RESULTS SUMMARY

SAMPLE DESCRIPTION

"BIOWAVE GRANULAR" test sample which are made of compounded natural minerals.

TEST METHOD

Fish acute toxicity test according to OECD Guidelines for Testing of Chemicals – Method 203 Fish Acute Toxicity test.

RESULT:

Mixture of solid/liquid (%, w/w)	Cumulative mortality (%)					Initial, 0 hr	Final, 96 hr
	2 hr	24 hr	48 hr	72 hr	96 hr	pH	pH
0.472	0	0	0	0	0	6.52	6.97

INFERENCE:

The classification system for substances hazardous to the aquatic environment according to The Globally Harmonised System (GHS) of Classification and Labeling of Chemicals (2005) is shown below.

Toxicity Category (Acute toxicity for 96 hr LC_{50} for crustacea)	Classification
Acute I	< 1 mg/L or ppm
Acute II	>1 - ≤10 mg/L or ppm
Acute III	>10 - ≤100 mg/L or ppm

Based on the criteria for the harmonized classification system for the test substance, the "Biowave" is classified as "NOT HAZARDOUS to the aquatic environment" as the LC_{50} value is above 100 mg/L or ppm.

Approved By:

Leong Man Loong

The inferences expressed herein are outside the scope of accreditation.

TITLE

96 hour Fish (tilapia nilotica) Acute Toxicity Test of BIOWAVE Granulars

TESTING FACILITY

In-House Laboratory Testing

109A, Jalan Gebeng 1/6, 26080 Kuantan, Pahang, Malaysia.

PURPOSE OF STUDY

The purpose of this study was to determine the acute toxicity effect of "BIOWAVE Granulars" on tilapia nilotica by obtaining the 96-hour lethal concentration in which 50 percent of the fish would be killed (96-hr LC_{50}).

TESTING METHOD

This study was conducted in accordance with the modification of Method 203, Fish Acute Toxicity Test (Adopted 17th July 1992), OECD Guideline for Testing of Chemicals 1998.

PERIOD OF STUDY

Receipt of sample: 15TH AUGUST 2013

Commencement of study: 15TH AUGUST 2013

Completion of observation: 19TH AUGUST 2013

PROJECT RESEARCH TEAM

Ng Kee Wee

Ainudin Sabri Bin Hanapi

Sugu Kutty

REVIEWED AND APPROVED

Leong Man Loong

TABLE OF CONTENTS

SUMMARY

The 96-hr static acute toxicity test using tilapia nilotica was conducted on a sample coded as “BIOWAVE Granulars”. The test was obtained from a definitive test with a sample weight of 500g. The test fish were exposed for a period of 96 hours. Mortality and toxicity responses were recorded at 2, 24, 48, 72 and 96 hour. The evaluation of LC_{50} was conducted based on the observation of mortality results. The LC_{50} value at 96-hr exposure of tilapia is 4,717 mg/kg.

MATERIALS AND METHODS

1. TEST SUBSTANCE

As per Material Safety Data Sheet (MSDS)

2. TEST ORGANISM

2.1 Test Fish selected

Tilapia (Tilapia nilotica)

2.2 Source

Tilapia was bought from fish shop and has been kept in laboratory for at least 7 days.

2.3 Number of test organisms

10 test fish were used per exposure level.

2.4 Characterization of the test organism

Test Fish	Weight	Body Length
Tilapia nilotica	0.54 ±0.15 g	3.27 ± 0.23 cm

3. CONDITION OF EXPOSURE

3.1 Type of test

Static, no renewal of test sample.

3.2 Exposure duration

96-hour exposure with observation recorded at 2, 24, 48, 72 and 96 hours.

3.3 Water

Dechlorinated pipe water was used.

3.4 Test Sample

A 500 g of BIOWAVE Granulars was filled in a glass cone was used in the test. The cone will protect test sample from being eaten by fish.

3.5 Aeration

Aeration was applied.

4. CONDITION OF TEST ENVIRONMENT

4.1 Test Vessel

A rectangular glass aquarium with a size of 914 mm x 457 mm x 457 mm to contain 0.106 m^3 of water.

4.2 Weight of test sample

500 g per 0.106 m^3 of water

4.3 Temperature

25 ± 3^{o}C was maintained daily throughout the exposure.

4.4 Renewal of test sample

No.

4.5 Light

A 24-hour ceiling white light was used throughout the test.

5. PREPARATION OF TEST SUBSTANCE

5.1 Test substance

5.1.1 Preparation

The test sample was filled into a glass cone and was put into the test vessel filled with water.

5.1.2 Stability of test sample

Some test granulars turned into powder when exposed to water.

5.1.3 Preparation time

New test sample was used prior to test fish exposure.

6. METHODS

The test was carried out using tilapia as the test fish and no replicates.

6.1 Procedures

500 g of test sample granular was filled in a glass cone. Ten (10) test fish of uniform size were put into test vessel for exposure. Observations were recorded after the first 2-hour exposure followed by 24 hourly mortality observation until 96 hours of exposure. Every dead fish was removed from the test vessel and recorded accordingly as percent mortality.

7. OBSERVATION AND MEASUREMENT

7.1 Mortality

The test fish was considered dead if no reaction was observed when touching its caudal penducle.

7.2 pH

The pH of the water was measured at the beginning and end of test using a pH meter.

7.3 Visual observation on abnormal response

General observation was done but no specific observation for abnormal response was conducted for this test.

RESULTS

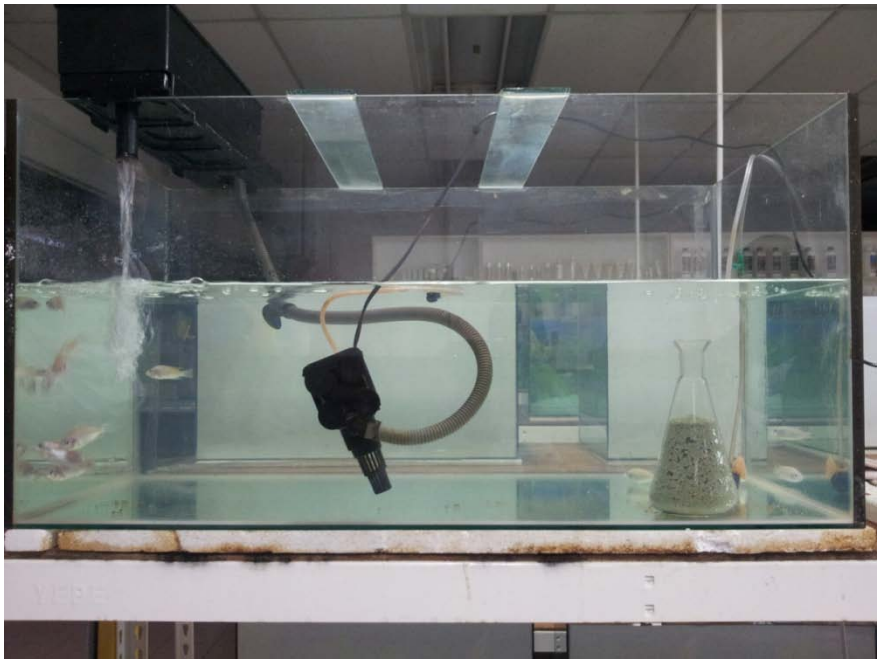
The mortality results for the definitive test are in Table 1: Results of the Definitive Test – Cumulative mortality and pH.

Table 1: Results of the Definitive Test – Cumulative mortality and pH.

Mixture of solid/liquid (%, w/w)	Cumulative mortality (%)					Initial, 0 hr	Final, 96 hr
	2 hr	24 hr	48 hr	72 hr	96 hr	pH	pH
0.472	0	0	0	0	0	6.52	6.97

0 hour (2.00 PM, 15/8/13)

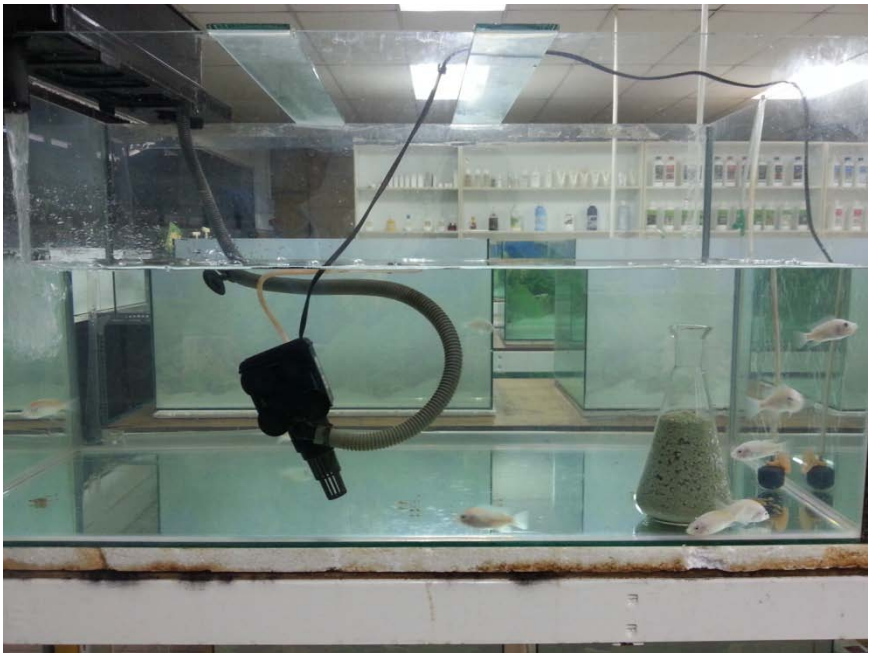
Water is cloudy.



2 hours (4.05 PM, 15/8/13)

Water is less cloudy. No dead fish was found.

IN-HOUSE TESTING



24 hours (2.00 PM, 16/8/13)

Water is clear. No dead fish was found.

48 hours (2.09 PM, 17/8/13)

Water is clear. No dead fish was found.

72 hours (1.57 PM, 18/8/13)

Water is clear. No dead fish was found.

96 hours (2.23 PM, 19/8/13)

Water is clear. No dead fish was found.

CONCLUSION

Based on the results of definitive test and mortality results, the 96 hour - LC_{50} value was established as 0.472% (w/w) or 4,717 mg/kg (w/w) or equivalent to 4,717 ppm.

INFERENCE:

The classification system for substances hazardous to the aquatic environment according to The Globally Harmonised System (GHS) of Classification and Labeling of Chemicals (2005) is shown below.

Toxicity Category (Acute toxicity for 96 hr LC_{50} for crustacea)	Classification
Acute I	< 1 mg/L or ppm
Acute II	>1 - ≤10 mg/L or ppm
Acute III	>10 - ≤100 mg/L or ppm

Based on the criteria for the harmonized classification system for the test substance, the “Biowave” is classified as “NOT HAZARDOUS to the aquatic environment” as the LC_{50} value is above 100 mg/L or ppm.

REFERENCES

1. OECD Guideline for Testing of Chemicals Method 203 Fish, Acute Toxicity Test, OECD 1998.

2. Acute Toxicity is expressed as the Median Lethal Concentration (LC_{50}) that is the concentration in water which kills 50% of a test batch of fish within a continuous period of exposure.

   Biowave test sample = 500 g = 500,000 mg

   Volume of water in test vessel = 0.914 m x 0.457 m x 0.254 m = 0.106 m^3

   1 m^3 of water = 1000 kg

   Median Lethal Concentration (LC_{50}) = 500,000 mg /[0.106 m^3 x (1000 kg/1 m^3)]
   = 4,717 mg/kg

3. Weight of test sample (Biowave) = 500 g = 0.5 kg

   Weight of water in test vessel = 0.106 m^3 x (1000 kg/1 m^3) = 106 kg

   Mixture of solid/liquid (%, w/w) = (0.5 kg/ 106 kg) x 100% = 0.472%