Syngene

Study Report
(Original 1/2)

Study Number: U-15005

Study Title

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

OECD Test Guideline: 402

Study Completion Date: 29 Sep 2015

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 Dermal Toxicity Study of GFS HG-AM GFS BioProtect TM Hospital Grade Disinfectant- GFS BioProtect TM Anti Mould in Sprague Dawley Rats
Study Number	:	U-15005
Test Item Name	:	GFS BIOPROTECT -HG-GM
Name to be used in the study plan and report	:	GFS HG-AM GFS BioProtect TM Hospital Grade Disinfectant- GFS BioProtect TM Anti Mould
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 Solutions Ltd. 41 Magnesium Street Narangba, QLD, Australia-4504
Monitoring Scientist	:	Brian Rhoades

1.2 RESPONSIBILITIES

Study Director	:	Lakshmi Narayana M, M.Pharm Syngene International Limited Biocon Park, Plot No. 2 & 3 Bommasandra IV Phase, Jigani Link Road Bangalore – 560099, India Email: Lakshmi.Narayana@syngeneintl.com Tel: +9180 6633 4788
Study Personnel	:	Prabhakar Bhoite MVSc DABT Raghu N, MSc STG Ranganath, MSc Satynarayana CH, MPharm Parikshit R.D MVSc Zabiullah A.J MSc
Study Pathologist	:	Vishal M. Mahajan, MVSc E-mail: vishal.mahajan@syngeneintl.com Tel: +91 80 – 6633 4283

1.3 STUDY SCHEDULE

Study Initiation Date	:	22 May, 2015
Experiment Start	:	22 May, 2015
Acclimatization Start	:	22 May, 2015
Dosing	:	27 May, 2015
Experiment Completion	:	10 Jun, 2015
Draft Report to Sponsor	:	28 Jul, 2015
Study Completion Date	:	29 Sep, 2015

2.0 ABBREVIATIONS

%	:	Percent
±	:	Plus or minus
°C	:	Degree Celsius
CAS	:	Chemical Abstract Service
COA	:	Certificate of Analysis
g	:	gram
GHS	:	Globally Harmonized System of Classification and Labelling of Chemicals
GLP	:	Good Laboratory Practice
ISO	:	International Organisation for Standardization
L x B x H	:	Length x Breadth x Height
L	:	Liter
LD_{50}	:	Median Lethal Dose
mg /kg	:	milligram per kilogram
mL	:	millilitre
MSDS	:	Material Safety Data Sheet
OECD	:	Organization for Economic Co-operation and Development
QAU	:	Quality Assurance Unit
SOP	:	Standard Operating Procedure
TICO	:	Test Item Control Office
TRIDS	:	Test/Reference Item Data Sheet

3.0 GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

Study Number	:	U-15005
Test Item	:	GFS HG-AM GFS BioProtect TM Hospital Grade Disinfectant- GFS BioProtect TM Anti Mould
Study Title	:	Acute Dermal 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 OECD Guideline for testing of chemicals; No. 402; Acute Dermal Toxicity adopted on 24th February, 1987.

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 Decision-Recommendation of the Council on Compliance with Principles of Good Laboratory Practice [C (95)8 (FINAL)] and the Standard Operating Procedures prevailing during the period of the experiment. The study plan was signed by the Study Director on 22 May, 2015 and by Monitoring Scientist on 26 May, 2015. Study plan amendment no. 1 was signed by the Study Director on Jun 25, 2015 and by Monitoring Scientist on 29 Jun, 2015 and a study Plan deviation was filed on 21 Jul, 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 truly. The study director takes the entire responsibility for the conduct of study, documentation of raw data, interpretation of the results, preparation and finalization of the report.

Study Director:

Name: **LAKSHMI NARAYANA M**

Signature: _____

Date: 29/09/15

4.0 AFFIRMATION STATEMENT

Study Number : U-15005

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

Study Director : Lakshmi Narayana M,

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: 29 Sep 2015

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

Study Number : U-15005

Test Item : GFS HG-AM GFS BioProtect TM Hospital Grade Disinfectant- GFS BioProtect TM Anti Mould

Study Director : Lakshmi Narayana M

Details of Inspection:

S. No.	Inspection Phase	Date of Inspection		Date of Reporting To	
		From	To	Study Director	Management
1	Draft Study plan	14/05/2015	14/05/2015	14/05/2015	14/05/2015
2	Test item administration	27/05/2015	27/05/2015	27/05/2015	27/05/2015
3	Draft Study plan amendment No 1	24/06/2015	24/06/2015	24/06/2015	24/06/2015
4	Draft Study report	16/07/2015	16/07/2015	16/07/2015	16/07/2015
5	Final Study report	29/09/2015	29/09/2015	29/09/2015	29/09/2015

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.

4	Test item dispensing	15/06/2015	15/06/2015	18/06/2015	18/06/2015
5	Body weight	17/06/2015	17/06/2015	17/06/2015	17/06/2015
6	Acclimatization	01/06/2015	01/06/2015	01/06/2015	01/06/2015

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: **JAYDEEP BHOLE**

Signature: _____

Date: 29 Sep 2015

6.0 SUMMARY

The Study entitled "Acute Dermal Toxicity Study of GFS HG-AM GFS BioProtect TM Hospital Grade Disinfectant- GFS BioProtect TM Anti Mould in Sprague Dawley Rats" was conducted to categorise the test item according to fixed LD_{50} cut-off values as per Globally Harmonized System for Classification of Chemicals (GHS) and OECD Harmonized Integrated Classification System for Human Health and Environmental Hazards of Chemical Substances and Mixtures.

A limit test was carried out using 5 male and 5 female rats at a single dose level of 2000 mg/kg body weight. Approximately 24 hours before test item application, fur was removed from the dorso-lumbar region of the trunk of all test animals by clipping with an electric clipper. In order to expose approximately 10% of the body surface, the fur was clipped starting at the scapulae (shoulders) to the wing of the Ilium (hipbone) and half way down the flank on each side of the animal. Care was taken to avoid abrasions on the skin and only those animals without any signs of injury or irritation on the skin were used in the study.

On Day 1, the required quantity of test item was calculated based on application volume and body weight of each animal. Calculated quantity of test item for each animal was applied evenly on the intact skin with a syringe and covered with a gauze patch. This gauze patch was anchored with a non-irritating adhesive tape.

After 24-hours application period (from last time of application on the day of dosing), the dressing was removed and the skin was gently cleaned / wiped with purified water.

Animals were observed individually for clinical signs during acclimatization and at 30 minutes, 1, 2, 3 and 4 hours from the last time of application on the day of dosing and once daily thereafter for 14 days.

All animals were individually observed and recorded for local signs of skin reactions such as erythema and oedema at the exposure site one day after patch removal (Day 3) till the end of experimental period. Skin reactions were assessed following Grading of Skin Reactions (OECD Test Guideline 404).

Body weights were recorded individually for all animals on Day 1 (prior to application), on Day 8 and Day 15. All animals were sacrificed and examined for gross pathological abnormalities on Day 15.

No mortality or morbidity was observed throughout the experimental period. All the animals were observed to be normal throughout the experimental period.

No erythema or oedema was observed at the application site in any animals throughout the study. All the animals gained body weights by Day 15 when compared with Day 1and Day 8.

Gross pathological examination did not reveal any abnormalities in any of the animals at terminal sacrifice.

Based on the results obtained, LD_{50} of **GFS HG-AM GFS BioProtect TM Hospital Grade Disinfectant- GFS BioProtect TM Anti Mould** is **Greater than 2000 mg/kg body weight** after single dermal exposure to Sprague Dawley Rats. Hence, **GFS HG-AM GFS BioProtect TM Hospital Grade Disinfectant- GFS BioProtect TM Anti Mould** is classified under **Category 5** as per Globally Harmonized System for Classification of Chemicals (UN GHS, 2013) and OECD Harmonized Integrated Classification System for Human Health and Environment Hazards of Chemical Substances and Mixtures (14th Aug, 2001).

7.0 OBJECTIVES

a) To assess the acute toxicity dose range of GFS HG-AM GFS BioProtect TM Hospital Grade Disinfectant- GFS BioProtect TM Anti Mould where lethality is expected following a single dermal application to rats.

b) To categorize the test item according to fixed LD_{50} cut-off values as per Globally Harmonized System of Classification and Labeling of Chemicals (GHS) and OECD Harmonized Integrated Classification System for Human Health and Environmental Hazards of Chemical Substances and Mixtures.

8.0 PRINCIPLE

The test item is applied to the intact skin in graduated doses to various groups of experimental animals, to observe the effects obtained at each dose level per group by single dermal exposure.

9.0 ANIMAL WELFARE

All animals were handled humanely with due regard for their welfare. Care of animals 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/496/06-2014).

10.0 SAFETY PRECAUTIONS

The test item was handled with all recommended personal protective equipment and safety measures as per the relevant SOP, MSDS and TRIDS. Personal protective equipment such as lab coat, mask, cap, gloves and shoes were used, wherever applicable to ensure adequate personal health and safety.

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
Density/Specific gravity	:	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^{0}C)

The test item details and identity of GFS HG-AM GFS BioProtect TM Hospital Grade Disinfectant- GFS BioProtect TM Anti Mould provided are based on the information provided by study sponsor in TRIDS, CoA and MSDS. Sponsor is responsible for purity and 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	:	Males and Females (Females used were nulliparous and non-pregnant)
Source	:	Vivo biotech, Hyderabad
Age at treatment	:	Males: 8-9 Weeks Females: 10-11 Weeks
Number of animals for limit test	:	10 (5 Males + 5 Females)
Extra animals for random selection	:	2 (1 Male and 1 Female)
Body Weight of animals	:	At start of treatment: Males: 248.20 – 274.48g Females: 222.76g – 229.78g
Justification	:	Rat is the most preferred rodent species by the OECD test guidelines.

11.3 ANIMAL HUSBANDRY

11.3.1 ENVIRONMENTAL CONDITIONS

Animals were maintained in a controlled environment with temperature range of 19.8°C to 24.4°C (acceptable range 22 ± 3°C), relative humidity in between 45 to 66% (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 20 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 experimental room was decontaminated and microbial load was checked. The results were found to be within acceptable limits and copies were filed in the raw data. The experimental room floor was cleaned every day.

11.3.3 HOUSING

Animals were housed individually in clean sterilized polycarbonate cages (with stainless steel cage grills) of dimensions approximately 41.0 x 26.5 x 20.0 cm (L x B x H). Enrichment was provided in each cage. Autoclaved corncob was used as the bedding and was changed along with the cage twice a week. Bedding material is analyzed for chemical contaminants annually in an ISO certified laboratory. Each batch of the bedding material is analyzed 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
Bedding material	Sparconn Life Sciences	SPAR 28/2015	January 2015	December 2015

11.3.4 DIET

The animals were fed, *ad libitum*, with Irradiated Laboratory Rodent Diet. 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 global 18% protein rodent diet	Harlan	2918-032315M	23 Mar 2015	18 Dec 2015

11.3.5 WATER

Potable water, filtered through Reverse Osmosis, was provided *ad libitum* to all animals after autoclaving, *via* polycarbonate bottles fitted with stainless steel nozzles. Water analysis for microbiological load and chemical contaminants is conducted in-house once every month. Chemical and microbiological 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 and relevant photocopies were kept in the raw data.

11.4 PREPARATION OF ANIMALS

11.4.1 ACCLIMATIZATION AND SELECTION

The animals were acclimatized in the experimental room (SB 24) for a period of five days. Animals were selected and grouped manually for limit test. Animals selected for dosing were examined for clinical signs before treatment.

11.4.2 ANIMAL IDENTIFICATION

On receipt, animals were assigned a temporary number (Animal No.: 1-6 for males and 7-12 for females) using a black color indelible marker pen towards the tip of the tail. Immediately after selection, the animals were assigned a unique permanent number from Ra8664 to Ra8668 for males and Ra8669 to Ra8673 for females by tattooing towards the base of the tail using a tattoo machine without any prefix (8664 to 8673). Cage cards indicating the Study Number, Animal Number, Group Number and other relevant details were displayed on the corresponding cages. Animals not selected (Animal No. 6 & 10) for the limit test were sacrificed by Carbon dioxide asphyxiation.

11.5 LIST OF MATERIALS AND INSTRUMENTS USED

Name of Material	Make	Batch Number	Date of Manufacture	Date of Expiry
1 mL Syringe	BD	0294090	11-2010	10-2015
Absorbent Gauze (5cm x 10m)	M/s. Gomathi Shankar Surgicals	197	Feb 2015	Feb 2018
Micropore Surgical Tape	3M India Ltd	R12120512	Dec 2012	Nov 2017

Name of Instrument	Make	In-House ID
Weighing Balance	Sartorius	SLAR-LI-157
Weighing Balance	Sartorius	SLAR-LI-177
Weighing Balance	Sartorius	SLAR-LI-236
Standard Weight	Cabfit	SLAR-LI-078
Thermo hygrometer	HTC	SLAR-AHU-09/28
Thermo hygrometer	Mextech TM1	SLAR-AHU-04/065
Tattooing Machine	AIMS	SLAR-LI-066
Electrical Clipper	Oster	SLAR-LI-182
Water Purification system	Millipore Elix	SLAR-LI-264

11.6 TEST ITEM PREPARATION

The test item was used as supplied by the sponsor. The specific gravity of test item was measured before study initiation and average (*n=3*) value was found to be 996.67 mg/mL.

11.7 ROUTE, VOLUME AND JUSTIFICATION OF ADMINISTRATION

Topical route of application was employed. The application volume calculated was 2.01 mL/kg. Topical exposure is one of the potential route of human exposure during manufacture, handling and use of the test item.

11.8 TREATMENT

A limit test was carried out using 5 male and 5 female rats at single dose level of 2000 mg/kg body weight based on the information provided by the Study Sponsor in MSDS. Approximately 24 hours before the test item application, fur was removed from the dorso-lumbar region of the trunk of all test animals by clipping with an electric clipper. In order to expose approximately 10% of the body surface, the fur starting at the scapulae (shoulders) to the wing of the Ilium (hipbone) and half way down the flank on each side of the animal were clipped. Care was taken to avoid abrasions on the skin and only those animals without any signs of injury or irritation on the skin were used in the study.

On Day 1, the required quantity of test item was calculated based on the body weight of each individual animal and dose level. The required amount of test item was transferred from TICO in a labeled beaker. Calculated quantity of test item for each animal was applied evenly on the intact skin with a syringe and covered with a gauze patch. This gauze patch was anchored with non-irritating adhesive tape. The area of skin covered by the gauze patch was about 5 cm x 5 cm. After 24-hours application period (from last time of application on the day of dosing), the dressing was removed and the skin was gently cleaned with purified water (Millipore Elix®).The skin reactions were assessed from one day after patch removal (Day 3) till the end of experimental period (Day 15).

11.9 DURATION OF STUDY

All animals were dosed once and observed for fourteen days.

12.0 OBSERVATIONS

12.1 MORBIDITY & MORTALITY

All animals were checked twice daily for morbidity and mortality and once daily on weekends (since the animals were observed to be normal) and at terminal sacrifice.

12.2 CLINICAL SIGNS

Animals were observed individually for visible clinical signs during acclimatization, pre-dose, 30 minutes, 1, 2, 3 and 4 hours from last time of application on the day of dosing and daily once thereafter for 14 days.

12.3 SKIN REACTIONS

All animals were individually observed and recorded for local signs of skin reactions such as erythema, oedema or any other reaction at the exposure site one day after patch removal (Day 3) till the end of experiment period (Day 15). Skin reactions were assessed following Grading of Skin Reactions (OECD Test Guideline 404) presented in Annexure 1.

12.4 BODY WEIGHT

The body weights were recorded individually for all animals at receipt, prior to application, at weekly interval (on Day 8) and at terminal sacrifice (on Day 15). Body weights recorded prior to application, at weekly interval (on Day 8) and at terminal sacrifice were reported. Body weight changes (Day 1-8 and Day 1-15) were calculated.

13.0 PATHOLOGY

13.1 NECROPSY

At the end of the observation period, all the animals were sacrificed using Carbon dioxide asphyxiation and subjected to gross necropsy.

14.0 RESULTS

14.1 MORBIDITY & MORTALITY

Refer Table 1

No mortality or morbidity was observed throughout the experimental period in any of the treated animals.

14.2 CLINICAL SIGNS

Animals were observed to be normal throughout the acclimatization period.

All the animals were observed to be normal pre and post dosing (Refer Table 1).

14.3 SKIN REACTIONS

Refer Table 2

No erythema or oedema was observed in any of the treated male and female animals throughout the experimental period.

14.4 BODY WEIGHT

Refer Table 3, and Table 4.

All animal gained body weights by Day 15 when compared with Day 1 and Day 8.

14.5 NECROPSY

Refer Table 5

Gross pathological examination did not reveal any abnormalities in any of the animals at terminal sacrifice.

15.0 CONCLUSION

Based on the results obtained, LD_{50} of **GFS HG-AM GFS BioProtect TM Hospital Grade Disinfectant- GFS BioProtect TM Anti Mould** is **Greater than 2000 mg/kg body weight** after single dermal exposure to Sprague Dawley Rats. Hence, **GFS HG-AM GFS BioProtect TM Hospital Grade Disinfectant- GFS BioProtect TM Anti Mould** is classified under **Category 5** as per Globally Harmonized System for Classification of Chemicals (UN GHS, 2013) and OECD Harmonized Integrated Classification System for Human Health and Environment Hazards of Chemical Substances and Mixtures (14th Aug, 2001).

16.0 ARCHIVING

On completion of the study, all raw data, the study plan, QA reviewed draft report and a final report (Original 2/2) 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 return the archived material to the Sponsor or for discarding the material.

17.0 REPORT DISTRIBUTION

Two originals are distributed as follows:
Original 1/2 – Sponsor
Original 2/2 – Archives of the test facility

18.0 REFERENCES

1. 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).
2. OECD Guidelines for the Testing of Chemicals, No.402, Acute Dermal Toxicity, Adopted on 24th February, 1987.
3. OECD Guidelines for the Testing of Chemicals, No.404, Acute Dermal Irritation/Corrosion, Adopted on 24thApril, 2002.
4. OECD Series on Testing and Assessment, Number 33 – Harmonized Integrated Classification System for Human Health and Environment Hazards of Chemical Substances and Mixtures. ENV/JM/MONO (2001)6, 14thAugust 2001.
5. Globally Harmonized System of Classification and Labeling of Chemicals (GHS) Fourth Revised Edition, United Nations (2013). ST/SG/AC.10/30/Rev.5.

19.0 TABLES

TABLE 1. INDIVIDUAL ANIMAL CLINICAL SIGNS AND MORTALITY

Animal Number	Dose*	Sex	Day																		
			1 (hours post dosing)						2	3	4	5	6	7	8	9	10	11	12	13	14	15
			#	0.5	1	2	3	4														
Ra8664	2000	M	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Ra8665		M	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Ra8666		M	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Ra8667		M	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Ra8668		M	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Ra8669		F	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Ra8670		F	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Ra8671		F	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Ra8672		F	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Ra8673		F	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

Key: h – hours, M – Male, F – Female, 1 Normal, *– mg/kg body weight, # – Pre dose

TABLE 2. GRADING SKIN REACTIONS BY DRAIZE SCALE

Animal Number	Dose*/ group	Sex	Day																									
			3		4		5		6		7		8		9		10		11		12		13		14		15	
			E	O	E	O	E	O	E	O	E	O	E	O	E	O	E	O	E	O	E	O	E	O	E	O	E	O
Ra8664	2000 (G1)	M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ra8665		M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ra8666		M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ra8667		M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ra8668		M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ra8669		F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ra8670		F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ra8671		F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ra8672		F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ra8673		F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Key: h – hours, M – Male, F – Female, *– mg/kg body weight
Refer Annexure 01 for grading of skin reactions.

TABLE 3. INDIVIDUAL ANIMAL BODY WEIGHTS

Group Number	Dose	Animal Numbers	Sex	Day 1 (g)	Day 8 (g)	Day 15 (g)
G1	2000 mg/kg	Ra8664	Male	274.48	292.66	306.18
		Ra8665		263.17	279.45	296.64
		Ra8666		248.20	265.16	281.48
		Ra8667		258.73	272.38	291.95
		Ra8668		263.28	281.48	300.01
Mean				**261.57**	**278.23**	**295.25**
SD				**9.47**	**10.31**	**9.28**
G1	2000 mg/kg	Ra8669	Female	226.35	237.18	251.15
		Ra8670		229.78	241.93	260.18
		Ra8671		222.76	235.65	245.18
		Ra8672		226.45	240.78	252.36
		Ra8673		228.92	243.55	258.18
Mean				**226.85**	**239.82**	**253.41**
SD				**2.74**	**3.30**	**5.97**

Key: mg/kg – Milligram per Kilogram, g – grams, SD – Standard Deviation

TABLE 4. INDIVIDUAL ANIMAL BODY WEIGHT CHANGES

Group Number	Dose	Animal Numbers	Sex	Day 1 - 8 (g)	Day 1 - 15 (g)
G1	2000 mg/kg	Ra8664	Male	18.18	31.70
		Ra8665		16.28	33.47
		Ra8666		16.96	33.28
		Ra8667		13.65	33.22
		Ra8668		18.20	36.73
Mean				**16.65**	**33.68**
SD				**1.87**	**1.85**
G1	2000 mg/kg	Ra8669	Female	10.83	24.80
		Ra8670		12.15	30.40
		Ra8671		12.89	22.42
		Ra8672		14.33	25.91
		Ra8673		14.63	29.26
Mean				**12.97**	**26.56**
SD				**1.57**	**3.27**

Key: mg/kg – Milligram per Kilogram, g – grams, SD – Standard Deviation

TABLE 5. INDIVIDUAL ANIMAL NECROPSY FINDINGS

Animal Numbers	Sex	Dose (mg/kg body weight)	Mode of death	Macroscopic findings	
				External	Internal
Ra8664	Male	2000	TS	NAD	NAD
Ra8665	Male		TS	NAD	NAD
Ra8666	Male		TS	NAD	NAD
Ra8667	Male		TS	NAD	NAD
Ra8668	Male		TS	NAD	NAD
Ra8669	Female		TS	NAD	NAD
Ra8670	Female		TS	NAD	NAD
Ra8671	Female		TS	NAD	NAD
Ra8672	Female		TS	NAD	NAD
Ra8673	Female		TS	NAD	NAD

Key: mg/kg – Milligram per Kilogram, TS – Terminal Sacrifice, NAD – No Abnormalities Detected

20.0 APPENDIX

APPENDIX 1. STUDY PLAN DEVIATION

Deviation No.	As in Study Plan	Deviation from Study Plan	Impact of the deviation on study
1.	**Page No. 1 of 2** **Section 1.3 PROPOSED STUDY SCHEDULE** Experiment completion date May 2015	Experiment completion date June, 2015	There is no impact on the study and results

Study Director:

Name : **LASKHMI NARAYANA M**

Signature : _____

Date : 29/09/15

21.0 ANNEXURES

ANNEXURE 1. GRADING OF SKIN REACTIONS

Grading of Skin Reactions

Erythema and Eschar Formation

No erythema 0

Very slight erythema (barely perceptible) 1

Well defined erythema 2

Moderate to severe erythema 3

Severe erythema (beef/beet redness) to slight eschar formation (injuries in depth)

Preventing grading of erythema 4

Maximum possible: 4

Oedema Formation

No oedema 0

Very slight oedema (barely perceptible) 1

Slight oedema (edges of area well defined by definite raising) 2

Moderate oedema (raised approximately 1 mm) 3

Severe oedema (raised more than 1 mm and extending beyond area of exposure) 4

Maximum possible: 4

ANNEXURE 2. GLOBALLY HARMONIZED SYSTEM OF CLASSIFICATION AND LABELING OF CHEMICALS

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 2 contd.GLOBALLY HARMONIZED SYSTEM OF CLASSIFICATION AND LABELING OF CHEMICALS

(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.

ANNEXURE 3. 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 : SG-0003

GFS AUSTRALASIA
ABN: 69 160 581 760

+617 3203 1200 +617 3399 2497 globalfuturesolutions.com
U1/41 Magnesium St, Narangba, QLD 4504 Australia admin@gfscorporation.net

ANNEXURE 4. 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 4 contd. GLP CERTIFICATE

National GLP Compliance Monitoring Authority

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

Areas of Expertise:

	Physical – Chemical Studies
o	Characterisation
o	5 Batch analysis
o	Analytical Method Development & Validation
o	Stability and Homogeneity Testing
o	Dose Confirmation Analysis
	Toxicity Studies
o	Acute toxicity studies (Repeat dose 7, 14, 21, 28 & 42 day studies)
o	Sub-chronic studies (Repeat dose 90 day studies)
o	Chronic Toxicity (Repeat Dose 120 & 180 day studies)
o	Local tolerance, Skin irritation, Guinea pig maximization studies
o	Reproductive and Development Toxicity
o	Neurotoxicity Studies
	Mutagenicity Studies
o	Bacterial Reverse Mutation Test (Ames Test)
o	Micronucleus Test (In vivo)
o	Chromosome aberration (In vitro)
	Others
o	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.

(D R PRASADA RAJU)
Head, NGCMA