Study No. J17000621-4

TEST REPORT

Impact assessment study for marine organisms
on a test plate coated with AQUATERRAS
—Saltwater Bivalve 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 Bivalve Acute Toxicity Test—
Study Number	J17000621-4

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 稙治

Laboratory & Study Manager

SUMMARY

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

This study was conducted to evaluate the acute toxicity of the test substance provided to us by the sponsor with embryos of the Pacific oyster (*Crassostrea gigas*) under the test conditions described below.

<Test Conditions>

1) Test substance:	Test plate coated with AQUATERRACE
2) Test organism:	Pacific oyster (*Crassostrea gigas*)
3) Test duration:	48 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:	15-30 embryos/mL
9) Number of replicates:	3 replicates/section
10) Test type:	Static
11) Temperature:	20±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 observed effect concentration (NOEC): 31.4 cm^2/L by painted area
No inhibition of growth of Pacific oyster (*Crassostrea gigas*) embryos was observed in the test section. Therefore, the test plate coated with AQUATERRACE showed no toxicity to the test organisms during the test period (P>0.05).

FINAL REPORT

1. Study Title

Impact assessment study for marine organisms on a test plate coated with AQUATERRAS —Saltwater Bivalve 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 embryos of saltwater bivalve.

5. Test Period

Start date: September 11, 2017
End date: September 13, 2017

6. Study manager: Tomoharu Nakamura (Laboratory Manager)

7. Experimental Staff: Jun-ichi Ueno (Deputy Manager, Technical Fellow)
Yuusuke Ishida (Research Engineer of Bivalve production section)
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

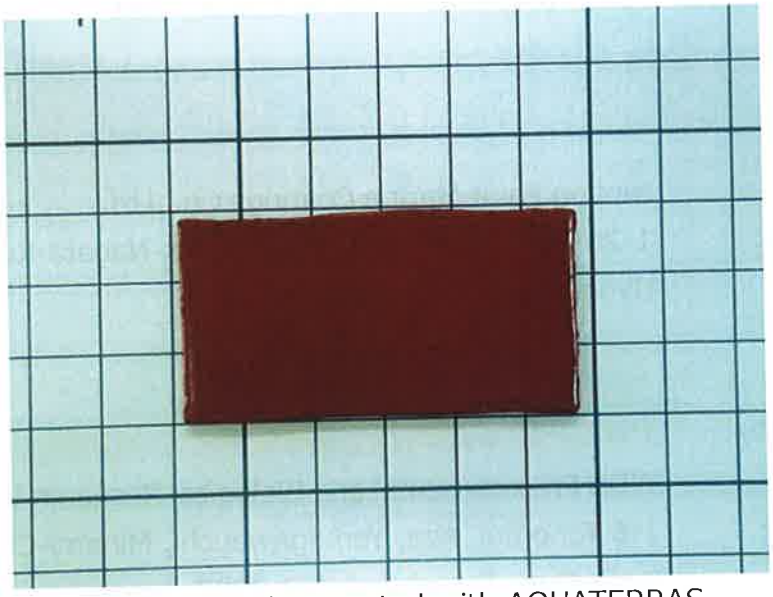

Fig. 1. Test plate coated with AQUATERRAS

9. Materials and Methods

1) Test organism

(1) Common name: Pacific oyster

(2) Scientific name: *Crassostrea gigas*

(3) Source: In-house production

(4) Other: Within 4 hours of fertilization, 2- to 8-cell stages

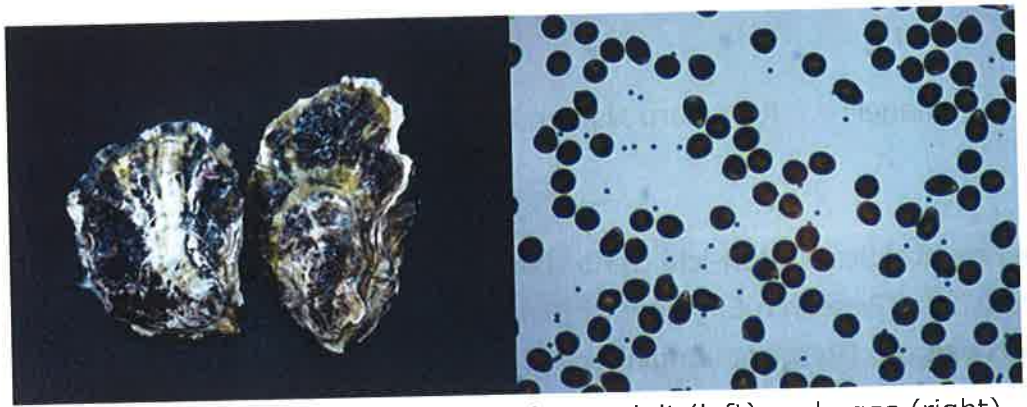

Fig. 2. Pacific oyster *Crassostrea gigas*, adult (left) and eggs (right)

2) Testing devices

Test vessel:	1-L glass beaker
Lighting system:	Fluorescent lighting with timer
Temperature control:	Thermostatic room

3) Test conditions

Test type:	Static
Test duration:	48 hours
Test water:	Filtered dilution water (20±2‰)
Test volume:	1 L
Test concentration*:	31.4 cm^2/L by painted area
Test section:	Addition section and control section
Number of test organisms:	15-30 embryos/mL
Number of replicates:	3 replicates/section
Temperature:	20±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 the 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) Observation of embryonic development

The state of embryonic development was observed using a biological microscope, and the percentage of embryos with normal development was calculated at 24, 48, 72, and 96 hours.

(2) Measurement of test conditions

Water quality parameters (temperature, dissolved oxygen, and pH) during the test were measured at 24, 48, 72, and 96 hours.

6) Treatment of results

The average percentage of embryos with normal development in the addition section and the control section were calculated and plotted against time.

(1) NOEC

In the case that no significant difference (5% level) was observed between the addition and control sections in the statistical analysis, the arbitrary addition amount was taken as the NOEC (NOEC ≥ addition amount) for the limit test.

10. Test Results

1) Percentage of embryos with normal development

At the end of the test period, the percentage of embryos with normal development in the control section was 82.03%. Inhibition of embryo development was not observed in the addition section. This means that the percentage of embryos with normal development in the addition section was almost the same as that in the control section (Table 1, Fig. 3).

Based on the results of the statistical analysis (*F*-test and t-test), the NOEC based on growth was ≥31.4 cm^2/L (P>0.05).

Table 1. Changes in *Crassostrea gigas* embryo development

Section	No.	At the start		24 h		48 h	
		Normal (No. embryos)	%	Normal (No. embryos)	%	Normal (No. embryos)	%
Test beakers	1	19 (19)	100.00	16 (22)	72.73	16 (22)	72.73
	2	27 (28)	96.43	18 (22)	81.82	21 (26)	80.77
	3	22 (22)	100.00	20 (24)	83.33	18 (22)	77.27
	Average	22.67 (23.00)	98.81	18.00 (22.67)	79.29	18.33 (23.33)	76.92
	SD	4.04 (4.58)	2.06	2.00 (1.15)	5.74	2.52 (2.31)	4.03
Control	1	22 (22)	100.00	17 (19)	89.47	19 (23)	82.61
	2	19 (20)	95.00	19 (21)	90.48	22 (28)	85.71
	3	17 (17)	100.00	16 (21)	76.19	14 (18)	77.78
	Average	19.33 (19.67)	98.33	17.33 (20.33)	85.38	18.33 (23.00)	82.03
	SD	2.52 (2.52)	2.89	1.53 (1.15)	7.97	4.04 (5.00)	4.00

SD, Standard deviation

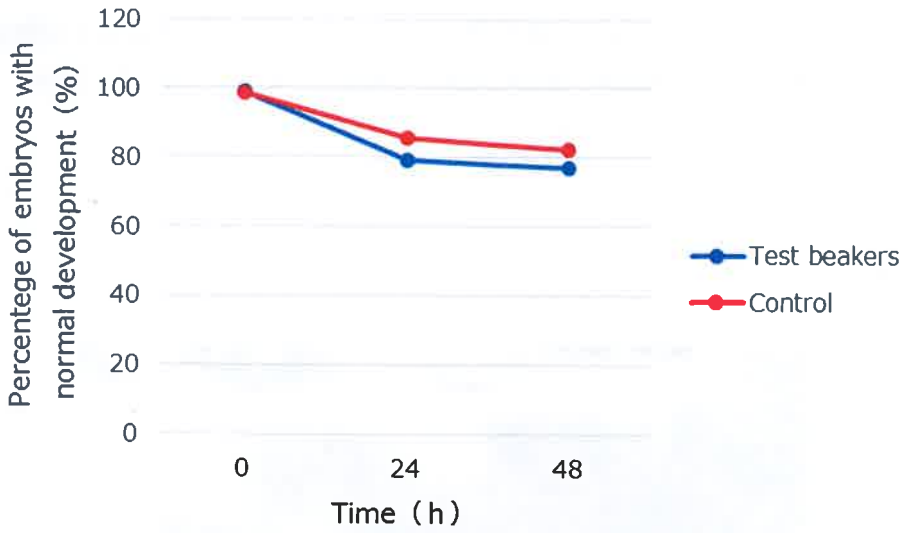

Fig. 3. Percentage of *Crassostrea gigas* embryos with normal development

2) Water quality

Water quality parameters (temperature, dissolved oxygen, and pH) in the test solution during the test period are shown in Table 2.

Table 2. Dissolved oxygen (DO, mg/L) and pH and water temperature (°C)

Time	Section	At the start	24 h	48 h
DO	Test beakers	7.4	7.1	6.9
	Control	7.3	7.1	6.9
pH	Test beakers	7.9	8.0	7.9
	Control	7.9	7.9	7.9
°C	Test beakers	20.1	20.1	20.2
	Control	20.1	20.1	20.2

3) 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 percentage of embryos with normal development in the control section was more than 70%, and the water quality and conditions were good. Therefore, the validity of this test was confirmed.

12. References

- United States Environmental Protection Agency. Ecological Effects Test Guidelines. OCSPP 850.1055: Bivalve Acute Toxicity Test (Embryo-Larval) (2016).

13. Images

Fig. 4. Overhead view of the test