

TEST REPORT

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

Study Number J17000621-1

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 智治

Laboratory & Study Manager

SUMMARY

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

This study was conducted to evaluate the acute toxicity of the test substance provided to us by the sponsor with Clown anemonefish (*Amphiprion ocellaris*) under the test conditions described below.

<Test Conditions>

- | | |
|------------------------------|---|
| 1) Test substance: | Test plate coated with AQUATERRAS |
| 2) Test organism: | Crown anemonefish (<i>Amphiprion ocellaris</i>) |
| 3) Test duration: | 96 hours |
| 4) Test vessel: | 30-L polycarbonate round tank |
| 5) Test water: | Filtered natural sea water (32±2‰) |
| 6) Test sections: | 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: | No feeding during test |
| 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 —Saltwater Fish 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 saltwater fish.

5. Test Period

Start date: October 2, 2017
End date: October 6, 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

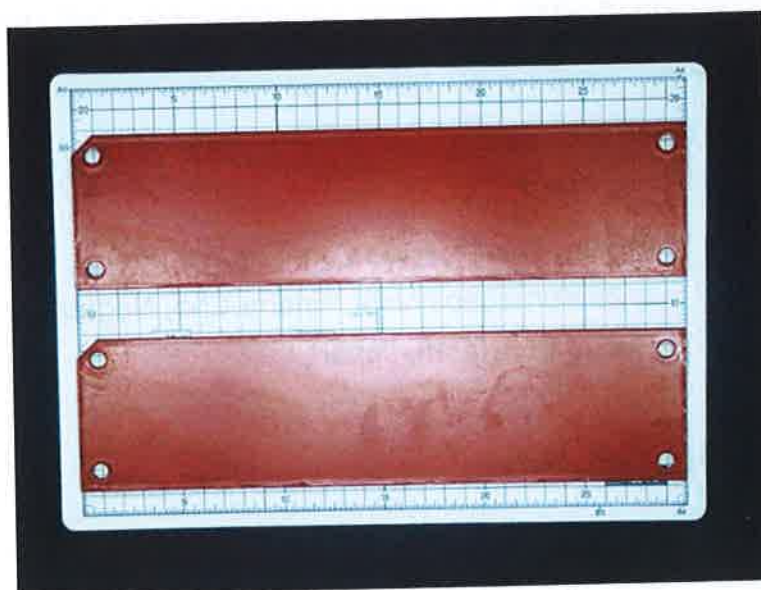


Fig. 1. Test plate coated with AQUATERRAS

9. Materials and Methods

1) Test organism

- (1) Common name: Clown anemonefish
- (2) Scientific name: *Amphiprion ocellaris*
- (3) Average length: 19.9 mm
- (4) Average weight: 126.6 mg
- (5) Source: In-house cultivation



Fig. 2. *Amphiprion ocellaris*

2) Testing devices

Test vessel:	30-L polycarbonate round tank
Lighting system:	Fluorescent lighting with timer

3) Test conditions

Test type:	Static
Test duration:	96 hours
Test water:	Filtered natural sea water (32±2‰)
Test volume:	25 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:	No feeding during test

*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 fish in each test vessel was counted and recorded at 24, 48, 72, and 96 hours. The number of fish 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, LC₅₀

The 24-, 48-, 72-, and 96-h LC₅₀ values were calculated by statistical procedures based on mortality.

10. Test Results

1) LC₅₀ values

The LC₅₀ values could not be calculated because there was no death of the test organisms.

Table 1. LC₅₀ values for *Amphiprion ocellaris* in the addition section

Section	24h LC ₅₀	48h LC ₅₀	72h LC ₅₀	96h LC ₅₀
Test tanks	-*	-*	-*	-*

*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 tanks	0	0	0	0	⊙	⊙	⊙	⊙
Control	0	0	0	0	⊙	⊙	⊙	⊙

⊙Indicates that no abnormality was observed.

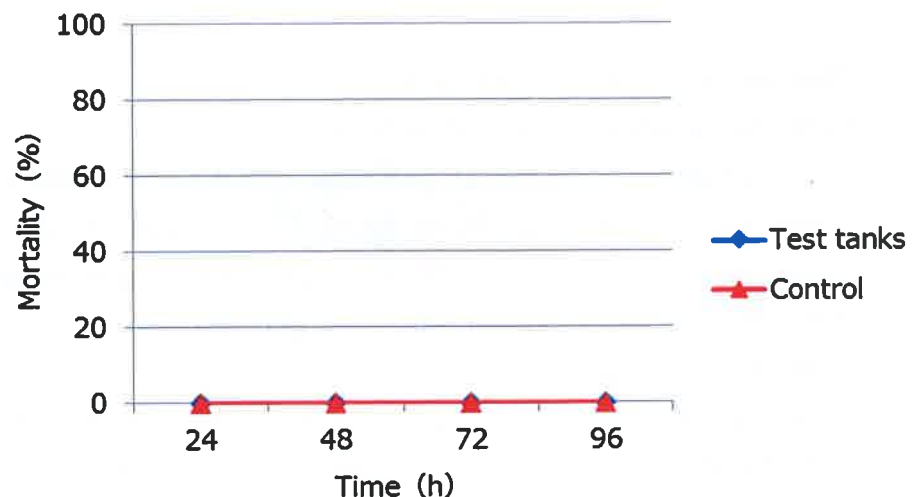


Fig. 3. Cumulative mortality at 24, 48, 72, and 96 hours

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 $25 \pm 1^\circ\text{C}$, and the dissolved oxygen was more than 60% of the saturated concentration. Also, no abnormal pH change was observed.

Table 3. Water temperature ($^\circ\text{C}$) at 24, 48, 72, and 96 hours

Section	24 h	48 h	72 h	96 h
Test tanks	24.6	25.0	25.0	25.1
Control	24.7	25.1	25.1	25.2

Table 4. Dissolved oxygen concentration (mg/L) at 24, 48, 72, and 96 hours

Section	24 h	48 h	72 h	96 h
Test tanks	7.5	7.6	7.6	7.6
Control	7.6	7.6	7.5	7.6

Table 5. pH values at 24, 48, 72, and 96 hours

Section	24 h	48 h	72 h	96 h
Test tanks	7.9	7.9	7.8	7.7
Control	7.9	7.8	7.8	7.8

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 850.1075: Freshwater and Saltwater Fish 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