TEST REPORT

Impact assessment study for marine organisms
on a test plate coated with AQUATERRAS
—Mysid Acute Toxicity Test—

October 6, 2017

WDB Environmental & Biological Research Institute Co., Ltd.
Sponsor: Nippon Paint Marine Coatings Co., Ltd.

Test substance: Test plate coated with AQUATERRAS

Study Title: Impact assessment study for marine organisms on a test plate coated with AQUATERRAS—Mysid Acute Toxicity Test—

Study Number: J17000621-2

This study was conducted with the test substance provided to us on September 11, 2017.

I hereby certify that the reported results reflect accurately the raw data of testing, and that the test results are valid.

Date: October 6, 2017

Approved by: [Signature]

Laboratory & Study Manager
SUMMARY

Impact assessment study for marine organisms on a test plate coated with AQUATERRAS
—Mysid Acute Toxicity Test—

This study was conducted to evaluate the acute toxicity of the test substance provided to us by the sponsor with *Americamysis bahia* under the test conditions described below.

<Test Conditions>

1) Test substance: Test plate coated with AQUATERRAS
2) Test organism: *Americamysis bahia* (<24 hours post release)
3) Test duration: 96 hours
4) Test vessel: 1-L glass beaker
5) Test water: Filtered dilution water (20±2‰)
6) Test section: Addition section and control section
7) Test concentration*: 31.4 cm²/L by painted area
8) Number of test organisms: 10 individuals/vessel
9) Number of replicates: 3 replicates/section
10) Test type: Static
11) Temperature: 25±1°C
12) Photoperiod: 12 hours light: 12 hours dark
13) Feeding: *Artemia* spp. Nauplii, once a day
14) Observation: 24, 48, 72 and 96 hours

*The test concentration was set based on the assumption of seawater being in a stationary state within a distance of 30 cm from the coated object.

<Test Results>

No mortality of the test organisms was observed in the test section. Therefore, the test plate coated with AQUATERRAS showed no acute toxicity to the test organisms during the test period.
FINAL REPORT

1. Study Title
   Impact assessment study for marine organisms on a test plate coated with AQUATERRAS —Mysid Acute Toxicity Test—

2. Sponsor
   Name: Nippon Paint Marine Coatings Co., Ltd.
   Address: 1-26 Komagabayashi Minami-Cho, Nagata-Ku, Kobe
            Hyogo 653-0045, Japan

3. Testing Facility
   Name: WDB Environmental and Biological Research Institute Co., Ltd.
   Address: 1-6 Tonomui, Aza, Yamagawauchi, Minami-Cho,
            Kaifu-Gun, Tokushima 779-2307, Japan

4. Purpose of the Study
   This study was conducted to evaluate the acute toxicity of the test substance with the mysids.

5. Test Period
   Start date: September 11, 2017
   End date: September 15, 2017

6. Study manager: Tomoharu Nakamura (Laboratory Manager)

7. Experimental Staff: Jun-ichi Ueno (Deputy Manager, Technical Fellow)
                       Kensuke Iwamoto (Research Engineer)
8. Test Substance
   1) Name: Test plate coated with AQUATERRAS
   2) Date received: September 11, 2017
   3) Storage conditions: Dark place at room temperature

   ![Test plate coated with AQUATERRAS](image)

   Fig. 1. Test plate coated with AQUATERRAS

9. Materials and Methods
   1) Test organism
      (1) Common name: Mysid shrimp
      (2) Scientific name: *Americamysis bahia*
      (3) Origin: National Institute for Environmental Studies, Japan
      (4) Source: In-house cultivation
      (5) Acclimation: Cultured under the same conditions as testing
      (6) Other: <24 hours post release

   ![Americamysis bahia](image)

   Fig. 2. *Americamysis bahia*
2) Testing devices
   Test vessel: 1-L glass beaker
   Lighting system: Fluorescent lighting with timer

3) Test conditions
   Test type: Static
   Test duration: 96 hours
   Test water: Filtered dilution water (20±2\%)
   Test volume: 1 L
   Test concentration*: 31.4 cm\(^2\)/L by painted area
   Test sections: Addition section and control section
   Number of test organisms: 10 individuals/vessel
   Number of replicates: 3 replicates/section
   Temperature: 25±1\°C
   Dissolved oxygen: More than 60\% of saturation
   pH: Between 7.5 and 8.5
   Photoperiod: 12 hours light: 12 hours dark
   Feeding: *Artemia* spp. Nauplii, once a day

*The test concentration was set based on the assumption of seawater being in a stationary state within a distance of 30 cm from the coated object.

4) Preparation of test substance and test solution
   The test substance provided by the sponsor was washed lightly with test water and immersed in the test vessel containing the test organisms to prepare the test solution.

5) Observations and measurements
   (1) Mortality, appearance and behavior
       The number of dead mysid in each test vessel was counted and recorded at 24, 48, 72, and 96 hours. The number of mysid exhibiting abnormal appearance or behavioral symptoms was summarized by time of observation, treatment, and replicate.
   (2) Measurement of test conditions
       Water quality parameters (temperature, dissolved oxygen, pH, and salinity) during the test were measured at 24, 48, 72, and 96 hours.
(3) Calculation of median lethal concentration, LC\textsubscript{50}

The 24-, 48-, 72-, and 96-h LC\textsubscript{50} values were calculated by statistical procedures based on mortality.

10. Test Results

1) LC\textsubscript{50} values

The LC\textsubscript{50} values could not be calculated because there was no death of the test organisms.

<table>
<thead>
<tr>
<th>Section</th>
<th>24 h LC\textsubscript{50}</th>
<th>48 h LC\textsubscript{50}</th>
<th>72 h LC\textsubscript{50}</th>
<th>96 h LC\textsubscript{50}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test beakers</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
</tbody>
</table>

*Indicates that the mortality was less than 50% and could not be calculated.

2) Mortality, appearance and behavior

No death, no abnormal appearance and no behavioral symptoms were observed during the test period.

Table 2. Cumulative mortality and observation at 24, 48, 72, and 96 hours

<table>
<thead>
<tr>
<th>Section</th>
<th>Cumulative rate (%)</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 h</td>
<td>48 h</td>
</tr>
<tr>
<td>Test beakers</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*Indicates that no abnormality was observed.

![Graph of cumulative mortality at 24, 48, 72, and 96 hours](image)

Fig. 3. Cumulative mortality at 24, 48, 72, and 96 hours
3) Water quality

Water quality parameters (temperature, dissolved oxygen, and pH) in the test solution during the test period are shown in Tables 3-5. The water temperature was within 25±1°C, and the dissolved oxygen was more than 60% of the saturated concentration. Also, no abnormal pH change was observed.

| Table 3. Water temperature (°C) at 24, 48, 72, and 96 hours |
|----------------|---------|-------|-------|-------|
| Section       | 24 h    | 48 h  | 72 h  | 96 h  |
| Test beakers  | 25.0    | 25.0  | 25.0  | 25.0  |
| Control       | 25.0    | 25.0  | 25.0  | 25.0  |

| Table 4. Dissolved oxygen concentration (mg/L) at 24, 48, 72, and 96 hours |
|----------------|---------|-------|-------|-------|
| Section       | 24 h    | 48 h  | 72 h  | 96 h  |
| Test beakers  | 7.2     | 7.1   | 7.1   | 7.1   |
| Control       | 7.2     | 7.1   | 7.0   | 7.1   |

| Table 5. pH values at 24, 48, 72, and 96 hours |
|----------------|---------|-------|-------|-------|
| Section       | 24 h    | 48 h  | 72 h  | 96 h  |
| Test beakers  | 7.9     | 7.9   | 7.9   | 7.9   |
| Control       | 7.9     | 7.9   | 7.9   | 7.9   |

4) Factors affecting the reliability of the test results

There were no factors that might have affected the reliability of the test results.

11. Validity of the Test

The mortality of the control section at the end of the test duration was less than 10%, and the water quality and test condition were good. Therefore, the validity of this test was confirmed.

12. References

13. Images

Fig. 4. Overhead view of the test

Fig. 5. Addition section (left) and control section (right) at the start of the test

Fig. 6. Addition section (left) and control section (right) at the end of the test