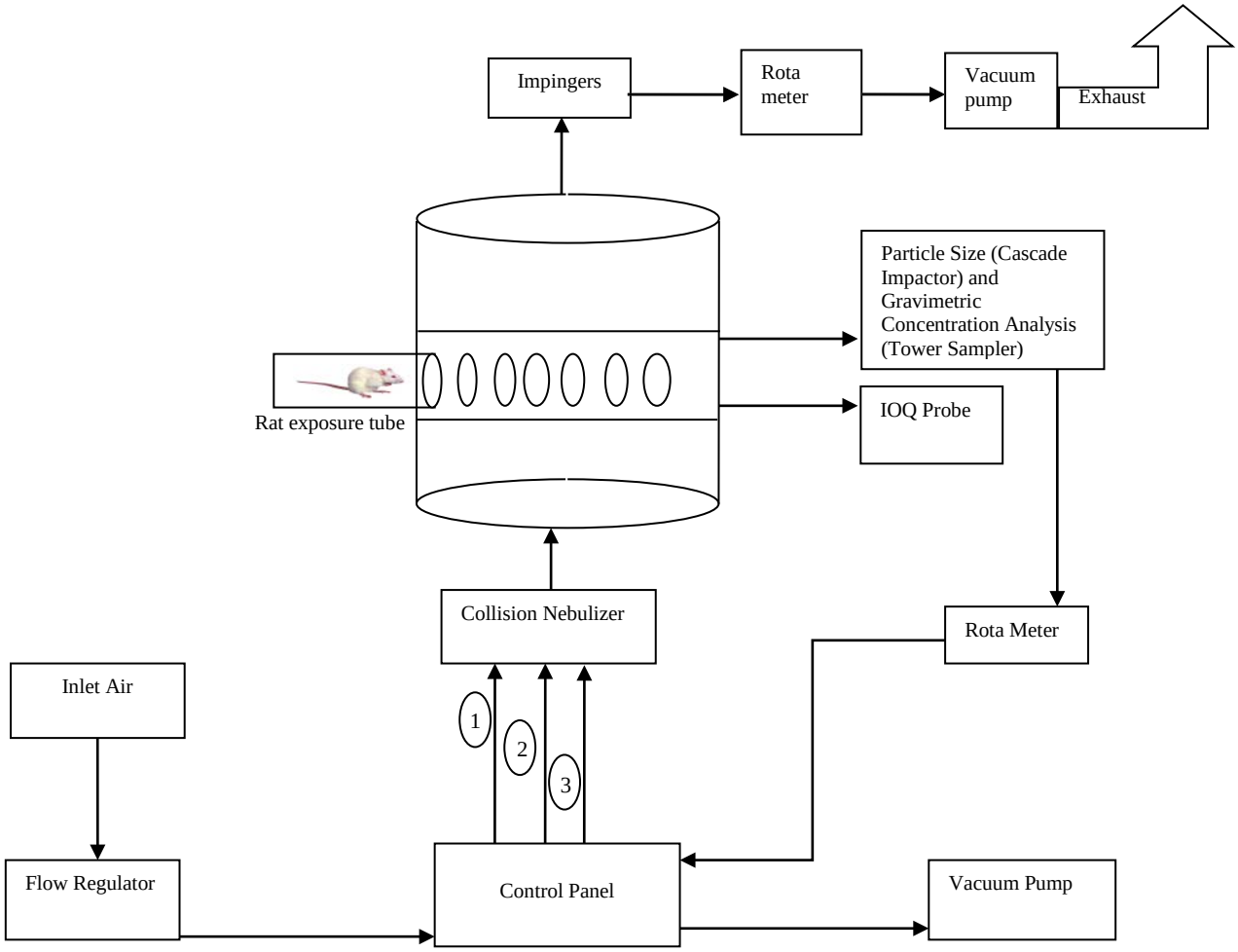
ANNEXURE 01: PARTICLE SIZE DISTRIBUTION CURVE



Mass Median Aerodynamic Diameter (MMAD) = 2.37 µm

$$\text{Geometric Standard Deviation} = \frac{\text{Size at 84.1 \% mass}}{\text{Size at 50\% mass}} = \frac{6.94}{2.37} = 2.93$$

ANNEXURE 02: SYSTEMIC LAYOUT OF LIQUID INHALATION SYSTEM



(1) And (2) = Dilution Air

(3) = Aerosol Generator Air

ANNEXURE 03: CERTIFICATE OF ANALYSIS

Certificate of Analysis

Product: GFS BioProtect - HG – AM (GFS BioProtect TM Hospital Grade Disinfectant – GFS BioProtect TM Anti Mould)

Batch No: 1005

Lot No: N/A

Date: 15/09/2014

Technical Results:

Appearance:	Clear, liquid
Odour:	Odourless
Boiling Point:	100 C
Solubility in water:	Miscible with water
Specific Gravity: (0.99 – 1.01 g/ml)	0.996 g/ml
pH: (3.00 – 5.00)	4.9
Flash point:	None
Flammability:	Non-flammable

Approved by: Tommy Yang B.S. Microbiology, M. Biotechnology

Please refer to the MSDS for additional product information

Test / ~~Reference~~ / ~~Other~~
Item Code : 56-0003

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Syngene International Ltd. Bangalore

ANNEXURE 04: CLASSIFICATION CRITERIA*

Table 3.1.1: Acute toxicity hazard categories and acute toxicity estimate (ATE) values defining the respective categories

Exposure route	Category 1	Category 2	Category 3	Category 4	Category 5
Oral (mg/kg bodyweight) *See notes (a) and (b)*	5	50	300	2000	5000 *See detailed criteria in Note (g)*
Dermal (mg/kg bodyweight) *See notes (a) and (b)*	50	200	1000	2000	
Gases (ppmV) *See notes (a), (b) and (c)*	100	500	2500	20000	*See detailed criteria in Note (g)*
Vapours (mg/l) *See notes (a), (b), (c), (d) and (e)*	0.5	2.0	10	20	
Dusts and Mists (mg/l) *See notes (a), (b), (c) and (f)*	0.05	0.5	1.0	5	

Note: *Gases concentration are expressed in parts per million per volume (ppmV).*

Notes to Table 3.1.1:

(a) *The acute toxicity estimate (ATE) for the classification of a substance is derived using the LD_{50}/LC_{50} where available;*

(b) *The acute toxicity estimate (ATE) for a substance in a mixture is derived using:*

(i) *the LD_{50}/LC_{50} where available; otherwise,*

(ii) *the appropriate conversion value from Table 3.1.2 that relates to the results of a range test; or*

(iii) *the appropriate conversion value from Table 3.1.2 that relates to a classification category;*

(c) *Inhalation cut-off values in the table are based on 4 hour testing exposures. Conversion of existing inhalation toxicity data which has been generated according to 1 hour exposures should be by dividing by a factor of 2 for gases and vapours and 4 for dusts and mists;*

ANNEXURE 04: CLASSIFICATION CRITERIA* (CONTINUED)

(d) It is recognized that saturated vapour concentration may be used as an additional element by some regulatory systems to provide for specific health and safety protection (e.g. UN Recommendations for the Transport of Dangerous Goods);

(e) For some substances the test atmosphere will not just be a vapour but will consist of a mixture of liquid and vapour phases. For other substances the test atmosphere may consist of a vapour which is near the gaseous phase. In these latter cases, classification should be based on ppmV as follows: Category 1 (100 ppmV), Category 2 (500 ppmV), Category 3 (2500 ppmV), Category 4 (20000 ppmV).

The terms "dust", "mist" and "vapour" are defined as follows:

(i) Dust: solid particles of a substance or mixture suspended in a gas (usually air);

(ii Mist: liquid droplets of a substance or mixture suspended in a gas (usually air);

(iii) Vapour: the gaseous form of a substance or mixture released from its liquid or solid state.

Dust is generally formed by mechanical processes. Mist is generally formed by condensation of supersatured vapours or by physical shearing of liquids. Dusts and mists generally have sizes ranging from less than 1 to about 100 µm;

(f) The values for dusts and mists should be reviewed to adapt to any future changes to OECD Test Guidelines with respect to technical limitation in generating, maintaining and measuring dust and mist concentrations in respirable form;

(g) Criteria for Category 5 are intended to enable the identification of substances which are of relatively low acute toxicity hazard but which under certain circumstances may present a danger to vulnerable populations. These substances are anticipated to have an oral or dermal LD_{50} in the range of 2000-5000 mg/kg bodyweight and equivalent doses for inhalation. The specific criteria for Category 5 are:

(i) The substance is classified in this category if reliable evidence is already available that indicates the LD_{50} (or LC_{50}) to be in the range of Category 5 values or other animal studies or toxic effects in humans indicate a concern for human health of an acute nature.

(ii) The substance is classified in this category, through extrapolation, estimation or measurement of data, if assignment to a more hazardous category is not warranted, and:

- *reliable information is available indicating significant toxic effects in humans; or*
- *any mortality is observed when tested up to Category 4 values by the oral, inhalation, or dermal routes; or*
- *where expert judgement confirms significant clinical signs of toxicity, when tested up to Category 4 values, except for diarrhoea, piloerection or an ungroomed appearance; or*
- *where expert judgement confirms reliable information indicating the potential for significant acute effects from other animal studies.*

Recognizing the need to protect animal welfare, testing in animals in Category 5 ranges is discouraged and should only be considered when there is a strong likelihood that results of such a test would have a direct relevance for protecting human health.

* Excerpt from UN GHS 2011

ANNEXURE 05: GLP CERTIFICATE

सत्यमेव जयते

GOVERNMENT OF INDIA

Department of Science and Technology

National Good Laboratory Practice (GLP) Compliance Monitoring Authority (NGCMA)

Certificate of GLP Compliance

Based on the Inspection and the subsequent follow-up actions

Syngene International Limited

Biocon Park, Plot No. 2 & 3, Bommasandra IV Phase,
Jigani Link Road, Bengaluru – 560099

is certified capable of conducting the below-mentioned tests/studies in compliance with Organization for Economic Co-operation & Development (OECD) Principles of GLP:

- **Physical – Chemical Studies**
- **Toxicity Studies**
- **Mutagenicity Studies**
- **Others: Bio analytical (TK analysis) for pre-clinical Studies**

The specific areas of expertise, types of chemicals and test systems are listed in annexure overleaf.

Validity: October 25, 2013 – October 24, 2016

This is subject to the test facility complying with the Terms and Conditions of the NGCMA's Document No. GLP-101 and OECD Principles of GLP.

Certificate No.: GLP/C-055/2013
Issue Date : 09-01-2014

(D R PRASADA RAJU)
Head, NGCMA

ANNEXURE 05: GLP CERTIFICATE (Continued)

National GLP Compliance Monitoring Authority

Annexure to Certificate of GLP Compliance No. GLP/C-055/2013

Areas of Expertise:

Physical – Chemical Studies

- Characterisation
- 5 Batch analysis
- Analytical Method Development & Validation
- Stability and Homogeneity Testing
- Dose Confirmation Analysis

Toxicity Studies

- Acute toxicity studies (Repeat dose 7, 14, 21, 28 & 42 day studies)
- Sub-chronic studies (Repeat dose 90 day studies)
- Chronic Toxicity (Repeat Dose 120 & 180 day studies)
- Local tolerance, Skin irritation, Guinea pig maximization studies
- Reproductive and Development Toxicity
- Neurotoxicity Studies

Mutagenicity Studies

- Bacterial Reverse Mutation Test (Ames Test)
- Micronucleus Test (In vivo)
- Chromosome aberration (In vitro)

Others

- Bio analytical (TK analysis) for pre-clinical studies

Types of Chemicals:

Industrial Chemicals, Pesticides, Pharmaceuticals, Veterinary drugs, Cosmetics, Food Additives and Feed Additives

Test Systems:

Rats, Mice, Rabbit, Guinea Pig, Bacterial tester strains for AMES test, CHO cell line, Human peripheral blood lymphocytes, Plasma and tissues.

भारत सरकार ★ Govt. of India
राष्ट्रीय जी एल पी अनुपालन निगरानी प्राधिकरण
National GLP Compliance Monitoring Authority
विज्ञान और प्रौद्योगिकी

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