Syngene

# Study Report

## (Original 1/2)

## Study Number: U-15004

### Study Title

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

**OECD Test Guideline: 423**

**Study Completion Date: 21 September 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-560 099, India**

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# 1.0 STUDY DETAILS

## 1.1 GENERAL

| | | |
|---|---|---|
| **Study Title** | : | Acute Oral Toxicity Study of GFS HG-AM GFS BioProtect TM Hospital Grade Disinfectant - GFS BioProtect TM Anti Mould in Sprague Dawley Rats |
| **Study Number** | : | U-15004 |
| **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<br>Biocon Park, Plot No. 2 & 3<br>Bommasandra IV Phase<br>Jigani Link Road<br>Bangalore – 560099, India |
| **Study Sponsor** | : | GFS Australasia Pty Ltd.<br>41 Magnesium Street<br>Narangba, QLD, Australia-4504 |
| **Monitoring Scientist** | : | Brian Rhoades |

## 1.2 RESPONSIBILITIES

Study Director : Lakshmi Narayana M, M.Pharm
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## 1.3 STUDY SCHEDULE

| | | |
|---|---|---|
| Study Initiation Date | : | May 22, 2015 |
| Experiment Start Date | : | May 22, 2015 |
| Acclimatization Start Date | : | May 22, 2015 |
| Treatment (Step I) | : | May 27, 2015 |
| Treatment (Step II) | : | May 29, 2015 |
| Treatment (Step III) | : | June 02, 2015 |
| Treatment (Step IV) | : | June 04, 2015 |
| Experiment Completion Date | : | June 18, 2015 |
| Draft Report to Sponsor | : | July 28, 2015 |
| Study Completion Date | : | September 21, 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 |
| QA | : | Quality Assurance |
| QAU | : | Quality Assurance Unit |
| SOP | : | Standard Operating Procedure |
| TICO | : | Test Item Control Office |
| TRIDS | : | Test/Reference Item Data Sheet |
| UN | : | United Nation |

## 3.0 GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

| | | |
|---|---|---|
| Study Number | : | U-15004 |
| Test Item | : | GFS HG-AM GFS BioProtect TM Hospital Grade Disinfectant- GFS BioProtect TM Anti Mould |
| Study Title | : | Acute Oral 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. 423; Acute oral toxicity – Acute toxic class method; adopted on 17th December, 2001.

This 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 as per test facility standard operating procedures prevailing during the experiment. The study plan was signed by the Study Director on May 22, 2015 and Study Monitor on May 26, 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 of conduct of the study, documentation of raw data, interpretation of the results, preparation and finalization of the report.

**Study Director:**

Name: **Lakshmi Narayana M, M.Pharm**

Signature: _____

Date: 21 Sep 2015

## 4.0 AFFIRMATION STATEMENT

Study Number : U-15004

Study Title : Acute Oral 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, M.Pharm

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

Study Number : U-15004

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

Study Director : Lakshmi Narayana M, M.Pharm

Details of Inspection :

| S. No. | Inspection Phase | Date of | | | |
|---|---|---|---|---|---|
| | | Inspection | | 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 | 29/05/2015 | 29/05/2015 | 29/05/2015 | 29/05/2015 |
| 3 | Draft Study Report | 16/07/2015 | 16/07/2015 | 16/07/2015 | 16/07/2015 |
| 4 | Final Study Report | 21/09/2015 | 21/09/2015 | 21/09/2015 | 21/09/2015 |

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.

| | | | | | |
|---|---|---|---|---|---|
| 5. | Animal Body Weighing | 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: **ANANTA KUMAR M**

Signature: _____

Date: 21 Sep 2015

## 6.0 SUMMARY

The study titled "Acute Oral Toxicity Study of GFS HG-AM GFS BioProtect TM Hospital Grade Disinfectant- GFS BioProtect TM Anti Mould in Sprague Dawley Rats" was conducted to determine and categorise the test item according to median lethal dose ($LD_{50}$) 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.

The test item was formulated in purified water at a concentration of 30 mg/mL (Step I and Step II) and 200 mg/mL (Step III and Step IV) and administered as a single dose at the dose volume of 10 mL/kg

Three female rats were administered at the dose level of 300 mg/kg body weight (Step I) as single oral gavage. No mortality was observed in this Step. To confirm the findings, further three female rats (Step II) were dosed at the same dose level of 300 mg/kg as no mortality was observed in the second Step. Step III animals were dosed at next higher dose 2000 mg/kg all the animals survived, again three animals were dosed at the same dose level (Step IV). Since mortality was not observed, the study was concluded. All survival animals were observed for 14 days following single dose administration.

All the animals gained body weights by Day 8 and Day 15 when compared to Day 1.

Gross pathological examination terminal sacrificed animals did not reveal any abnormalities.

Gross pathological examination of all the surviving animals at terminal sacrifice did not revealed any external and internal gross pathological changes.

Based on the results, the median lethal dose of **GFS HG-AM GFS BioProtect TM Hospital Grade Disinfectant- GFS BioProtect TM Anti Mould** after single oral administration to female rats is greater than **2000 mg/kg body weight**. Hence, **GFS HG-AM GFS BioProtect TM Hospital Grade Disinfectant- GFS BioProtect TM Anti Mould** is classified under **Category 5 or Unclassfied** 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 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.

## 8.0 PRINCIPLE

The test item is administered orally to a group of experimental animals at one of the defined doses. The test item is tested using a stepwise procedure, each step using three animals of single sex (females). Absence or presence of compound-related mortality of the animals dosed at one step will determine the next step, i.e.

- no further testing is needed
- dosing of three additional animals, with the same dose
- dosing of three additional animals at the next higher or the next lower dose level

Based on a stepwise procedure with the use of a minimum number of animals per step, sufficient information is obtained on the acute toxicity of the test item to enable its classification.

## 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/495/06-2014).

## 10.0 OBJECTIVE

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

b) To classify 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.

# 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.<br>41 Magnesium Street<br>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 and identity of GFS HG-AM GFS BioProtect TM Hospital Grade Disinfectant- GFS BioProtect TM Anti Mould provided here were based on the information provided by study Sponsor in TIDS, 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 | : | Female (Nulliparous and Non-Pregnant) |
| Source | : | Vivo Biotech, Hyderabad |
| Age at treatment | : | 10-12 Weeks |

| | | |
|---|---|---|
| Number of animals | : | 12 |
| Number of animals per step | : | 3 |
| Body Weight of animals | : | Treatment Start: 211.15 g to 241.45 g |
| 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 sterilized cage grills) of dimensions approximately 41.0 x 26.5 x 20.0 cm (L x B x H). 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 screened in-house for the microbial load. Animals were placed over sterilized stainless steel grills on the day of fasting and the grills were removed during feed restoration. 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 | Jan 2015 | Dec 2015 |

| | | | | |
|---|---|---|---|---|
| Bedding material | Sparconn Life Sciences | SPAR 29/2015 | Apr 2015 | Mar 2015 |

### 11.3.4 DIET

The animals were fed, *ad libitum*, with Irradiated Laboratory Rodent Diet (Teklad global 18% protein rodent diet). On receipt of each batch, a sample was screened in-house for microbial load. Chemical contaminants 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 also 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, reports of tests and records pertaining to the study 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 kept under acclimatization in experimental room SB 24 for a period of 5 days (Step I), 7 days (Step II), 12 days (Step III), and 13 days (Step IV). Animals were selected and grouped manually. Animals selected for dosing were examined for clinical signs before treatment. At the time of subsequent dosing (Step II), the weight variation of the animals was ensured to be within range of ± 20% of the mean body weight of previously dosed animals.

### 11.4.2 ANIMAL IDENTIFICATION

On receipt into the experimental room, animals were assigned a temporary number (1-12) towards the tip of tail using a red indelible marker pen. Immediately after selection, the animals were assigned a permanent number (Ra8652 to Ra8663) and was tattooed

towards the base of the tail (8652 to 8663). Cage cards indicating the Study Number, Animal Number, Step Number and other relevant details were displayed on the corresponding cages.

## 11.5 LIST OF MATERIALS AND INSTRUMENTS USED

| Name of Material | Make | Batch Number | Date of Manufacture | Date of Expiry |
|---|---|---|---|---|
| Syringes (3 mL) | Dispovan | 18036SK1 | APR 2011 | MAR 2016 |

| 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 |
| Thermo hygrometer | HTC | SLAR-AHU-09/28 |
| Thermo hygrometer | Mextech TM1 | SLAR-AHU-04/065 |
| Standard Weight | Cabfit | SLAR-LI-078 |
| Tattoo Machine | AIMS | SLAR-LI-182 |
| Magnetic Stirrer | Shalom instruments | SLAR-LI-214 |
| Magnetic Stirrer | Shalom instruments | SLAR-LI-215 |
| Fume hood | Kewaunee Scientific corporation | SLAR-LI-029 |
| Water Purification System | Millipore Elix | SLAR-LI-264 |

## 11.6 VEHICLE

Purified Water was selected as vehicle based on preliminary solubility test which was performed at the test facility before study initiation.

## 11.7 JUSTIFICATION FOR ROUTE OF ADMINISTRATION

The oral route of administration was used as it is recommended in the guideline and it is a potential route of human exposure during manufacture, handling and use of the test item.

## 11.8 DOSE FORMULATION PREPARATION

The formulations was prepared with the test item as supplied by the Sponsor. The test item formulations was prepared shortly before dosing. Required quantity i.e., 0.30080g and 0.30025g (Step I and Step II) and 2.00077g and 2.00060g (Step III and Step IV) of test item was transferred in a labeled beaker from TICO. A small quantity of the vehicle

was added to the beaker, mixed well and transferred to a volumetric flask (10.0 mL). The beaker was rinsed with a small quantity of vehicle and transferred to the volumetric flask. The total volume required was made up in the volumetric flask by adding sufficient quantity of vehicle. The formulation was mixed well and was transferred into the labeled beaker.

The amount of the test item, volume of the formulation prepared was varied based on the dose and volume of administration depending on the body weights of the animals.

## 11.9 TREATMENT

Three female animals were used at each step. The starting dose selected was 300 mg/kg body weight, based on information provided by Study Sponsor in MSDS about the test item. The test procedure followed the scheme as described in Annexure 1 (Test Procedure with a starting dose of 300 mg/kg bodyweight based on Annex 2c, OECD Guideline 423, adopted on 17th December 2001). Three female rats were administered at the dose level of 300 mg/kg body weight (Step I) as single oral gavage. No mortality was observed in this Step. To confirm the findings, further three female rats (Step II) were dosed at the same dose level of 300 mg/kg as no mortality was observed in the second Step. Step III animals were dosed at next higher dose 2000 mg/kg all the animals survived, again three animals were dosed at the same dose level (Step IV). Since mortality was not observed, the study was concluded.

| STEP | NUMBER OF ANIMALS PER STEP (Females) | ANIMAL NUMBERS | | |
|---|---|---|---|---|
| Step I | 3 | Ra8652 | - | Ra8654 |
| Step II | 3 | Ra8655 | - | Ra8657 |
| Step III | 3 | Ra8658 | - | Ra8660 |
| Step IV | 3 | Ra8661 | - | Ra8663 |

## 11.10 DURATION OF STUDY

All the animals were dosed by oral gavage once and surviving animals were observed for fourteen days.

# 12.0 OBSERVATIONS

## 12.1 MORBIDITY & MORTALITY

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

## 12.2 CLINICAL SIGNS

Animals were observed individually for visible clinical signs during acclimatization, at 30 minutes, 1, 2, 3 and 4 hours (±5 minutes) after test item administration and once daily thereafter for 14 days.

## 12.3 BODY WEIGHT

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

# 13.0 PATHOLOGY

## 13.1 NECROPSY

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

# 14.0 RESULTS

## 14.1 MORBIDITY & MORTALITY

No mortality or morbidity was observed throughout the experimental period in any of the treated animals (Refer Table 1).

## 14.2 CLINICAL SIGNS

All the animals during acclimatization were found to be normal

**Step I and Step II (300 mg/kg)**

All the treated animals of Step I and Step II were observed to be normal till the end of the experimental period (Refer Table 1).

**Step III and Step IV (2000 mg/kg)**

All the treated animals of Step III and Step IV were observed to be normal till the end of the experimental period (Refer Table 1).

## 14.3 BODY WEIGHT

Refer Table 2 and Table 3

All the animals gained body weights by Day 15 when compared to Day 1 and Day 8.

## 14.4 NECROPSY

Refer Table 4

Gross pathological examination of all the animals at terminal sacrifice (Day 15) did not reveal any external and internal gross pathological changes.

## 15.0 CONCLUSION

Based on the results, the median lethal dose of **GFS HG-AM GFS BioProtect TM Hospital Grade Disinfectant- GFS BioProtect TM Anti Mould** after single oral administration to female rats is greater than **2000-5000 mg/kg body weight**. Hence, **GFS BIOPROTECT -HG-GM** is classified under **Category 5** or **unclassified** 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 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 were 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.423, Acute Oral Toxicity – Acute Toxic Class Method, Adopted on 17th December, 2001.
3. 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, 14 August 2001.

4. Globally Harmonized System of Classification and Labeling of Chemicals (GHS) Fifth Revised Edition, United Nations (2013). ST/SG/AC.10/30/Rev.5.

# 19.0 TABLES

## TABLE 1. INDIVIDUAL ANIMAL CLINICAL SIGNS AND MORTALITY

| Step Number | Dose (mg/kg) | Animal Numbers | Sex | Day | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 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 | | | | | | | | | | | | | | |
| Step I | 300 | Ra8652 | F | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | | Ra8653 | F | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | | Ra8654 | F | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Step II | 300 | Ra8655 | F | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | | Ra8656 | F | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | | Ra8657 | F | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Step III | 2000 | Ra8658 | F | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | | Ra8659 | F. | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | | Ra8660 | F | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Step IV | 2000 | Ra8661 | F | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | | Ra8662 | F | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | | Ra8663 | F | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |

**Key:** mg/kg – milligram per kilogram; F – Female; # – Pre dose, 1– Normal

## TABLE 2. INDIVIDUAL ANIMAL BODY WEIGHTS

| Step Number | Dose | Animal Numbers | Sex | Day 1 (g) | Day 8 (g) | Day 15 (g) |
|---|---|---|---|---|---|---|
| Step I | 300 mg/kg | Ra8652 | Female | 239.60 | 256.48 | 271.25 |
| | | Ra8653 | | 230.63 | 247.75 | 269.66 |
| | | Ra8654 | | 230.65 | 245.91 | 264.35 |
| **Mean** | | | | **233.63** | **250.05** | **268.42** |
| **SD** | | | | **5.17** | **5.65** | **3.61** |
| Step II | 300 mg/kg | Ra8655 | Female | 241.45 | 260.16 | 282.81 |
| | | Ra8656 | | 231.65 | 252.60 | 279.33 |
| | | Ra8657 | | 226.78 | 249.71 | 268.41 |
| **Mean** | | | | **233.29** | **254.16** | **276.85** |
| **SD** | | | | **7.47** | **5.40** | **7.51** |
| Step III | 2000 mg/kg | Ra8658 | Female | 240.71 | 261.21 | 286.18 |
| | | Ra8659 | | 237.12 | 258.35 | 274.92 |
| | | Ra8660 | | 239.69 | 259.88 | 278.19 |
| **Mean** | | | | **239.17** | **259.81** | **279.76** |
| **SD** | | | | **1.85** | **1.43** | **5.79** |
| Step IV | 2000 mg/kg | Ra8661 | Female | 211.15 | 236.28 | 261.28 |
| | | Ra8662 | | 221.28 | 247.68 | 269.74 |
| | | Ra8663 | | 226.69 | 252.18 | 278.92 |
| **Mean** | | | | **219.71** | **245.38** | **269.98** |
| **SD** | | | | **7.89** | **8.20** | **8.82** |

**Key:** mg/kg – Milligram per kilogram, g – grams

## TABLE 3. INDIVIDUAL ANIMAL BODY WEIGHT CHANGES

| Step Number | Dose | Animal Numbers | Sex | Day 1 - 8 (g) | Day 1 - 15 (g) |
|---|---|---|---|---|---|
| Step I | 300 mg/kg | Ra8652 | Female | 16.88 | 31.65 |
| | | Ra8653 | | 17.12 | 39.03 |
| | | Ra8654 | | 15.26 | 33.44 |
| **Mean** | | | | **16.42** | **34.71** |
| **SD** | | | | **0.90** | **3.44** |
| Step II | 300 mg/kg | Ra8655 | Female | 18.71 | 41.36 |
| | | Ra8656 | | 20.95 | 47.68 |
| | | Ra8657 | | 22.93 | 41.63 |
| **Mean** | | | | **20.86** | **43.56** |
| **SD** | | | | **1.89** | **3.20** |
| Step III | 2000 mg/kg | Ra8658 | Female | 20.50 | 45.47 |
| | | Ra8659 | | 21.23 | 37.80 |
| | | Ra8660 | | 20.19 | 38.50 |
| **Mean** | | | | **20.64** | **40.59** |
| **SD** | | | | **0.48** | **3.79** |
| Step IV | 2000 mg/kg | Ra8661 | Female | 25.13 | 50.13 |
| | | Ra8662 | | 26.40 | 48.46 |
| | | Ra8663 | | 25.49 | 52.23 |
| **Mean** | | | | **25.67** | **50.27** |
| **SD** | | | | **0.59** | **1.69** |

**Key:** mg/kg – Milligram per kilogram, g – grams

## TABLE 4. INDIVIDUAL ANIMAL NECROPSY FINDINGS

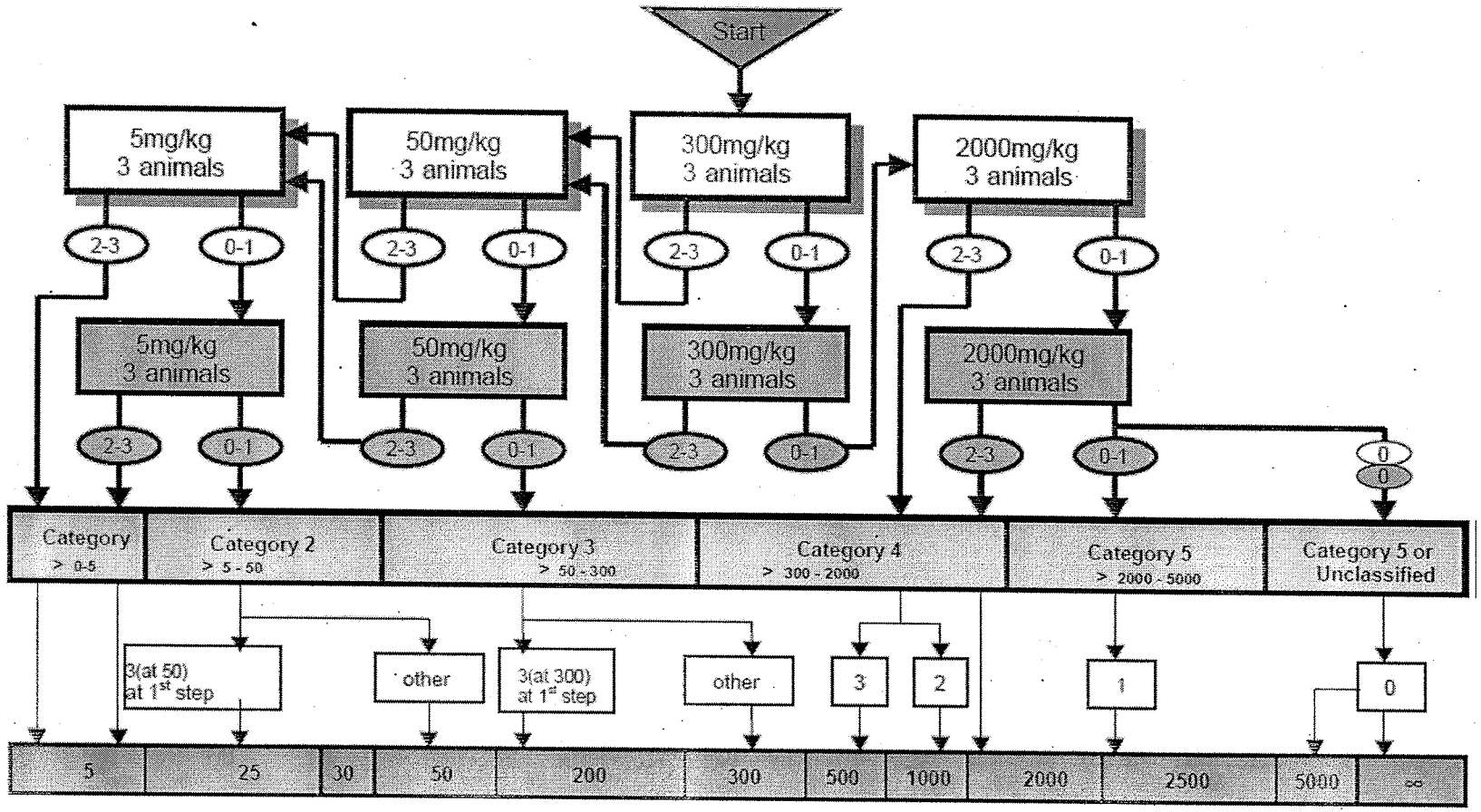
| Step Number | Dose | Animal Numbers. | Sex | Mode of death | Gross Pathological Findings | |
|---|---|---|---|---|---|---|
| | | | | | External | Internal |
| Step I | 300 mg/kg | Ra8652 | Female | TS | NAD | NAD |
| | | Ra8653 | | TS | NAD | NAD |
| | | Ra8654 | | TS | NAD | NAD |
| | | | | | | |
| Step II | 300 mg/kg | Ra8655 | Female | TS | NAD | NAD |
| | | Ra8656 | | TS | NAD | NAD |
| | | Ra8657 | | TS | NAD | NAD |
| | | | | | | |
| Step III | 2000 mg/kg | Ra8658 | Female | TS | NAD | NAD |
| | | Ra8659 | | TS | NAD | NAD |
| | | Ra8660 | | TS | NAD | NAD |
| | | | | | | |
| Step IV | 2000 mg/kg | Ra8661 | Female | TS | NAD | NAD |
| | | Ra8662 | | TS | NAD | NAD |
| | | Ra8663 | | TS | NAD | NAD |

NAD - No Abnormality Detected; TS - Terminal Sacrifice

# 20.0 ANNEXURES

## ANNEXURE 1. TEST PROCEDURE – GUIDELINE EXCERPT

## PROCEDURE FOR STARTING DOSE OF 300 mg/kg BODY WEIGHT



# 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 : 56-0003

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 3 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**

- 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.

(D R PRASADA RAJU)
Head, NGCMA