Study No. J17000621-2

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:* 中村 翔治

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 cm^2/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

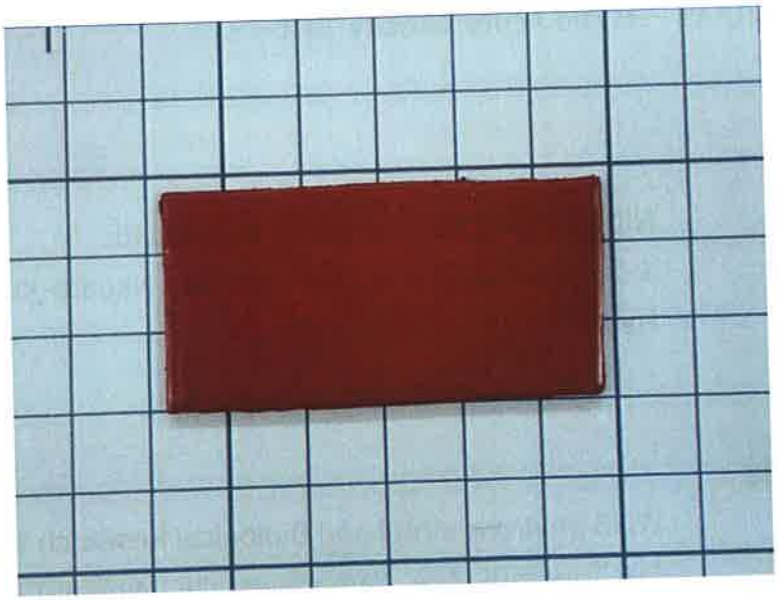

Fig. 1. Test plate coated with AQUATERRAS

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

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_{50}

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

10. Test Results

1) LC_{50} values

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

Table 1. LC_{50} values for *Americamysid bahia* in the addition section

Section	24h LC_{50}	48h LC_{50}	72h LC_{50}	96h LC_{50}
Test beakers	-*	-*	-*	-*

*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

Section	Cumulative rate (%)				Observation			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
Test beakers	0	0	0	0	◎	◎	◎	◎
Control	0	0	0	0	◎	◎	◎	◎

◎Indicates that no abnormality was observed.

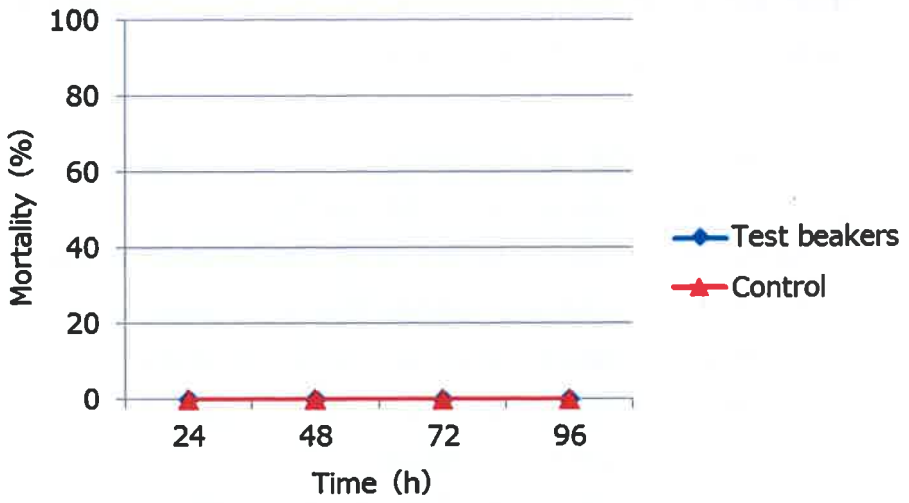

Fig. 3. Cumulative mortality at 24, 48, 72, and 96 hours

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

- United States Environmental Protection Agency. Ecological Effects Test Guidelines. OCSPP 850.1035: Mysid Acute Toxicity Test (2016).

13. Images

Fig.4. Overhead view of the test

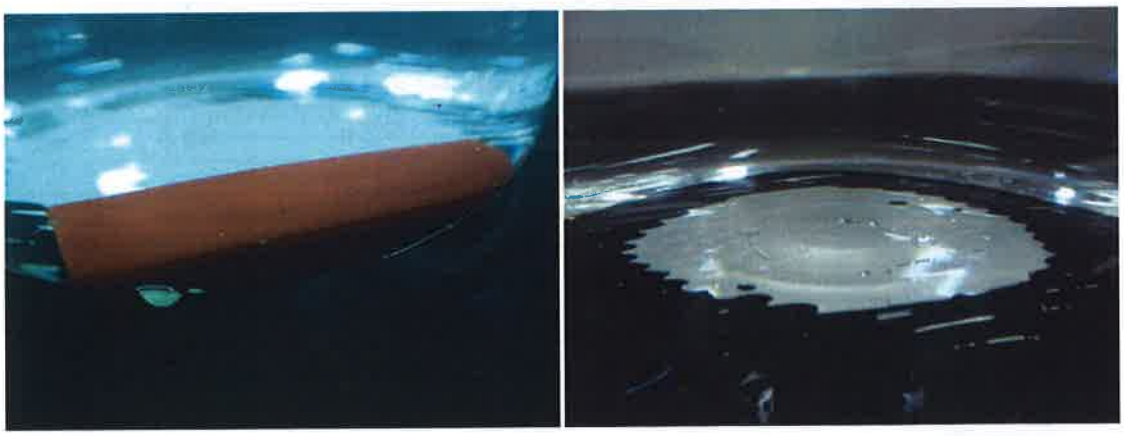

Fig.5. Addition section (left) and control section (right) at the start of the test

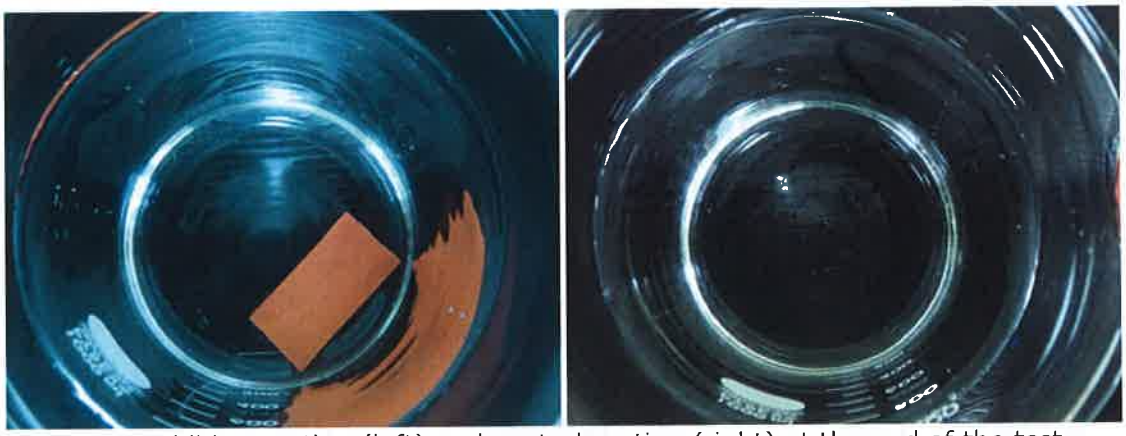

Fig.6. Addition section (left) and control section (right) at the end of the test