*Study Code: J076/2013/46*
*Reference No: ETRC257/16/1127(R0117/2013)*

SIRIM

# TEST REPORT

# DAPHNIA MAGNA ACUTE IMMOBILISATION TEST OF NANO COLLOIDAL ARGENTUM

***Prepared for:***

Trumer Medicare Sdn. Bhd.
No.16-2, Jalan SS19/1G
47500 Subang Jaya,
Selangor

***Prepared by:***
Karimah Muhamad
Environmental Technology Research Centre (ETRC)
Building 15, SIRIM Berhad
No 1, Persiaran Dato Menteri,
Section 2, 40700 Shah Alam,
Selangor Darul Ehsan.

**29 AUGUST, 2013**

## Table of Contents

**Study Information**

**Study Title:**
*Daphnia magna,* Acute Immobilisation Test of 'Nano colloidal argentum'

**Study Director:**
Karimah Muhamad
Environmental Technology Research Centre
Building 15, SIRIM Berhad
No. 1, Persiaran Dato' Menteri
Section 2, 40700 Shah Alam, Selangor
Phone: 603-5544 6583
Fax: 603-5544 6590

**Study personnel:**
i. Nor Syafika Adua Mohamad Bakri

**Study period:**
i. Experimental start date: 2 July 2013
ii. Experimental completion date: 11 July 2013

**Sponsor**
Trumer Medicare Sdn. Bhd.
No.16-2, Jalan SS19/1G
47500 Subang Jaya,
Selangor

**Testing Facility**
Ecotoxicity Laboratory 2
Environmental Technology Research Centre
Block 15, SIRIM Berhad
No. 1, Persiaran Dato' Menteri, Section 2
40700 Shah Alam, Selangor

**Statement of ISO/IEC 17025 STR 1.2 Compliance**

I, the undersigned, hereby declare that this report constitutes a true and complete representation of the procedures followed and of the result obtained in this study by ETRC, SIRIM Berhad, and that the study was carried out under my supervision.

The study was conducted according to the ISO/IEC 17025 STR 1.2 requirements.

Karimah Muhamad
Study Director
Environmental Technology Research Centre
SIRIM Berhad

29/8/2013
Signature and Date

**Report Information**

**Study Title:**
*Daphnia magna,* Acute Immobilisation Test of 'Nano colloidal argentum'

**This report was prepared by:**
Karimah Muhamad
Environmental Technology Research Centre
Block 15, SIRIM Berhad
No. 1, Persiaran Dato' Menteri,
Section 2, 40700 Shah Alam, Selangor.
Phone: 603-5544 6583
Fax: 603-5544 6590

Name: Karimah Muhamad
Study Director

Date: 29/8/2013

**Summary**

The *Daphnia magna,* Acute Immobilization test of Nano colloidal argentum is described in this report. The range finding and definitive tests were conducted from 2 July, 2013 to July 11, 2013 according to Method 202, *Daphnia magna,* Acute Immobilization Test (Adopted 13 April 2004), OECD Guideline for Testing of Chemicals and ETRC Standard Operating Procedures manual.

The test was started with a range finding test with a series of concentrations 700 mg/L, 500 mg/L, 300 mg/L, 100 mg/L and 50 mg/L.

Definitive test was conducted in a series concentration of 300 mg/L, 250 mg/L, 208 mg/L, 174 mg/L and 145 mg/L. The daphnids were exposed to the test concentration for a period of 48 hours. The $EC_{50}$ of the Nano colloidal argentum is determined at 261 mg/L. Nano colloidal argentum is not classified based on the classification limit according to Globally Harmonized System of Classification and Labeling of Chemicals (GHS).

## 1 Introduction

Nano colloidal argentum is a product from Trumer Medicare Sdn Bhd. This study was conducted in accordance with Method 202 (Adopted 13 April 2004), *Daphnia magna*, Acute immobilization Test, OECD Guideline for Testing of Chemicals.

## 2 Materials and Methods

### 2.1 Test Item

(Information as provided by the sponsor)

| | |
|---|---|
| Name: | Nano colloidal argentum |
| Other name: | Not applicable |
| CAS No.: | Not applicable |
| Sponsor: | Trumer Medicare Sdn. Bhd. |
| Lot/Batch No.: | Not applicable |
| Date of receipt: | 18 June 2013 |
| Expiry date: | No information |
| Structural formula or rational formula: | Not applicable |
| Purity: | No information |
| Contents of Impurities: | No information |
| Physicochemical properties as follows: | |
| Appearance at ordinary temperature: | Colourless clear liquid |
| Molecular weight: | Not applicable |
| Stability: | No information |
| Melting Point: | Not applicable |
| Boiling Point: | No information |
| Flash Point: | No information |
| Vapor Pressure: | No information |
| Partition Coefficient: | Not applicable |
| Solubility: | Insoluble in water |
| Degree of Solubility: | |
| Storage condition: | Room temperature |
| Stability of test concentrations: | No information |
| Handling care: | Wear gloves and lab coat |

### 2.2 Reference Items

(Not applicable)

## 2.3 Test Methods

Method 202 (Adopted 13 April 2004) *Daphnia magna,* Acute Immobilization Test, OECD Guideline for Testing of Chemicals.

## 2.4 Test System

The *Daphnia magna* stock culture was sourced from the Pusat Penyelidikan Perikanan Air Tawar (PPPAT), Batu Berendam, Melaka on April 2000 which was originated from Rijksuniversiteit Gent, Laboratorium voor Ecologie der Dieren, Belgium. Since then, the stock culture was cultured at SIRIM ETRC's hatchery and acclimatised in the Ecotoxicity Laboratory 2 at controlled environment condition. The *Daphnia magna* was confirmed for taxonomy as *Daphnia magna* by Mr. Chuah Hean Peng, the Research Officer from Freshwater Fisheries Research Division, Department of Fisheries Malaysia, Negeri Sembilan.

*Daphnia magna* stock culture was placed in water bath maintained at 20 ± 1°C with controlled photoperiod at 16 hrs light and 8 hours dark. Ten (10) adult daphnia was cultured in one beaker containing 100 mL holding water. Daphnids were fed once daily with 1.5 mL combination of *Chlorella vulgaris*, yeast and fish pellet. Any sign of stress were observed and recorded every day except weekend. Progeny will be removed every day except weekend.

Daphnids of less than 24 hours old derived from healthy stock of batch no. B8 (GN6) was selected as test systems.

## 2.5 Holding and dilution water

Dechlorinated tap water with adjusted hardness of batch no DW170613 was used as holding and dilution water for range finding and definitive tests. The water was stored in glass tank and aerated prior to use for the test so that the dissolved oxygen concentration has reached the saturation level. The characteristics of the holding and dilution water as shown below:

**Table 1: Holding and dilution water characteristic**

| Parameter | Unit | Value |
|---|---|---|
| Free chlorine | mg/L | <0.001 |
| Total hardness (as $CaCO_3$) | mg/L | 158 |
| Dissolved oxygen (DO) | mg/L | 8.68 |
| pH | N.A | 7.42 |
| Alkalinity | mg/L | not conducted |
| Conductivity | µs/cm | 247 |
| Ca/Mg ratio | N.A | 0.4:1 |
| Na/K ratio | N.A | not conducted |

*Note: n. a: not applicable*

**Table 2: Dechlorinated tap water chemicals characteristic**

| Substance | Acceptable Concentration | Measured Concentration |
|---|---|---|
| Particulate matter | < 20 mg/L | not conducted |
| Total organic carbon | < 2 mg/L | 1.1 mg/L |
| Unionised ammonia | < 1 µg/L | not conducted |
| Residual chlorine | < 10 µg/L | < 1 µg/L |
| Total organophosphorus pesticide | < 50 ng/L | 19 ng/L |
| Total organochlorine pesticide plus polychlorinated biphenyl | < 50 ng/L | 19 ng/L |
| Total organic chlorine | < 25 ng/L | not detected |

## 3 Experimental Design and Procedures

### 3.1 Preparation of test item

As the test item is soluble in water the stock solution for range finding was prepared by dissolving 0.5g test item and top up to 500 mL with deionized water which equivalent to 1000 mg/L.The stock solution was used for the preparation of test solution for range finding test and definitive test by diluting with aerated dechlorinated tap water according to the specified concentration.

### 3.2 Condition of exposure

The daphnids aged less than 24 hours and derived from healthy stock were exposed in 100 mL test solutions for 48 hours. The test was conducted in 250 mL glass beaker. Twenty (20) daphnids divided into four (4) groups of five (5) daphnids were used for each concentration and for the control. The test beaker were placed in water bath with the temperature at 20°C throughout the test and maintained under a 16 hours photoperiod daily. Light intensity measured was 627 to 568 LUX using lux meter. The test solutions were not aerated and the daphnids were not fed during the test.

### 3.3 Range finding test

Range finding test was conducted to determine the suitable range of concentration for the definitive test. Test solutions were prepared by diluting stock, 1000 mg/L with aerated dilution water. Five (5) test concentrations prepared were 700 mg/L, 500 mg/L, 300 mg/L, 100 mg/L and 50 mg/L. Five (5) daphnids were introduced per 100 mL test solution for each concentration.

### 3.4 Definitive test

Based on the result of the range finding test, five (5) test concentrations were prepared with constant factor of 1.2 in a geometric series of 300 mg/L, 250 mg/L, 208 mg/L, 174 mg/L and 145 mg/L. Twenty (20) daphnids divided into four (4) groups of five (5) daphnids each were introduced for each test vessel for each concentration and control. The daphnids were exposed at each test solution for a period of 48 hours and were observed at 24 and 48 hours interval.

### 3.5 Observation and examination

The test organism was considered immobilised if the organism was not able to swim within 15 seconds after gentle agitation of the test vessel. Any abnormal behaviors were recorded. Physical-chemical measurements were conducted for dissolved oxygen and pH at the beginning and end of test using DO meter YSL Model 52 and pH meter Hanna model pH 211.

### 3.6 Statistical method

The $EC_{50}$ value of the test substance was evaluated using Probit Analysis of MINITAB 5 statistical software programme.

## 4 Results

The results and measurements made for the study are represented in Appendix A; Table 3, Table 4 and Table 5. The graph for probability plot for immobility at 24 hours and 48 hours are shown in Appendix B.

## 5 Conclusion

The $EC_{50}$ value at 48-hour exposure of *Daphnia magna* was 261 mg/L. Nano colloidal argentum is not classified based on the classification limit according to Globally Harmonized System of Classification and Labeling of Chemicals (GHS).

## 6. Storage & Retention of Records/Materials

Data sheets and reports shall be retained in the Filling Cabinet of the Environmental Technology Research Centre, SIRIM Berhad for 10 years following the date of completion of the study.

## 7. Reference Documents

Method 202 (Adopted 13 April 2004), *Daphnia magna,* Acute Immobilization Test OECD Guideline for Testing of Chemicals.

*Appendix A*

**Table 3: pH and DO Measurement**

| Concentration (mg/L) | pH | | DO (mg/L) | |
|---|---|---|---|---|
| | Initial | Final | Initial | Final |
| Control | 7.03 | 7.39 | 8.49 | 9.14 |
| 300 | 7.74 | 7.58 | 8.43 | 9.22 |
| 250 | 7.73 | 7.59 | 8.45 | 9.28 |
| 208 | 7.67 | 7.53 | 8.46 | 9.27 |
| 174 | 7.60 | 7.51 | 8.47 | 9.22 |
| 145 | 7.52 | 7.46 | 8.51 | 9.24 |

**Table 4: $EC_{50}$ for 24 hours and 48 hours with Confidence Limit and Standard Error**

| Exposure | $EC_{50}$ (mg/L) | 95% Confidence Limit (mg/L) | Standard Error |
|---|---|---|---|
| 24 | 275 | N.A | N.A |
| 48 | 261 | N.A | N.A |

*Note: N.A-not applicable due to data limitation*

**Table 5: Definitive Test – Cumulative Immobility and Abnormal Behaviour**

| Conc. of Test Solution (mg/L) | Replicates | Immobility | | Abnormal Behaviour<br>**AS** At the surface **HYP** Hyper active<br>**AB** At the bottom **DC** Discoloured | |
|---|---|---|---|---|---|
| | | 24hr | 48hr | 24hr | 48hr |
| Control | A | 0 | 0 | 5:AS | 5:AS |
| | B | 0 | 0 | 5:AB | 5:AB |
| | C | 0 | 0 | 4:AB, 1:AS | 1:AS, 4:AB |
| | D | 0 | 0 | 5:AB | 5:AB |
| | Total | 0 | 0 | | |
| | % | 0 | 0 | | |
| 300 | A | 5 | 5 | 5: DEAD | 5: DEAD |
| | B | 5 | 5 | 5: DEAD | 5: DEAD |
| | C | 5 | 5 | 5: DEAD | 5: DEAD |
| | D | 5 | 5 | 5: DEAD | 5: DEAD |
| | Total | 20 | 20 | | |
| | % | 100 | 100 | | |
| 250 | A | 0 | 2 | 3:AB, 2:AS | 2: DEAD, 3:AS |
| | B | 0 | 2 | 4:AB, 1:AS | 2: DEAD, 3:AB |
| | C | 0 | 0 | 3:AS, 2:AB | 4:AS, 1:AB |
| | D | 0 | 1 | 5: AB | 1: DEAD, 4:AS |
| | Total | 0 | 5 | | |
| | % | 0 | 25 | | |
| 208 | A | 0 | 0 | 5: AS | 4:AS, 1:AB |
| | B | 0 | 0 | 3:AS, 2:AB | 5: AB |
| | C | 0 | 0 | 2:AB, 3:AS | 4:AS, 1:AB |
| | D | 0 | 0 | 3:AS, 2:AB | 3:AS, 2:AB |
| | Total | 0 | 0 | | |
| | % | 0 | 0 | | |
| 174 | A | 0 | 0 | 2:AB, 3:AS | 4:AS, 1:AB |
| | B | 0 | 0 | 2:AS, 3:AB | 5: AS |
| | C | 0 | 0 | 2:AS, 3:AB | 4:AS, 1:AB |
| | D | 0 | 0 | 4:AS, 1:AB | 5: AS |
| | Total | 0 | 0 | | |
| | % | 0 | 0 | | |
| 145 | A | 0 | 0 | 5: AB | 5: AS |
| | B | 0 | 0 | 4:AB, 1:AS | 5: AB |
| | C | 0 | 0 | 5: AB | 5: AB |
| | D | 0 | 0 | 4:AB, 1:AS | 3:AB, 2:AS |
| | Total | 0 | 0 | | |
| | % | 0 | 0 | | |

*Appendix B*

**Figure 1: Probability Plot for Immobility at 24 Hour**

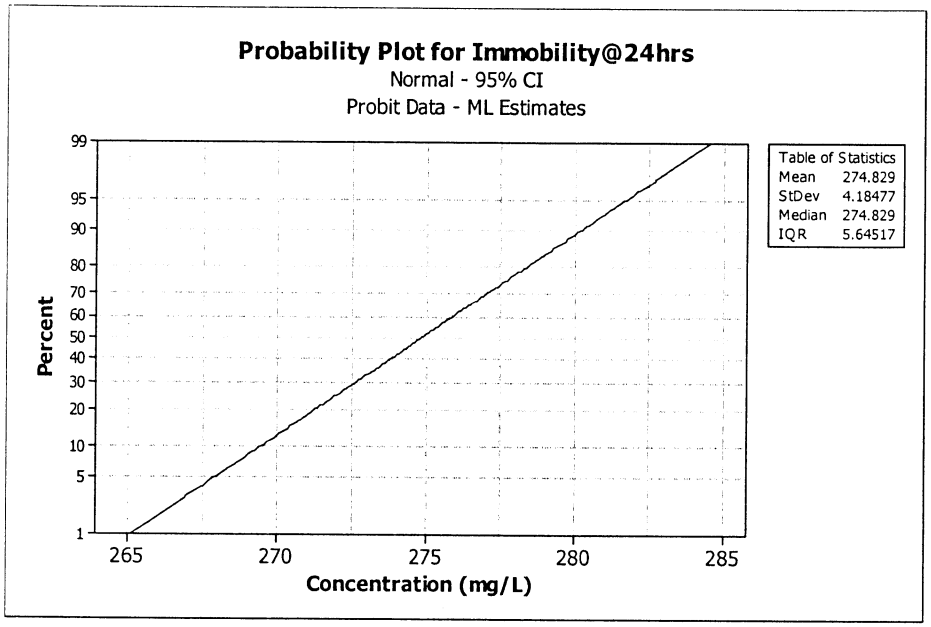


**Figure 2: Probability Plot for Immobility at 48 Hour**