



**CMA Testing  
and Certification  
Laboratories**  
廠商會檢定中心

**Term Contract for Provision of Sampling and Analyzing of  
Wastewater and Sludge Samples for Various Sewage  
Treatment Facilities and Marine Water Samples in Urban  
Area, Lantau and Outlying Islands to the Drainage Services  
Department**

**Whole Effluent Toxicity Test (WETT) at SCISTW  
Report for the Month of August 2020**

Contract No. : DE/2018/02

Applicant : DRAINAGE SERVICES DEPT. - DIVISION 2

Address : STONECUTTERS ISLAND SEWAGE  
TREATMENT WORKS, NGONG SHUEN CHAU,  
KOWLOON, HONG KONG

Application No. : LZ014562(7)

Report No. : AZ0038170(8)

Report Issued Date : 28 Aug 2020

*For and on behalf of*  
CMA Industrial Development Foundation Limited

Authorized Signature :

Lau Yan Kin  
Senior Manager  
Environmental Division



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## 1. Introduction

### 1.1. Background

The whole effluent toxicity tests (WETT) were carried out under the requirements of Drainage Service Department (DSD).

### 1.2. Testing laboratory and investigator

The following tests were carried out in the Coastal Marine Laboratory (CML), Hong Kong University of Science and Technology.

**Principle investigator:** Prof. Wen-Xiong WANG

**Phone number:** (852) 2358-7346

**Fax number:** (852) 2358-1552

**Address:** Department of Ocean Science, Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong

### 1.3. Sample

A 24-hour flow-weighted composite effluent sample was collected from Stonecutters Island Sewage Treatment Works (SCISTW) on July 30, 2020. Effluent sample was shipped immediately to the testing laboratory on the same day of collection and stored at 4 °C until use.

### 1.4. Test species

The following test species were included in the WETT:

- Amphipod (*Melita longidactyla*)
- Fish (*Lutjanus malabaricus*)
- Barnacle larvae (*Balanus amphitrite*)
- Diatom (*Skeletonema costatum*)
- Shrimp (*Metapenaeus ensis*)

### 1.5. Test protocols

The WETT testing methods and procedures follow those documented in “Consultancy Study on Fisheries and Marine Ecological Criteria for Impact Assessment-Final Report” commissioned by Agriculture, Fisheries and Conservation Department (AFCD), as indicated in tender addendum No. 1 by Drainage Services Department (DSD).



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## 2. Report on Amphipod Acute Toxicity Test



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## Test report

### 2.1. Samples storage and pretreatment

Effluent sample was thoroughly mixed and passed through 2-mm mesh to remove the large debris. Effluent was added with ocean salt in order to raise the salinity to the required level (30‰) and then aerated moderately such that the dissolved oxygen (DO) reached saturation prior to use. Salinity control was set up to monitor if there was adverse effect on the test organisms.

### 2.2. Test organism

Species: Amphipod (*Melita longidactyla*).  
Source: Collected from local coastal waters from Sai Kung  
Size/age: 0.5-0.7 cm  
Acclimatization: Acclimatized in fully aerated seawater (temperature: 22±1°C, salinity: 30‰) at least 48 hours in the laboratory prior to test. Fed with green algae *Ulva lactuca*.

### 2.3. Summary of test conditions

Type of test: Static  
Duration: 48 h, 30/7/2020-1/8/2020  
Dilution seawater source: Seawater collected from a pristine site in Clear Water Bay, Sai Kung, Hong Kong  
Dilution seawater pretreatment: Filtered through 0.22 µm membrane  
Testing temperature: 22±1 °C  
Lighting: Continuous  
Salinity: 30‰  
Testing chamber: Pre-cleaned 150 mL glass flask  
Feeding: None  
Number of organisms per replicate: 10  
Replicate number: 4  
Volume of test medium: 100 mL  
Aeration: Moderate, around 100 bubbles/min  
Reference toxicant: CdCl<sub>2</sub>  
Positive control: 48 h acute toxicity test  
Salinity control: Prepared with ocean salt adding into de-ionized water, salinity: 30‰



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**2.4. Test results**

Table 1. Survival of amphipods after 48 hours.

| Treatment        | Effluent concentration (%) | Number of living amphipods after 48 hour (individuals) |             |             |             |      |      |
|------------------|----------------------------|--|-------------|-------------|-------------|------|------|
|                  |                            | Replicate 1  | Replicate 2 | Replicate 3 | Replicate 4 | Mean | SD   |
| Negative control | 0                          | 10   | 10          | 9           | 10          | 9.75 | 0.50 |
| Salinity control | 0                          | 10   | 9           | 10          | 10          | 9.75 | 0.50 |
| Concentration 1  | 6.5                        | 10   | 9           | 9           | 9           | 9.25 | 0.50 |
| Concentration 2  | 12.5                       | 8  | 9           | 8           | 9           | 8.50 | 0.58 |
| Concentration 3  | 25                         | 7  | 8           | 8           | 6           | 7.25 | 0.96 |
| Concentration 4  | 50                         | 4  | 5           | 3           | 5           | 4.25 | 0.96 |
| Concentration 5  | 100                        | 0  | 0           | 0           | 0           | 0.00 | 0.00 |



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Table 2. Survival percentage of amphipods after 48 hours.

| Treatment        | Effluent concentration (%) | Percentage of living amphipods after 48 hour (%) |             |             |             |       |      |
|------------------|----------------------------|--|-------------|-------------|-------------|-------|------|
|                  |                            | Replicate 1                                      | Replicate 2 | Replicate 3 | Replicate 4 | Mean  | SD   |
| Negative control | 0                          | 100  | 100         | 90          | 100         | 97.50 | 5.00 |
| Salinity control | 0                          | 100  | 90          | 100         | 100         | 97.50 | 5.00 |
| Concentration 1  | 6.5                        | 100  | 90          | 90          | 90          | 92.50 | 5.00 |
| Concentration 2  | 12.5                       | 80   | 90          | 80          | 90          | 85.00 | 5.77 |
| Concentration 3  | 25                         | 70   | 80          | 80          | 60          | 72.50 | 9.57 |
| Concentration 4  | 50                         | 40   | 50          | 30          | 50          | 42.50 | 9.57 |
| Concentration 5  | 100                        | 0  | 0           | 0           | 0           | 0.00  | 0.00 |





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**2.5 Summary of water quality parameters monitoring during test.**

Table 3. Summary of water quality parameters during amphipod acute toxicity test.

| Water quality parameters                                 | Effluent concentration (%) |                  |           |           |           |           |           |
|--|----------------------------|------------------|-----------|-----------|-----------|-----------|-----------|
|  | Negative control           | Salinity control | 6.5       | 12.5      | 25        | 50        | 100       |
| Salinity (‰)   | 30.0                       | 30.0             | 30.0      | 30.0      | 30.0      | 30.0      | 30.0      |
| Dissolved oxygen (mg L <sup>-1</sup> )                   | 7.0-7.4                    | 7.0-7.1          | 6.9-7.1   | 6.9-7.1   | 6.9-7.2   | 7.0-7.1   | 6.8-7.0   |
| Temperature (°C)   | 22.0                       | 22.0             | 22.0      | 22.0      | 22.0      | 22.0      | 22.0      |
| pH   | 7.9-8.0                    | 7.9-8.1          | 7.9-8.1   | 7.9-8.1   | 7.9-8.1   | 7.7-7.9   | 7.7-8.0   |
| Total ammonia (start/end, mg L <sup>-1</sup> )           | <0.01/<0.01                | 0.05/0.04        | 1.52/1.63 | 3.10/3.06 | 5.07/5.29 | 12.8/13.6 | 23.1/24.3 |
| Total sulfide (start/end, mg L <sup>-1</sup> )           | <0.1                       | <0.1             | <0.1      | <0.1      | <0.1      | <0.1      | <0.1      |
| Total residual chlorine (start/end, mg L <sup>-1</sup> ) | <0.02                      | <0.02            | <0.02     | <0.02     | <0.02     | <0.02     | <0.02     |
| Total suspended solid (start/end, mg L <sup>-1</sup> )   | <2                         | <2               | <2        | <2        | <2        | <2        | <2        |



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**2.5. LC<sub>50</sub> for the amphipod *Melita longidactyla* and test acceptability**

Table 4. LC<sub>50</sub> for the amphipods and test acceptability.

| Parameter  | Value                        | Control limit                              |
|--|------------------------------|--|
| Calculated LC <sub>50</sub>                        | 40.89%                       | NA   |
| Negative control survival                          | 97.50%                       | >90%                                       |
| Reference toxicant 48-h acute test                 | 1.30 mg L <sup>-1</sup>      | 1.25±0.15 mg L <sup>-1</sup>               |
| 95% of confidence range of reference toxicant test | 1.11-1.42 mg L <sup>-1</sup> | NA   |
| Daily temperature variation                        | <0.5 °C                      | Average daily temperature variation: ±1 °C |
| Dissolved oxygen concentration                     | >6.8 mg L <sup>-1</sup>      | >4 mg L <sup>-1</sup>                      |

NA: Not applicable



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## 3. Report on Fish Acute Toxicity Test



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## Test report

### 3.1. Samples storage and pretreatment

Effluent sample was thoroughly mixed and passed through 2-mm mesh to remove large debris. Effluent was added with ocean salt in order to raise the salinity to the required level (30‰) and then aerated moderately such that the dissolved oxygen (DO) reached saturation prior to use. Salinity control was set up to monitor if there was adverse effect on the test organisms.

### 3.2. Test organism

Species: Fish (*Lutjanus malabaricus*)  
Source: Purchased from local contracted fish farm  
Size/age: 2-3 cm  
Acclimatization: Acclimatized in fully aerated seawater (temperature: 22±1°C, salinity: 30‰) at least 48 hours in laboratory prior to test. Fed with fresh shrimp purchased from local market.

### 3.3. Summary of test conditions

Type of test: Static  
Duration: 48 h, 30/7/2020-1/8/2020  
Dilution seawater source: Seawater collected from a pristine site in Clear Water Bay, Sai Kung, Hong Kong  
Dilution seawater pretreatment: Filtered through 5 µm filtration bag  
Testing temperature: 22±1 °C  
Lighting: Continuous  
Salinity: 30‰  
Testing chamber: Pre-cleaned 20 L tank  
Feeding: None  
Number of organisms per replicate: 20  
Replicate number: 4  
Volume of test medium: 20 L  
Aeration: Moderate, with air stone  
Reference toxicant: CdCl<sub>2</sub>  
Positive control: 48 h acute toxicity test  
Salinity control: Prepared with ocean salt adding into de-ionized water, salinity: 30‰



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**3.4. Test results**

Table 1. Survival of fish after 48 hours.

| Treatment        | Effluent concentration (%) | Number of living fish after 48 hour (individuals) |             |             |             |       |      |
|------------------|----------------------------|---|-------------|-------------|-------------|-------|------|
|                  |                            | Replicate 1                                       | Replicate 2 | Replicate 3 | Replicate 4 | Mean  | SD   |
| Negative control | 0                          | 20  | 20          | 18          | 18          | 19.00 | 1.15 |
| Salinity control | 0                          | 18  | 18          | 18          | 20          | 18.50 | 1.00 |
| Concentration 1  | 6.5                        | 20  | 16          | 16          | 16          | 17.00 | 2.00 |
| Concentration 2  | 12.5                       | 14  | 16          | 12          | 12          | 13.50 | 1.91 |
| Concentration 3  | 25                         | 12  | 10          | 10          | 12          | 11.00 | 1.15 |
| Concentration 4  | 50                         | 8   | 6           | 4           | 4           | 5.50  | 1.91 |
| Concentration 5  | 100                        | 0   | 2           | 0           | 2           | 1.00  | 1.15 |



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Table 2. Survival percentage of fish after 48 hours.

| Treatment        | Effluent concentration (%) | Percentage of living fish after 48 hour (%) |             |             |             |       |       |
|------------------|----------------------------|---|-------------|-------------|-------------|-------|-------|
|                  |                            | Replicate 1                                 | Replicate 2 | Replicate 3 | Replicate 4 | Mean  | SD    |
| Negative control | 0                          | 100   | 100         | 90          | 90          | 95.00 | 5.77  |
| Salinity control | 0                          | 90  | 90          | 90          | 100         | 92.50 | 5.00  |
| Concentration 1  | 6.5                        | 100   | 80          | 80          | 80          | 85.00 | 10.00 |
| Concentration 2  | 12.5                       | 70  | 80          | 60          | 60          | 67.50 | 9.57  |
| Concentration 3  | 25                         | 60  | 50          | 50          | 60          | 55.00 | 5.77  |
| Concentration 4  | 50                         | 40  | 30          | 20          | 20          | 27.50 | 9.57  |
| Concentration 5  | 100                        | 0   | 10          | 0           | 10          | 5.00  | 5.77  |



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**3.5. Summary of water quality parameters monitoring during test**

Table 3. Summary of water quality parameters during fish acute toxicity test.

| Water quality parameters                                 | Effluent concentration (%) |                  |           |           |           |           |           |
|--|----------------------------|------------------|-----------|-----------|-----------|-----------|-----------|
|  | Negative control           | Salinity control | 6.5       | 12.5      | 25        | 50        | 100       |
| Salinity (‰)   | 30                         | 30               | 30        | 30        | 30        | 30        | 30        |
| Dissolved oxygen (mg L <sup>-1</sup> )                   | 6.8-6.9                    | 6.7-6.9          | 6.8-6.9   | 6.8-6.9   | 6.8-6.9   | 6.7-6.9   | 6.7-6.9   |
| Temperature (°C)   | 22                         | 22               | 22        | 22        | 22        | 22        | 22        |
| pH   | 7.8-7.9                    | 7.8-8.0          | 7.8-8.0   | 7.8-7.9   | 7.8-7.9   | 7.8-8.0   | 7.7-7.8   |
| Total ammonia (start/end, mg L <sup>-1</sup> )           | 0.14/0.22                  | 0.16/0.26        | 1.68/1.93 | 3.12/3.65 | 6.54/7.02 | 14.5/15.2 | 30.6/33.4 |
| Total sulfide (start/end, mg L <sup>-1</sup> )           | <0.1                       | <0.1             | <0.1      | <0.1      | <0.1      | <0.1      | <0.1      |
| Total residual chlorine (start/end, mg L <sup>-1</sup> ) | <0.02                      | <0.02            | <0.02     | <0.02     | <0.02     | <0.02     | <0.02     |
| Total suspended solid (mg L <sup>-1</sup> )              | 6/6                        | 7/8              | 12/18     | 16/17     | 28/30     | 37/42     | 61/65     |



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**3.6. LC<sub>50</sub> for the fish *Lutjanus malabaricus* and test acceptability**

Table 4. LC<sub>50</sub> for the fish and test acceptability.

a: The mortalities in all concentration groups were less than 50% of that in control group and thus LC<sub>50</sub> cannot be calculated.

| Parameter  | Value                          | Control limit                               |
|--|--------------------------------|---|
| Calculated LC <sub>50</sub>                        | 27.35%                         | NA  |
| Negative survival                                  | 95.00%                         | >90%  |
| Reference toxicant 48-h acute test                 | 15.15 mg L <sup>-1</sup>       | 14.6±1.78 mg L <sup>-1</sup>                |
| 95% of confidence range of reference toxicant test | 12.56-16.49 mg L <sup>-1</sup> | NA  |
| Daily temperature variation                        | <0.5 °C                        | Average daily temperature variation: ± 1 °C |
| Dissolved oxygen concentration                     | >6.7 mg L <sup>-1</sup>        | >4 mg L <sup>-1</sup>                       |

calculated.

NA: Not applicable





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## 4. Report on Barnacle Larvae Acute Toxicity Test



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## Test report

### 4.1. Samples storage and pretreatment

Effluent sample was thoroughly mixed and passed through 5 µm membrane filter to remove large debris. Effluent was added with ocean salt in order to raise the salinity to the required level (30‰) and then aerated moderately to dissolved oxygen (DO) saturation prior to use. Salinity control was set up to monitor if there was adverse effect on the test organisms.

### 4.2. Test organism

Species: Barnacle larvae (*Balanus amphitrite*).  
Source: Introduced from adult barnacles collected from Sai Kung  
Size/age: Stage II  
Acclimatization: Acclimatized in fully aerated seawater held in 500 mL glass beaker (temperature: 22±1°C, salinity: 30‰) for at least 24 hours in laboratory prior to test. Fed with diatom *Chaetoceros gracilis*.

### 4.3. Summary of test conditions

Type of test: Static  
Duration: 48 h, 30/7/2020-1/8/2020  
Dilution seawater source: Seawater collected from a pristine site in Clear Water Bay, Sai Kung, Hong Kong  
Dilution seawater pretreatment: Filtered through 0.22 µm membrane  
Testing temperature: 22±1 °C  
Lighting: Continuous  
Salinity: 30‰  
Testing chamber: Pre-cleaned 50 mL glass beaker  
Feeding: None  
Number of organisms per replicate: 20  
Replicate number: 4  
Volume of test medium: 20 mL  
Aeration: Moderate, around 100 bubbles/min  
Reference toxicant: CdCl<sub>2</sub>  
Positive control: 48 h acute toxicity test  
Salinity control: Prepared with ocean salt adding into de-ionized water, salinity: 30‰



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**4.4. Test results**

Table 1. Survival of barnacle larvae after 48 hours

| Treatment        | Effluent concentration (%) | Number of living barnacle larvae after 48 hour (individuals) |             |             |             |       |      |
|------------------|----------------------------|--|-------------|-------------|-------------|-------|------|
|                  |                            | Replicate 1  | Replicate 2 | Replicate 3 | Replicate 4 | Mean  | SD   |
| Negative control | 0                          | 20   | 20          | 20          | 19          | 19.75 | 0.50 |
| Salinity control | 0                          | 20   | 20          | 20          | 18          | 19.50 | 1.00 |
| Concentration 1  | 6.5                        | 16   | 18          | 16          | 15          | 16.25 | 1.26 |
| Concentration 2  | 12.5                       | 14   | 10          | 12          | 12          | 12.00 | 1.63 |
| Concentration 3  | 25                         | 8  | 7           | 5           | 5           | 6.25  | 1.50 |
| Concentration 4  | 50                         | 4  | 2           | 0           | 1           | 1.75  | 1.71 |
| Concentration 5  | 100                        | 0  | 0           | 0           | 0           | 0.00  | 0.00 |



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Table 2. Survival percentage of barnacle larvae after 48 hours.

| Treatment        | Effluent concentration (%) | Percentage of living barnacle larvae after 48 hour (%) |             |             |             |       |      |
|------------------|----------------------------|--|-------------|-------------|-------------|-------|------|
|                  |                            | Replicate 1  | Replicate 2 | Replicate 3 | Replicate 4 | Mean  | SD   |
| Negative control | 0                          | 100  | 100         | 100         | 95          | 98.75 | 2.50 |
| Salinity control | 0                          | 100  | 100         | 100         | 90          | 97.50 | 5.00 |
| Concentration 1  | 6.5                        | 80   | 90          | 80          | 75          | 81.25 | 6.29 |
| Concentration 2  | 12.5                       | 70   | 50          | 60          | 60          | 60.00 | 8.16 |
| Concentration 3  | 25                         | 40   | 35          | 25          | 25          | 31.25 | 7.50 |
| Concentration 4  | 50                         | 20   | 10          | 0           | 5           | 8.75  | 8.54 |
| Concentration 5  | 100                        | 0.   | 0           | 0           | 0           | 0.00  | 0.00 |



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**4.5. Summary of water quality parameters monitoring during test**

Table 3. Summary of water quality parameters during barnacle larvae acute toxicity test

| Water quality parameters                                 | Effluent concentration (%) |                  |           |           |           |           |           |
|--|----------------------------|------------------|-----------|-----------|-----------|-----------|-----------|
|  | Negative control           | Salinity control | 6.5       | 12.5      | 25        | 50        | 100       |
| Salinity (‰)   | 30.0                       | 30.0             | 30.0      | 30.0      | 30.0      | 30.0      | 30.0      |
| Dissolved oxygen (mg L <sup>-1</sup> )                   | 6.8-7.0                    | 6.9-7.1          | 6.9-7.0   | 6.9-7.1   | 7.0-7.7   | 6.9-7.0   | 7.0-7.0   |
| Temperature (°C)   | 22.0                       | 22.0             | 22.0      | 22.0      | 22.0      | 22.0      | 22.0      |
| pH   | 7.9-8.0                    | 7.8-8.0          | 7.9-8.0   | 7.8-8.0   | 7.8-7.9   | 7.7-7.9   | 7.7-7.9   |
| Total ammonia (start/end, mg L <sup>-1</sup> )           | 0.04/0.05                  | 0.06/0.08        | 1.93/2.17 | 3.67/4.06 | 5.94/6.37 | 12.6/14.6 | 22.4/23.5 |
| Total sulfide (start/end, mg L <sup>-1</sup> )           | <0.1                       | <0.1             | <0.1      | <0.1      | <0.1      | <0.1      | <0.1      |
| Total residual chlorine (start/end, mg L <sup>-1</sup> ) | <0.02                      | <0.02            | <0.02     | <0.02     | <0.02     | <0.02     | <0.02     |
| Total suspended solid (mg L <sup>-1</sup> )              | <2                         | <2               | <2        | <2        | <2        | <2        | <2        |



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**4.6. LC<sub>50</sub> for the barnacle larvae *Balanus amphitrite* and test acceptability**

Table 4. LC<sub>50</sub> for the barnacle larvae and test acceptability

| Parameter   | Value                        | Control limit                               |
|---|------------------------------|---|
| Calculated LC <sub>50</sub>                         | 15.82 %                      | NA  |
| Negative survival                                   | 98.75 %                      | >90%  |
| Reference toxicant 48-h acute test                  | 1.01 mg L <sup>-1</sup>      | 1.04±0.11 mg L <sup>-1</sup>                |
| 95% of confidence range of reference toxicant test: | 0.89-1.12 mg L <sup>-1</sup> | NA  |
| Daily temperature variation                         | <0.5°C                       | Average daily temperature variation: ± 1 °C |
| Dissolved oxygen concentration                      | >6.8 mg L <sup>-1</sup>      | >4 mg L <sup>-1</sup>                       |

NA: Not applicable



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## 5. Report on Diatom Growth Inhibition Test (Chronic toxicity test)



Report No. : AZ0038170(8)

Application No.: LZ014562(7)

## Test report

### 5.1. Samples storage and pretreatment

Effluent sample was thoroughly mixed and passed through 5 µm membrane filter to remove large debris. Effluent was added with ocean salt in order to raise the salinity to the required level (30‰) and then aerated moderately to dissolved oxygen (DO) saturation prior to use. Salinity control was set up to monitor if there was adverse effect on the test organisms.

### 5.2. Test organism

Species: Diatom (*Skeletonema costatum*)  
Source: Grown from laboratory culture obtained from Coastal Marine Lab, Hong Kong University of Science and Technology  
Size/age: Log growth phase  
Acclimatization: Grown in 250 mL glass flask (temperature: 22±1°C, salinity: 30‰, 3000 lux) for at least two weeks prior to test.

### 5.3. Summary of test conditions

Type of test: Static  
Duration: 7 days, 30/7/2020-6/8/2020  
Dilution seawater source: Seawater collected from a pristine site in Clear Water Bay, Sai Kung, Hong Kong  
Dilution seawater pretreatment: Filtered through 0.22 µm membrane  
Testing temperature: 22±1 °C  
Lighting: 12 h light/12 h dark cycle, 3000±500 lux  
Salinity: 30‰  
Testing chamber: Pre-cleaned 100 mL glass beaker  
Initial cell density:  $(5.0\pm 0.4)\times 10^4$  cell mL<sup>-1</sup>  
Replicate number: 4  
Volume of test medium: 25 mL  
Aeration: None  
Reference toxicant: CdCl<sub>2</sub>  
Positive control: 7-day IC<sub>50</sub> toxicity test  
Salinity control: Prepared with ocean salt adding into de-ionized water, salinity: 30‰





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**5.4. Test results**

Table 1. Cell density of diatom *Skeletonema costatum* at the beginning and end of growth inhibition test. Initial cell density:  $(5.0 \pm 0.4) \times 10^4$  cell mL<sup>-1</sup>.

| Treatment        | Effluent concentration (%) | Cell density after 7-day growth ( $\times 10^6$ cell mL <sup>-1</sup> ) |             |             |             |      |      |
|------------------|----------------------------|---|-------------|-------------|-------------|------|------|
|                  |                            | Replicate 1   | Replicate 2 | Replicate 3 | Replicate 4 | Mean | SD   |
| Negative control | 0                          | 1.06  | 0.99        | 1.01        | 1.12        | 1.05 | 0.06 |
| Salinity control | 0                          | 1.05  | 1.08        | 1.03        | 1.00        | 1.04 | 0.03 |
| Concentration 1  | 2.5                        | 1.18  | 1.20        | 1.23        | 1.24        | 1.21 | 0.03 |
| Concentration 2  | 5                          | 1.25  | 1.32        | 1.35        | 1.27        | 1.30 | 0.05 |
| Concentration 3  | 10                         | 1.09  | 1.11        | 1.06        | 1.02        | 1.07 | 0.04 |
| Concentration 4  | 25                         | 0.98  | 0.89        | 0.92        | 0.91        | 0.93 | 0.04 |
| Concentration 5  | 50                         | 0.62  | 0.43        | 0.36        | 0.40        | 0.45 | 0.12 |
| Concentration 6  | 100                        | 0.01  | 0.01        | 0.00        | 0.01        | 0.01 | 0.01 |



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Table 2. Growth rate of *Skeletonema costatum* within 7 days.

| Treatment        | Effluent concentration (%) | 7-day average growth rate (d <sup>-1</sup> ) |             |             |             |      |      |
|------------------|----------------------------|--|-------------|-------------|-------------|------|------|
|                  |                            | Replicate 1                                  | Replicate 2 | Replicate 3 | Replicate 4 | Mean | SD   |
| Negative control | 0                          | 0.44   | 0.43        | 0.43        | 0.44        | 0.43 | 0.01 |
| Salinity control | 0                          | 0.43   | 0.44        | 0.43        | 0.43        | 0.43 | 0.00 |
| Concentration 1  | 2.5                        | 0.45   | 0.45        | 0.46        | 0.46        | 0.46 | 0.00 |
| Concentration 2  | 5                          | 0.46   | 0.47        | 0.47        | 0.46        | 0.47 | 0.01 |
| Concentration 3  | 10                         | 0.44   | 0.44        | 0.44        | 0.43        | 0.44 | 0.01 |
| Concentration 4  | 25                         | 0.43   | 0.41        | 0.42        | 0.41        | 0.42 | 0.01 |
| Concentration 5  | 50                         | 0.36   | 0.31        | 0.28        | 0.30        | 0.31 | 0.03 |
| Concentration 6  | 100                        | 0.00   | 0.00        | 0.00        | 0.00        | 0.00 | 0.00 |



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**5.5. Summary of water quality parameters monitoring during test**

Table 3. Summary of water quality parameters during diatom growth inhibition test

| Water quality parameters                                 | Effluent concentration (%) |                  |           |           |           |           |           |           |
|--|----------------------------|------------------|-----------|-----------|-----------|-----------|-----------|-----------|
|  | Negative control           | Salinity control | 2.5       | 5.0       | 10        | 25        | 50        | 100       |
| Salinity (‰)   | 30.0                       | 30.0             | 30.0      | 30.0      | 30.0      | 30.0      | 30.0      | 30.0      |
| Dissolved oxygen (mg L <sup>-1</sup> )                   | 6.8-7.8                    | 6.8-7.9          | 6.8-7.9   | 6.8-8.0   | 6.8-8.1   | 6.8-7.9   | 6.7-7.9   | 6.7-7.1   |
| Temperature (°C)   | 22.0                       | 22.0             | 22.0      | 22.0      | 22.0      | 22.0      | 22.0      | 22.0      |
| pH   | 7.9-8.1                    | 7.9-8.1          | 7.9-8.1   | 7.9-8.1   | 7.9-8.2   | 7.8-8.2   | 7.7-8.1   | 7.7-8.0   |
| Total ammonia (start/end, mg L <sup>-1</sup> )           | 0.03/0.03                  | 0.05/0.04        | 1.37/1.43 | 2.44/2.68 | 4.62/4.71 | 7.37/7.56 | 12.5/12.7 | 24.2/25.4 |
| Total sulfide (start/end, mg L <sup>-1</sup> )           | <0.1                       | <0.1             | <0.1      | <0.1      | <0.1      | <0.1      | <0.1      | <0.1      |
| Total residual chlorine (start/end, mg L <sup>-1</sup> ) | <0.02                      | <0.02            | <0.02     | <0.02     | <0.02     | <0.02     | <0.02     | <0.02     |
| Total suspended solid (mg L <sup>-1</sup> )              | <2                         | <2               | <2        | <2        | <2        | <2        | <2        | <2        |



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**5.6. IC<sub>50</sub> for the diatom *Skeletonema costatum* and test acceptability**

Table 4. IC<sub>50</sub>, none observed effect concentration (NOEC) for the diatom and test acceptability

| Parameter  | Value                        | Control limit                               |
|--|------------------------------|---|
| Calculated IC <sub>50</sub>                        | 42.64%                       | NA  |
| None observed effect concentration (NOEC)          | 10%                          | -   |
| Reference toxicant 7-day test:                     | 0.12 mg L <sup>-1</sup>      | 0.13±0.02 mg L <sup>-1</sup>                |
| 95% of confidence range of reference toxicant test | 0.10-0.15 mg L <sup>-1</sup> | NA  |
| Temperature variation                              | <0.5 °C                      | Average daily temperature variation: ± 1 °C |

NA: Not applicable



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## 6. Report on Shrimp Acute Toxicity Test



Report No. : AZ0038170(8)

Application No.: LZ014562(7)

## Test report

### 6.1. Samples storage and pretreatment

Effluent sample was thoroughly mixed and passed through 2-mm mesh to remove the large debris. Effluent was added with ocean salt in order to raise the salinity to 25‰ and then aerated moderately such that the dissolved oxygen (DO) reached saturation prior to use. Salinity control was set up to monitor if there was adverse effect on the test organisms.

### 6.2. Test organism

Species: Shrimp (*Metapenaeus ensis*).  
Source: Purchased from contracted fish dealer  
Size/age: 5-7 cm  
Acclimatization: Acclimatized in fully aerated seawater (temperature: 22±1°C, salinity: 25‰) at least 48 hours in the laboratory prior to test. Fed with commercial shrimp feeds.

### 6.3. Summary of test conditions

Type of test: Static  
Duration: 48 h, 30/7/2020-1/8/2020  
Dilution seawater source: Seawater collected from a pristine site in Clear Water Bay, Sai Kung, Hong Kong  
Dilution seawater pretreatment: Filtered through 0.22 µm membrane  
Testing temperature: 22±1 °C  
Lighting: Continuous  
Salinity: 25‰  
Testing chamber: Pre-cleaned 20 L tank  
Feeding: None  
Number of organisms per replicate: 10  
Replicate number: 4  
Volume of test medium: 10 L  
Aeration: Moderate, with air stone  
Reference toxicant: CdCl<sub>2</sub>  
Positive control: 48 h acute toxicity test  
Salinity control: Prepared with ocean salt adding into de-ionized water, salinity: 25‰



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**6.4. Test results**

Table 1. Survival of shrimps after 48 hours.

| Treatment        | Effluent concentration (%) | Number of living shrimps after 48 hour (individuals) |             |             |             |      |      |
|------------------|----------------------------|--|-------------|-------------|-------------|------|------|
|                  |                            | Replicate 1  | Replicate 2 | Replicate 3 | Replicate 4 | Mean | SD   |
| Negative control | 0                          | 10   | 10          | 9           | 10          | 9.75 | 0.50 |
| Salinity control | 0                          | 9  | 9           | 9           | 10          | 9.25 | 0.50 |
| Concentration 1  | 6.5                        | 9  | 8           | 8           | 8           | 8.25 | 0.50 |
| Concentration 2  | 12.5                       | 6  | 5           | 5           | 3           | 4.75 | 1.26 |
| Concentration 3  | 25                         | 4  | 3           | 5           | 4           | 4.00 | 0.82 |
| Concentration 4  | 50                         | 0  | 3           | 1           | 2           | 1.50 | 1.29 |
| Concentration 5  | 100                        | 0  | 0           | 0           | 0           | 0.00 | 0.00 |



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Table 2. Survival percentage of shrimps after 48 hours.

| Treatment        | Effluent concentration (%) | Percentage of living shrimps after 48 hour (%) |             |             |             |       |       |
|------------------|----------------------------|--|-------------|-------------|-------------|-------|-------|
|                  |                            | Replicate 1                                    | Replicate 2 | Replicate 3 | Replicate 4 | Mean  | SD    |
| Negative control | 0                          | 100  | 100         | 90          | 100         | 97.50 | 5.00  |
| Salinity control | 0                          | 90   | 90          | 90          | 100         | 92.50 | 5.00  |
| Concentration 1  | 6.5                        | 90   | 80          | 80          | 80          | 82.50 | 5.00  |
| Concentration 2  | 12.5                       | 60   | 50          | 50          | 30          | 47.50 | 12.58 |
| Concentration 3  | 25                         | 40   | 30          | 50          | 40          | 40.00 | 8.16  |
| Concentration 4  | 50                         | 0  | 30          | 10          | 20          | 15.00 | 12.91 |
| Concentration 5  | 100                        | 0  | 0           | 0           | 0           | 0.00  | 0.00  |





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**6.5. Summary of water quality parameters monitoring during test.**

Table 3. Summary of water quality parameters during shrimp acute toxicity test.

| Water quality parameters                                 | Effluent concentration (%) |                  |           |           |           |           |           |
|--|----------------------------|------------------|-----------|-----------|-----------|-----------|-----------|
|  | Negative control           | Salinity control | 6.5       | 12.5      | 25        | 50        | 100       |
| Salinity (‰)   | 25.0                       | 25.0             | 25.0      | 25.0      | 25.0      | 25.0      | 25.0      |
| Dissolved oxygen (mg L <sup>-1</sup> )                   | 6.8-7.1                    | 6.9-7.1          | 6.9-7.1   | 6.9-7.0   | 6.8-6.9   | 6.8-6.9   | 6.7-6.9   |
| Temperature (°C)   | 22.0                       | 22.0             | 22.0      | 22.0      | 22.0      | 22.0      | 22.0      |
| pH   | 7.9-8.0                    | 7.8-7.9          | 7.8-7.9   | 7.8-8.0   | 7.7-7.9   | 7.7-7.8   | 7.7-7.9   |
| Total ammonia (start/end, mg L <sup>-1</sup> )           | 0.05/0.07                  | 0.05/0.09        | 1.07/1.24 | 2.40/2.66 | 5.87/6.07 | 10.6/11.3 | 22.6/24.0 |
| Total sulfide (start/end, mg L <sup>-1</sup> )           | <0.1                       | <0.1             | <0.1      | <0.1      | <0.1      | <0.1      | <0.1      |
| Total residual chlorine (start/end, mg L <sup>-1</sup> ) | <0.02                      | <0.02            | <0.02     | <0.02     | <0.02     | <0.02     | <0.02     |
| Total suspended solid (start/end, mg L <sup>-1</sup> )   | 8/10                       | 9/9              | 13/16     | 23/24     | 30/36     | 42/49     | 54/60     |



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**6.6 LC<sub>50</sub> for the shrimp *Metapenaeus ensis* and test acceptability**

Table 4. LC<sub>50</sub> for the *Metapenaeus ensis* and test acceptability.

| Parameter  | Value                        | Control limit                              |
|--|------------------------------|--|
| Calculated LC <sub>50</sub>                        | 17.27%                       | NA   |
| Negative control survival                          | 97.50%                       | >90%                                       |
| Reference toxicant 48-h acute test                 | 5.26 mg L <sup>-1</sup>      | 5.19±0.51 mg L <sup>-1</sup>               |
| 95% of confidence range of reference toxicant test | 4.57-5.70 mg L <sup>-1</sup> | NA   |
| Daily temperature variation                        | <0.5°C                       | Average daily temperature variation: ±1 °C |
| Dissolved oxygen concentration                     | >6.7 mg L <sup>-1</sup>      | >4 mg L <sup>-1</sup>                      |

NA: Not applicable



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## 7. Conclusion



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Table 1. Comparison of measured toxicity values with the target toxicity levels.

| Test species                                 | Measured LC <sub>50</sub> /IC <sub>50</sub> /NOEC | Target toxicity level |
|--|---|-----------------------|
| Amphipod<br><i>Melita longidactyla</i>       | 40.89 %   | ≥7.1%                 |
| Fish<br><i>Lutjanus malabaricus</i>          | 27.35 %   | ≥7.1%                 |
| Barnacle larvae<br><i>Balanus amphitrite</i> | 15.82 %   | ≥7.1%                 |
| Diatom<br><i>Skeletonema costatum</i>        | 42.64 %   | -                     |
| Diatom<br><i>Skeletonema costatum</i>        | 10 % (NOEC)                                       | ≥0.51%                |
| Shrimp<br><i>Metapenaeus ensis</i>           | 17.27 %   | ≥7.1%                 |

Conclusion: all the measured values met the target toxicity levels as indicated in the EM&A Manual.



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## **Appendix A**

### **Monitoring Data for Amphipod Acute Toxicity Test**



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Table 1. Dissolved oxygen concentration and pH in each concentration treatment in amphipod acute toxicity test.

| Concentration treatment (%) | Dissolved oxygen (mg L <sup>-1</sup> ) |      |      |      |      | pH  |      |      |      |      |
|-----------------------------|--|------|------|------|------|-----|------|------|------|------|
|                             | 0 h                                    | 12 h | 24 h | 36 h | 48 h | 0 h | 12 h | 24 h | 36 h | 48 h |
| Negative control            | 7.1                                    | 7.1  | 7.4  | 7.2  | 7.0  | 8.0 | 8.0  | 7.9  | 7.9  | 8.0  |
| Salinity control            | 7.0                                    | 7.0  | 7.0  | 7.1  | 7.1  | 7.9 | 8.0  | 7.9  | 7.9  | 8.1  |
| 6.5                         | 6.9                                    | 6.9  | 7.0  | 7.0  | 7.1  | 7.9 | 8.0  | 8.0  | 8.0  | 8.1  |
| 12.5                        | 6.9                                    | 7.0  | 7.0  | 7.0  | 7.1  | 8.0 | 7.9  | 7.9  | 8.1  | 8.0  |
| 25                          | 6.9                                    | 7.1  | 7.1  | 7.1  | 7.2  | 7.9 | 8.1  | 8.0  | 8.1  | 8.0  |
| 50                          | 7.0                                    | 7.1  | 7.0  | 7.0  | 7.0  | 7.7 | 7.7  | 7.9  | 7.9  | 7.9  |
| 100                         | 6.8                                    | 7.0  | 7.0  | 7.0  | 6.9  | 7.7 | 7.8  | 7.9  | 7.9  | 8.0  |



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Table 2. Salinity and temperature in each concentration treatment in amphipod acute toxicity test.

| Concentration treatment (%) | Salinity (‰) |      |      |      |      | Temperature (°C) |      |      |      |      |
|-----------------------------|--------------|------|------|------|------|------------------|------|------|------|------|
|                             | 0 h          | 12 h | 24 h | 36 h | 48 h | 0 h              | 12 h | 24 h | 36 h | 48 h |
| Negative control            | 30.0         | 30.0 | 30.0 | 30.0 | 30.0 | 22.0             | 22.0 | 22.0 | 22.0 | 22.0 |
| Salinity control            | 30.0         | 30.0 | 30.0 | 30.0 | 30.0 | 22.0             | 22.0 | 22.0 | 22.0 | 22.0 |
| 6.5                         | 30.0         | 30.0 | 30.0 | 30.0 | 30.0 | 22.0             | 22.0 | 22.0 | 22.0 | 22.0 |
| 12.5                        | 30.0         | 30.0 | 30.0 | 30.0 | 30.0 | 22.0             | 22.0 | 22.0 | 22.0 | 22.0 |
| 25                          | 30.0         | 30.0 | 30.0 | 30.0 | 30.0 | 22.0             | 22.0 | 22.0 | 22.0 | 22.0 |
| 50                          | 30.0         | 30.0 | 30.0 | 30.0 | 30.0 | 22.0             | 22.0 | 22.0 | 22.0 | 22.0 |
| 100                         | 30.0         | 30.0 | 30.0 | 30.0 | 30.0 | 22.0             | 22.0 | 22.0 | 22.0 | 22.0 |



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Table 3. Ammonia-N, sulphide, total suspended solids, total residual chlorine concentration at the beginning and ending of the amphipod acute toxicity test.

| Concentration treatment (%) | Ammonia-N (mg L <sup>-1</sup> ) |       | Sulphide (mg L <sup>-1</sup> ) |      | Total suspended solids (mg L <sup>-1</sup> ) |     | Total residual chlorine (mg L <sup>-1</sup> ) |       |
|-----------------------------|---------------------------------|-------|--------------------------------|------|--|-----|---|-------|
|                             | Initial                         | End   | Initial                        | End  | Initial                                      | End | Initial                                       | End   |
| Negative control            | <0.01                           | <0.01 | <0.1                           | <0.1 | <2   | <2  | <0.02   | <0.02 |
| Salinity control            | 0.05                            | 0.04  | >0.1                           | <0.1 | <2   | <2  | <0.02   | <0.02 |
| 6.5                         | 1.52                            | 1.63  | >0.1                           | <0.1 | <2   | <2  | <0.02   | <0.02 |
| 12.5                        | 3.10                            | 3.06  | >0.1                           | <0.1 | <2   | <2  | <0.02   | <0.02 |
| 25                          | 5.07                            | 5.29  | >0.1                           | <0.1 | <2   | <2  | <0.02   | <0.02 |
| 50                          | 12.8                            | 13.6  | >0.1                           | <0.1 | <2   | <2  | <0.02   | <0.02 |
| 100                         | 23.1                            | 24.3  | >0.1                           | <0.1 | <2   | <2  | <0.02   | <0.02 |





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## **Appendix B**

### **Monitoring Data for Fish Acute Toxicity Test**



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Table 1. Dissolved oxygen concentration and pH in each concentration treatment in fish acute toxicity test.

| Concentration treatment (%) | Dissolved oxygen (mg L <sup>-1</sup> ) |      |      |      |      | pH  |      |      |      |      |
|-----------------------------|--|------|------|------|------|-----|------|------|------|------|
|                             | 0 h                                    | 12 h | 24 h | 36 h | 48 h | 0 h | 12 h | 24 h | 36 h | 48 h |
| Negative control            | 6.8                                    | 6.8  | 6.8  | 6.8  | 6.9  | 7.9 | 7.8  | 7.8  | 7.8  | 7.9  |
| Salinity control            | 6.8                                    | 6.7  | 6.8  | 6.8  | 6.9  | 7.8 | 8.0  | 7.8  | 7.9  | 7.9  |
| 6.5                         | 6.8                                    | 6.8  | 6.9  | 6.8  | 6.9  | 7.9 | 7.8  | 8.0  | 7.8  | 7.9  |
| 12.5                        | 6.9                                    | 6.8  | 6.8  | 6.9  | 6.8  | 7.9 | 7.9  | 7.9  | 7.9  | 7.8  |
| 25                          | 6.9                                    | 6.8  | 6.9  | 6.9  | 6.9  | 7.9 | 7.9  | 7.8  | 7.9  | 7.8  |
| 50                          | 6.7                                    | 6.8  | 6.8  | 6.9  | 6.9  | 7.8 | 8.0  | 7.9  | 7.8  | 7.8  |
| 100                         | 6.8                                    | 6.7  | 6.7  | 6.8  | 6.9  | 7.7 | 7.8  | 7.8  | 7.7  | 7.7  |



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Report No. : AZ0038170(8)

Application No.: LZ014562(7)

Table 2. Salinity and temperature in each concentration treatment in fish acute toxicity test.

| Concentration treatment (%) | Salinity (‰) |      |      |      |      | Temperature (°C) |      |      |      |      |
|-----------------------------|--------------|------|------|------|------|------------------|------|------|------|------|
|                             | 0 h          | 12 h | 24 h | 36 h | 48 h | 0 h              | 12 h | 24 h | 36 h | 48 h |
| Negative control            | 30.0         | 30.0 | 30.0 | 30.0 | 30.0 | 22.0             | 22.0 | 22.0 | 22.0 | 22.0 |
| Salinity control            | 30.0         | 30.0 | 30.0 | 30.0 | 30.0 | 22.0             | 22.0 | 22.0 | 22.0 | 22.0 |
| 6.5                         | 30.0         | 30.0 | 30.0 | 30.0 | 30.0 | 22.0             | 22.0 | 22.0 | 22.0 | 22.0 |
| 12.5                        | 30.0         | 30.0 | 30.0 | 30.0 | 30.0 | 22.0             | 22.0 | 22.0 | 22.0 | 22.0 |
| 25                          | 30.0         | 30.0 | 30.0 | 30.0 | 30.0 | 22.0             | 22.0 | 22.0 | 22.0 | 22.0 |
| 50                          | 30.0         | 30.0 | 30.0 | 30.0 | 30.0 | 22.0             | 22.0 | 22.0 | 22.0 | 22.0 |
| 100                         | 30.0         | 30.0 | 30.0 | 30.0 | 30.0 | 22.0             | 22.0 | 22.0 | 22.0 | 22.0 |



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Report No. : AZ0038170(8)

Application No.: LZ014562(7)

Table 3. Ammonia-N, sulphide, total suspended solids, total residual chlorine concentration at the beginning and ending of the fish acute toxicity test.

| Concentration treatment (%) | Ammonia-N (mg L <sup>-1</sup> ) |      | Sulphide (mg L <sup>-1</sup> ) |      | Total suspended solids (mg L <sup>-1</sup> ) |     | Total residual chlorine (mg L <sup>-1</sup> ) |       |
|-----------------------------|---------------------------------|------|--------------------------------|------|--|-----|---|-------|
|                             | Initial                         | End  | Initial                        | End  | Initial                                      | End | Initial                                       | End   |
| Negative control            | 0.14                            | 0.22 | <0.1                           | <0.1 | 6  | 6   | <0.02   | <0.02 |
| Salinity control            | 0.16                            | 0.26 | <0.1                           | <0.1 | 7  | 8   | <0.02   | <0.02 |
| 6.5                         | 1.68                            | 1.93 | <0.1                           | <0.1 | 12   | 18  | <0.02   | <0.02 |
| 12.5                        | 3.12                            | 3.65 | <0.1                           | <0.1 | 16   | 17  | <0.02   | <0.02 |
| 25                          | 6.54                            | 7.02 | <0.1                           | <0.1 | 28   | 30  | <0.02   | <0.02 |
| 50                          | 14.5                            | 15.2 | <0.1                           | <0.1 | 37   | 42  | <0.02   | <0.02 |
| 100                         | 30.6                            | 33.4 | <0.1                           | <0.1 | 61   | 65  | <0.02   | <0.02 |



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Application No.: LZ014562(7)

## **Appendix C**

### **Monitoring Data for Barnacle Larvae Acute Toxicity Test**



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Report No. : AZ0038170(8)

Application No.: LZ014562(7)

Table 1. Dissolved oxygen concentration and pH in each concentration treatment in barnacle larvae acute toxicity test.

| Concentration treatment (%) | Dissolved oxygen (mg L <sup>-1</sup> ) |      |      |      |      | pH  |      |      |      |      |
|-----------------------------|--|------|------|------|------|-----|------|------|------|------|
|                             | 0 h                                    | 12 h | 24 h | 36 h | 48 h | 0 h | 12 h | 24 h | 36 h | 48 h |
| Negative control            | 6.8                                    | 6.8  | 6.9  | 7.0  | 7.0  | 8.0 | 7.9  | 7.9  | 7.9  | 8.0  |
| Salinity control            | 6.9                                    | 7.0  | 6.9  | 6.9  | 7.1  | 7.9 | 8.0  | 8.0  | 7.9  | 7.8  |
| 6.5                         | 7.0                                    | 7.0  | 7.0  | 7.0  | 6.9  | 7.9 | 8.0  | 7.9  | 8.0  | 7.9  |
| 12.5                        | 7.1                                    | 6.9  | 7.1  | 7.1  | 7.0  | 8.0 | 7.9  | 7.9  | 7.9  | 7.8  |
| 25                          | 7.0                                    | 7.0  | 7.0  | 7.1  | 7.0  | 7.9 | 7.8  | 7.8  | 7.8  | 7.9  |
| 50                          | 7.0                                    | 6.9  | 7.0  | 7.0  | 7.0  | 7.7 | 7.8  | 7.9  | 7.9  | 7.9  |
| 100                         | 7.0                                    | 7.0  | 7.0  | 7.0  | 7.0  | 7.7 | 7.8  | 7.8  | 7.9  | 7.8  |



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Report No. : AZ0038170(8)

Application No.: LZ014562(7)

Table 2. Salinity and temperature in each concentration treatment in barnacle larvae acute toxicity test.

| Concentration treatment (%) | Salinity (‰) |      |      |      |      | Temperature (°C) |      |      |      |      |
|-----------------------------|--------------|------|------|------|------|------------------|------|------|------|------|
|                             | 0 h          | 12 h | 24 h | 36 h | 48 h | 0 h              | 12 h | 24 h | 36 h | 48 h |
| Negative control            | 30.0         | 30.0 | 30.0 | 30.0 | 30.0 | 22.0             | 22.0 | 22.0 | 22.0 | 22.0 |
| Salinity control            | 30.0         | 30.0 | 30.0 | 30.0 | 30.0 | 22.0             | 22.0 | 22.0 | 22.0 | 22.0 |
| 6.5                         | 30.0         | 30.0 | 30.0 | 30.0 | 30.0 | 22.0             | 22.0 | 22.0 | 22.0 | 22.0 |
| 12.5                        | 30.0         | 30.0 | 30.0 | 30.0 | 30.0 | 22.0             | 22.0 | 22.0 | 22.0 | 22.0 |
| 25                          | 30.0         | 30.0 | 30.0 | 30.0 | 30.0 | 22.0             | 22.0 | 22.0 | 22.0 | 22.0 |
| 50                          | 30.0         | 30.0 | 30.0 | 30.0 | 30.0 | 22.0             | 22.0 | 22.0 | 22.0 | 22.0 |
| 100                         | 30.0         | 30.0 | 30.0 | 30.0 | 30.0 | 22.0             | 22.0 | 22.0 | 22.0 | 22.0 |



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Report No. : AZ0038170(8)

Application No.: LZ014562(7)

Table 3. Ammonia-N, sulphide, total suspended solids, total residual chlorine concentration at the beginning and ending of the barnacle larvae acute toxicity test.

| Concentration treatment (%) | Ammonia-N (mg L <sup>-1</sup> ) |      | Sulphide (mg L <sup>-1</sup> ) |      | Total suspended solids (mg L <sup>-1</sup> ) |     | Total residual chlorine (mg L <sup>-1</sup> ) |       |
|-----------------------------|---------------------------------|------|--------------------------------|------|--|-----|---|-------|
|                             | Initial                         | End  | Initial                        | End  | Initial                                      | End | Initial                                       | End   |
| Negative control            | 0.04                            | 0.05 | <0.1                           | <0.1 | <2   | <2  | <0.02   | <0.02 |
| Salinity control            | 0.06                            | 0.08 | <0.1                           | <0.1 | <2   | <2  | <0.02   | <0.02 |
| 6.5                         | 1.93                            | 2.17 | <0.1                           | <0.1 | <2   | <2  | <0.02   | <0.02 |
| 12.5                        | 3.67                            | 4.06 | <0.1                           | <0.1 | <2   | <2  | <0.02   | <0.02 |
| 25                          | 5.94                            | 6.37 | <0.1                           | <0.1 | <2   | <2  | <0.02   | <0.02 |
| 50                          | 12.6                            | 14.6 | <0.1                           | <0.1 | <2   | <2  | <0.02   | <0.02 |
| 100                         | 22.4                            | 23.5 | <0.1                           | <0.1 | <2   | <2  | <0.02   | <0.02 |





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## **Appendix D**

### **Monitoring Data for Diatom Growth Inhibition Test (Chronic toxicity test)**



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Report No. : AZ0038170(8)

Application No.: LZ014562(7)

Table 1. Dissolved oxygen concentration and pH in each concentration treatment in diatom growth inhibition test.

| Concentration treatment (%) | Dissolved oxygen (mg L <sup>-1</sup> ) |      |      |      |      |       |       |       | pH  |      |      |      |      |       |       |       |
|-----------------------------|--|------|------|------|------|-------|-------|-------|-----|------|------|------|------|-------|-------|-------|
|                             | 0 h                                    | 24 h | 48 h | 72 h | 96 h | 120 h | 144 h | 168 h | 0 h | 24 h | 48 h | 72 h | 96 h | 120 h | 144 h | 168 h |
| Negative control            | 6.8                                    | 6.9  | 7.0  | 7.4  | 7.5  | 7.6   | 7.8   | 7.8   | 8.0 | 7.9  | 8.0  | 8.1  | 8.0  | 8.0   | 8.1   | 8.1   |
| Salinity control            | 6.8                                    | 6.9  | 7.0  | 7.3  | 7.4  | 7.6   | 7.7   | 7.9   | 7.9 | 7.9  | 8.0  | 8.0  | 8.1  | 8.1   | 8.0   | 8.0   |
| 2.5                         | 6.8                                    | 6.8  | 7.2  | 7.2  | 7.4  | 7.6   | 7.8   | 7.9   | 7.9 | 8.0  | 8.0  | 8.0  | 8.0  | 8.1   | 8.1   | 8.1   |
| 5                           | 6.8                                    | 6.8  | 7.2  | 7.3  | 7.5  | 7.5   | 7.8   | 8.0   | 8.0 | 7.9  | 7.9  | 7.9  | 8.0  | 8.1   | 8.1   | 8.1   |
| 10                          | 6.8                                    | 6.9  | 7.1  | 7.3  | 7.4  | 7.6   | 7.7   | 8.1   | 7.9 | 7.8  | 7.9  | 8.0  | 8.1  | 8.1   | 8.2   | 8.2   |
| 25                          | 6.8                                    | 6.9  | 7.0  | 7.1  | 7.1  | 7.4   | 7.8   | 7.9   | 7.8 | 7.8  | 8.1  | 8.1  | 8.2  | 8.2   | 8.1   | 8.2   |
| 50                          | 6.7                                    | 6.8  | 7.1  | 7.2  | 7.5  | 7.9   | 7.9   | 7.9   | 7.7 | 7.7  | 7.8  | 7.9  | 8.0  | 8.0   | 8.0   | 8.1   |
| 100                         | 6.7                                    | 6.8  | 6.8  | 6.8  | 6.8  | 6.9   | 7.1   | 7.1   | 7.7 | 7.7  | 7.7  | 7.8  | 7.9  | 8.0   | 7.9   | 8.0   |



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Report No. : AZ0038170(8)

Application No.: LZ014562(7)

Table 2. Salinity and temperature in each concentration treatment in diatom growth inhibition test.

| Concentration treatment (%) | Salinity (‰) |      |      |      |      |      |      |      | Temperature (°C) |      |      |      |      |      |      |      |
|-----------------------------|--------------|------|------|------|------|------|------|------|------------------|------|------|------|------|------|------|------|
|                             | 0h           | 24h  | 48h  | 72h  | 96h  | 120h | 144h | 168h | 0h               | 24h  | 48h  | 72h  | 96h  | 120h | 144h | 168h |
| Negative control            | 30.0         | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 | 22.0             | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 |
| Salinity control            | 30.0         | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 | 22.0             | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 |
| 2.5                         | 30.0         | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 | 22.0             | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 |
| 5                           | 30.0         | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 | 22.0             | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 |
| 10                          | 30.0         | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 | 22.0             | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 |
| 25                          | 30.0         | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 | 22.0             | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 |
| 50                          | 30.0         | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 | 22.0             | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 |
| 100                         | 30.0         | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 | 22.0             | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 |



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Report No. : AZ0038170(8)

Application No.: LZ014562(7)

Table 3. Ammonia-N, sulphide, total suspended solids, total residual chlorine concentration at the beginning and ending of the diatom growth inhibition toxicity test.

| Concentration treatment (%) | Ammonia-N (mg L <sup>-1</sup> ) |      | Sulphide (mg L <sup>-1</sup> ) |      | Total suspended solids (mg L <sup>-1</sup> ) |     | Total residual chlorine (mg L <sup>-1</sup> ) |       |
|-----------------------------|---------------------------------|------|--------------------------------|------|--|-----|---|-------|
|                             | Initial                         | End  | Initial                        | End  | Initial                                      | End | Initial                                       | End   |
| Negative control            | 0.03                            | 0.03 | <0.1                           | <0.1 | <2   | <2  | <0.02   | <0.02 |
| Salinity control            | 0.05                            | 0.04 | <0.1                           | <0.1 | <2   | <2  | <0.02   | <0.02 |
| 2.5                         | 1.37                            | 1.43 | <0.1                           | <0.1 | <2   | <2  | <0.02   | <0.02 |
| 5                           | 2.44                            | 2.68 | <0.1                           | <0.1 | <2   | <2  | <0.02   | <0.02 |
| 10                          | 4.62                            | 4.71 | <0.1                           | <0.1 | <2   | <2  | <0.02   | <0.02 |
| 25                          | 7.37                            | 7.56 | <0.1                           | <0.1 | <2   | <2  | <0.02   | <0.02 |
| 50                          | 12.5                            | 12.7 | <0.1                           | <0.1 | <2   | <2  | <0.02   | <0.02 |
| 100                         | 24.2                            | 25.4 | <0.1                           | <0.1 | <2   | <2  | <0.02   | <0.02 |



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## **Appendix E**

### **Monitoring Data for Shrimp Acute Toxicity Test**



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Report No. : AZ0038170(8)

Application No.: LZ014562(7)

Table 1. Dissolved oxygen concentration and pH in each concentration treatment in shrimp acute toxicity test.

| Concentration treatment (%) | Dissolved oxygen (mg L <sup>-1</sup> ) |      |      |      |      | pH  |      |      |      |      |
|-----------------------------|--|------|------|------|------|-----|------|------|------|------|
|                             | 0 h                                    | 12 h | 24 h | 36 h | 48 h | 0 h | 12 h | 24 h | 36 h | 48 h |
| Negative control            | 6.8                                    | 6.8  | 6.9  | 7.0  | 7.1  | 8.0 | 7.9  | 8.0  | 7.9  | 7.9  |
| Salinity control            | 6.9                                    | 7.0  | 7.0  | 6.9  | 7.1  | 7.9 | 7.9  | 7.9  | 7.8  | 7.9  |
| 6.5                         | 7.0                                    | 6.9  | 7.1  | 7.1  | 7.0  | 7.9 | 7.8  | 7.9  | 7.8  | 7.8  |
| 12.5                        | 7.0                                    | 7.0  | 6.9  | 7.0  | 7.0  | 8.0 | 7.9  | 7.8  | 7.8  | 7.8  |
| 25                          | 6.9                                    | 6.9  | 6.8  | 6.8  | 6.9  | 7.9 | 7.8  | 7.8  | 7.7  | 7.7  |
| 50                          | 6.8                                    | 6.8  | 6.8  | 6.8  | 6.9  | 7.8 | 7.8  | 7.8  | 7.7  | 7.8  |
| 100                         | 6.8                                    | 6.8  | 6.9  | 6.9  | 6.7  | 7.7 | 7.9  | 7.8  | 7.8  | 7.7  |



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Report No. : AZ0038170(8)

Application No.: LZ014562(7)

Table 2. Salinity and temperature in each concentration treatment in shrimp acute toxicity test.

| Concentration treatment (%) | Salinity (‰) |      |      |      |      | Temperature (°C) |      |      |      |      |
|-----------------------------|--------------|------|------|------|------|------------------|------|------|------|------|
|                             | 0 h          | 12 h | 24 h | 36 h | 48 h | 0 h              | 12 h | 24 h | 36 h | 48 h |
| Negative control            | 25.0         | 25.0 | 25.0 | 25.0 | 25.0 | 22.0             | 22.0 | 22.0 | 22.0 | 22.0 |
| Salinity control            | 25.0         | 25.0 | 25.0 | 25.0 | 25.0 | 22.0             | 22.0 | 22.0 | 22.0 | 22.0 |
| 6.5                         | 25.0         | 25.0 | 25.0 | 25.0 | 25.0 | 22.0             | 22.0 | 22.0 | 22.0 | 22.0 |
| 12.5                        | 25.0         | 25.0 | 25.0 | 25.0 | 25.0 | 22.0             | 22.0 | 22.0 | 22.0 | 22.0 |
| 25                          | 25.0         | 25.0 | 25.0 | 25.0 | 25.0 | 22.0             | 22.0 | 22.0 | 22.0 | 22.0 |
| 50                          | 25.0         | 25.0 | 25.0 | 25.0 | 25.0 | 22.0             | 22.0 | 22.0 | 22.0 | 22.0 |
| 100                         | 25.0         | 25.0 | 25.0 | 25.0 | 25.0 | 22.0             | 22.0 | 22.0 | 22.0 | 22.0 |



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Table 3. Ammonia-N, sulphide, total suspended solids, total residual chlorine concentration at the beginning and ending of the shrimp acute toxicity test.

| Concentration treatment (%) | Ammonia-N (mg L <sup>-1</sup> ) |      | Sulphide (mg L <sup>-1</sup> ) |      | Total suspended solids (mg L <sup>-1</sup> ) |     | Total residual chlorine (mg L <sup>-1</sup> ) |       |
|-----------------------------|---------------------------------|------|--------------------------------|------|--|-----|---|-------|
|                             | Initial                         | End  | Initial                        | End  | Initial                                      | End | Initial                                       | End   |
| Negative control            | 0.05                            | 0.07 | <0.1                           | <0.1 | 8  | 10  | <0.02   | <0.02 |
| Salinity control            | 0.05                            | 0.09 | <0.1                           | <0.1 | 9  | 9   | <0.02   | <0.02 |
| 6.5                         | 1.07                            | 1.24 | <0.1                           | <0.1 | 13   | 16  | <0.02   | <0.02 |
| 12.5                        | 2.40                            | 2.66 | <0.1                           | <0.1 | 23   | 24  | <0.02   | <0.02 |
| 25                          | 5.87                            | 6.07 | <0.1                           | <0.1 | 30   | 36  | <0.02   | <0.02 |
| 50                          | 10.6                            | 11.3 | <0.1                           | <0.1 | 42   | 49  | <0.02   | <0.02 |
| 100                         | 22.6                            | 24.0 | <0.1                           | <0.1 | 54   | 60  | <0.02   | <0.02 |