



**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 Jan 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. : LY041453(6)

Report No. : AZ0007329(0)

Report Issued Date : 17 Jan 2020

For and on behalf of
CMA Industrial Development Foundation Limited

Authorized Signature :

Lau Yan Kin
Senior Manager
Environmental Division



Report No. : AZ0007329(0)

Application No.: LY041453(6)

Contents	2
1 Introduction	4
1.1 Background	4
1.2 Testing laboratory and investigator	4
1.3 Sample	4
1.4 Test species	4
1.5 Test protocols	4
2 Report on Amphipod Acute Toxicity Test	5
2.1 Samples storage and pretreatment	6
2.2 Test organism	6
2.3 Summary of test conditions	6
2.4 Test results	7-8
2.5 Summary of water quality parameters monitoring during test	9
2.6 LC ₅₀ for the amphipod <i>Melita longidactyla</i> and test acceptability	10
3 Report on Fish Acute Toxicity Test	11
3.1 Samples storage and pretreatment	12
3.2 Test organism	12
3.3 Summary of test conditions	12
3.4 Test results	13-15
3.5 Summary of water quality parameters monitoring during test	15
3.6 LC ₅₀ for the fish <i>Lutjanus malabaricus</i> and test acceptability	16
4 Report on Barnacle Larvae Acute Toxicity Test	17
4.1 Samples storage and pretreatment	18
4.2 Test organism	18
4.3 Summary of test conditions	18
4.4 Test results	19-20
4.5 Summary of water quality parameters monitoring during test	21
4.6 LC ₅₀ for the barnacle larvae <i>Balanus amphitrite</i> and test acceptability	22
5 Report on Diatom Growth Inhibition Test (Chronic toxicity test)	23
5.1 Samples storage and pretreatment	24
5.2 Test organism	24
5.3 Summary of test conditions	24
5.4 Test results	25-26
5.5 Summary of water quality parameters monitoring during test	27



Report No. : AZ0007329(0)

Application No.: LY041453(6)

5.6	IC ₅₀ for the diatom <i>Skeletonema costatum</i> and test acceptability	28
6	Report on Shrimp Acute Toxicity Test	29
6.1	Samples storage and pretreatment	30
6.2	Test organism	30
6.3	Summary of test conditions	30
6.4	Test results	31-32
6.5	Summary of water quality parameters monitoring during test	33
6.6	LC ₅₀ for the shrimp <i>Metapenaeus ensis</i> and test acceptability	34
7	Conclusion	35-36
	Appendix A Monitoring Data for Amphipod Acute Toxicity Test	37-40
	Appendix B Monitoring Data for Fish Acute Toxicity Test	41-44
	Appendix C Monitoring Data for Barnacle Larvae Acute Toxicity Test	45-48
	Appendix D Monitoring Data for Diatom Growth Inhibition Test	49-52
	Appendix E Monitoring Data for Shrimp Acute Toxicity Test	52-56



Report No. : AZ0007329(0)

Application No.: LY041453(6)

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 Jan 15th, 2020. Effluent sample was shipped immediately to the testing laboratory on the same day of collection and stored at 4 °C until use. Toxicity testings were started on the same day after sample collection.

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).



**CMA Testing
and Certification
Laboratories**

廠商會檢定中心

Report No. : AZ0007329(0)

Application No.: LY041453(6)

2. Report on Amphipod Acute Toxicity Test



Report No. : AZ0007329(0)

Application No.: LY041453(6)

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, 15/1/2020-17/1/2020
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: CdCl₂
Positive control: 48 h acute toxicity test
Salinity control: Prepared with ocean salt adding into de-ionized water, salinity: 30‰



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

Report No. : AZ0007329(0)

Application No.: LY041453(6)

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	10	10	10	10.00	0.00
Concentration 1	6.5	9	10	10	9	9.50	0.58
Concentration 2	12.5	9	9	10	8	9.00	0.82
Concentration 3	25	5	5	6	6	5.50	0.58
Concentration 4	50	2	3	3	4	3.00	0.82
Concentration 5	100	0	0	1	0	0.25	0.50



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

Report No. : AZ0007329(0)

Application No.: LY041453(6)

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	100	100	100	100.00	0.00
Concentration 1	6.5	90	100	100	90	95.00	5.77
Concentration 2	12.5	90	90	100	80	90.00	8.16
Concentration 3	25	50	50	60	60	55.00	5.77
Concentration 4	50	20	30	30	40	30.00	8.16
Concentration 5	100	0	0	10	0	2.50	5.00



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

Report No. : AZ0007329(0)

Application No.: LY041453(6)

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 ⁻¹)	6.7-7.5	6.7-7.6	6.7-7.6	6.8-7.5	6.8-7.5	6.6-7.6	6.7-7.6
Temperature (°C)	22.0	22.0	22.0	22.0	22.0	22.0	22.0
pH	7.8-8.2	7.7-8.2	7.7-8.2	7.8-8.2	7.8-8.1	7.7-8.0	7.6-8.1
Total ammonia (start/end, mg L ⁻¹)	0.08/0.08	0.09/0.10	1.52/1.60	2.86/2.90	6.11/6.18	11.8/12.1	22.5/23.2
Total sulfide (start/end, mg L ⁻¹)	<1	<1	<1	<1	<1	<1	<1
Total residual chlorine (start/end, mg L ⁻¹)	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02
Total suspended solid (start/end, mg L ⁻¹)	<2	<2	<2	<2	<2	<2	<2



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

Report No. : AZ0007329(0)

Application No.: LY041453(6)

2.5. LC₅₀ for the amphipod *Melita longidactyla* and test acceptability

Table 4. LC₅₀ for the amphipods and test acceptability.

Parameter	Value	Control limit
Calculated LC ₅₀	31.06%	NA
Negative control survival	97.50%	>90%
Reference toxicant 48-h acute test	1.32 mg L ⁻¹	1.25±0.15 mg L ⁻¹
95% of confidence range of reference toxicant test	1.11-1.42 mg L ⁻¹	NA
Daily temperature variation	<0.5 °C	Average daily temperature variation: ±1 °C
Dissolved oxygen concentration	>6.6 mg L ⁻¹	>4 mg L ⁻¹

NA: Not applicable



CMA Testing and Certification Laboratories

廠商會檢定中心

Report No. : AZ0007329(0)

Application No.: LY041453(6)

3. Report on Fish Acute Toxicity Test



Report No. : AZ0007329(0)

Application No.: LY041453(6)

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, 15/1/2020-17/1/2020
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: CdCl₂
Positive control: 48 h acute toxicity test
Salinity control: Prepared with ocean salt adding into de-ionized water, salinity: 30‰



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

Report No. : AZ0007329(0)

Application No.: LY041453(6)

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	20	20	20.00	0.00
Salinity control	0	20	20	20	19	19.75	0.50
Concentration 1	6.5	19	19	20	19	19.25	0.50
Concentration 2	12.5	19	19	18	18	18.50	0.58
Concentration 3	25	15	16	14	15	15.00	0.82
Concentration 4	50	12	11	13	10	11.50	1.29
Concentration 5	100	0	0	0	0	0.00	0.00



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

Report No. : AZ0007329(0)

Application No.: LY041453(6)

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	100	100	100.00	0.00
Salinity control	0	100	100	100	95	98.75	2.50
Concentration 1	6.5	95	95	100	95	96.25	2.50
Concentration 2	12.5	95	95	90	90	92.50	2.89
Concentration 3	25	75	80	70	75	75.00	4.08
Concentration 4	50	60	55	65	50	57.50	6.45
Concentration 5	100	0	0	0	0	0.00	0.00



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

Report No. : AZ0007329(0)

Application No.: LY041453(6)

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 ⁻¹)	6.7-6.9	6.8-7.0	6.8-7.0	6.8-7.0	6.8-7.0	6.7-7.0	6.8-6.9
Temperature (°C)	22	22	22	22	22	22	22
pH	7.9-8.0	7.8-8.0	7.8-8.0	7.8-8.1	7.8-8.0	7.8-8.0	7.7-7.9
Total ammonia (start/end, mg L ⁻¹)	0.11/0.11	0.10/0.09	1.37/1.40	2.77/2.82	5.06/5.14	10.7/11.4	19.8/21.6
Total sulfide (start/end, mg L ⁻¹)	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Total residual chlorine (start/end, mg L ⁻¹)	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02
Total suspended solid (mg L ⁻¹)	7/7	9/9	13/15	20/24	37/42	53/58	79/87



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

Report No. : AZ0007329(0)

Application No.: LY041453(6)

3.6. LC₅₀ for the fish *Lutjanus malabaricus* and test acceptability

Table 4. LC₅₀ for the fish and test acceptability.

a: The mortalities in all concentration groups were less than 50% of that in control group and thus LC₅₀ cannot be calculated.

Parameter	Value	Control limit
Calculated LC ₅₀	40.41%	NA
Negative survival	100.0 %	>90%
Reference toxicant 48-h acute test	13.29 mg L ⁻¹	14.6±1.78 mg L ⁻¹
95% of confidence range of reference toxicant test	11.26-15.17 mg L ⁻¹	NA
Daily temperature variation	<0.5 °C	Average daily temperature variation: ± 1 °C
Dissolved oxygen concentration	>6.7 mg L ⁻¹	>4 mg L ⁻¹

calculated.

NA: Not applicable



CMA Testing and Certification Laboratories

廠商會檢定中心

Report No. : AZ0007329(0)

Application No.: LY041453(6)

4. Report on Barnacle Larvae Acute Toxicity Test



Report No. : AZ0007329(0)

Application No.: LY041453(6)

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, 15/1/2020-17/1/2020
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: CdCl₂
Positive control: 48 h acute toxicity test
Salinity control: Prepared with ocean salt adding into de-ionized water, salinity: 30‰



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

Report No. : AZ0007329(0)

Application No.: LY041453(6)

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	19	19	19.50	0.58
Salinity control	0	20	19	19	19	19.25	0.50
Concentration 1	6.5	16	17	17	17	16.75	0.50
Concentration 2	12.5	15	13	11	12	12.75	1.71
Concentration 3	25	10	10	7	8	8.75	1.50
Concentration 4	50	3	5	2	2	3.00	1.41
Concentration 5	100	0	0	0	0	0.00	0.00



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

Report No. : AZ0007329(0)

Application No.: LY041453(6)

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	95	95	97.50	2.89
Salinity control	0	100	95	95	95	96.25	2.50
Concentration 1	6.5	80	85	85	85	83.75	2.50
Concentration 2	12.5	75	65	55	60	63.75	8.54
Concentration 3	25	50	50	35	40	43.75	7.50
Concentration 4	50	15	25	10	10	15.00	7.07
Concentration 5	100	0	0	0	0	0.00	0.00



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

Report No. : AZ0007329(0)

Application No.: LY041453(6)

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 ⁻¹)	7.0-7.2	7.0-7.2	7.0-7.2	6.9-7.1	6.9-7.1	7.0-7.1	7.0-7.1
Temperature (°C)	22.0	22.0	22.0	22.0	22.0	22.0	22.0
pH	7.9-8.1	7.8-8.0	7.9-8.1	7.8-8.0	7.8-8.0	7.9-8.0	7.9-8.0
Total ammonia (start/end, mg L ⁻¹)	0.12/0.10	0.12/0.14	1.23/1.35	2.46/2.59	5.23/5.45	13.6/13.4	25.5/26.0
Total sulfide (start/end, mg L ⁻¹)	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Total residual chlorine (start/end, mg L ⁻¹)	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02
Total suspended solid (mg L ⁻¹)	<2	<2	<2	<2	<2	<2	<2



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

Report No. : AZ0007329(0)

Application No.: LY041453(6)

4.6. LC₅₀ for the barnacle larvae *Balanus amphitrite* and test acceptability

Table 4. LC₅₀ for the barnacle larvae and test acceptability

Parameter	Value	Control limit
Calculated LC ₅₀	18.98 %	NA
Negative survival	97.50 %	>90%
Reference toxicant 48-h acute test	1.01 mg L ⁻¹	1.04±0.11 mg L ⁻¹
95% of confidence range of reference toxicant test:	0.93-1.04 mg L ⁻¹	NA
Daily temperature variation	<0.5°C	Average daily temperature variation: ± 1 °C
Dissolved oxygen concentration	>6.9 mg L ⁻¹	>4 mg L ⁻¹

NA: Not applicable



CMA Testing and Certification Laboratories

廠商會檢定中心

Report No. : AZ0007329(0)

Application No.: LY041453(6)

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



Report No. : AZ0007329(0)

Application No.: LY041453(6)

Test report

5.1. Samples storage and pretreatment

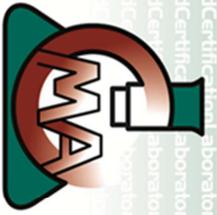
Effluent sample was thoroughly mixed and passed through 5 µm membrane filter to remove large debris. Effluent was added with ocean salt in order to raise the salinity to the required level (30‰) and then aerated moderately to dissolved oxygen (DO) saturation prior to use. Salinity control was set up 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, 15/1/2019-22/1/2019
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⁻¹
Replicate number: 4
Volume of test medium: 25 mL
Aeration: None
Reference toxicant: CdCl₂
Positive control: 7-day IC₅₀ toxicity test
Salinity control: Prepared with ocean salt adding into de-ionized water, salinity: 30‰



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

Report No. : AZ0007329(0)

Application No.: LY041453(6)

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⁻¹.

Treatment	Effluent concentration (%)	Cell density after 7-day growth ($\times 10^6$ cell mL ⁻¹)					
		Replicate 1	Replicate 2	Replicate 3	Replicate 4	Mean	SD
Negative control	0	1.09	1.22	1.10	1.16	1.14	0.06
Salinity control	0	1.32	1.36	1.34	1.24	1.32	0.05
Concentration 1	2.5	1.63	1.66	1.50	1.54	1.58	0.07
Concentration 2	5	1.43	1.36	1.38	1.27	1.36	0.07
Concentration 3	10	1.24	1.18	1.26	1.15	1.21	0.05
Concentration 4	25	0.89	0.76	0.74	0.82	0.80	0.07
Concentration 5	50	0.53	0.44	0.38	0.46	0.45	0.06
Concentration 6	100	0.00	0.00	0.00	0.00	0.00	0.00



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

Report No. : AZ0007329(0)

Application No.: LY041453(6)

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

Treatment	Effluent concentration (%)	7-day average growth rate (d ⁻¹)					
		Replicate 1	Replicate 2	Replicate 3	Replicate 4	Mean	SD
Negative control	0	0.44	0.46	0.44	0.45	0.45	0.01
Salinity control	0	0.47	0.47	0.47	0.46	0.47	0.01
Concentration 1	2.5	0.50	0.50	0.49	0.49	0.49	0.01
Concentration 2	5	0.48	0.47	0.47	0.46	0.47	0.01
Concentration 3	10	0.46	0.45	0.46	0.45	0.45	0.01
Concentration 4	25	0.41	0.39	0.38	0.40	0.40	0.01
Concentration 5	50	0.34	0.31	0.29	0.32	0.31	0.02
Concentration 6	100	0.00	0.00	0.00	0.00	0.00	0.00



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Report No. : AZ0007329(0)

Application No.: LY041453(6)

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 ⁻¹)	6.7-8.4	6.7-8.5	6.8-8.8	6.8-8.5	6.8-8.5	6.7-8.4	6.7-8.3	6.8-7.2
Temperature (°C)	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0
pH	7.8-8.4	7.7-8.4	7.8-8.4	7.7-8.3	7.8-8.3	7.7-8.3	7.6-8.3	7.5-8.2
Total ammonia (start/end, mg L ⁻¹)	0.16/0.15	0.17/0.18	1.41/1.44	2.61/2.65	4.26/4.35	6.82/7.03	12.4/13.0	23.7/25.2
Total sulfide (start/end, mg L ⁻¹)	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Total residual chlorine (start/end, mg L ⁻¹)	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02
Total suspended solid (mg L ⁻¹)	<2	<2	<2	<2	<2	<2	<2	<2



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Report No. : AZ0007329(0)

Application No.: LY041453(6)

5.6. IC₅₀ for the diatom *Skeletonema costatum* and test acceptability

Table 4. IC₅₀, none observed effect concentration (NOEC) for the diatom and test acceptability

Parameter	Value	Control limit
Calculated IC ₅₀	33.66%	NA
None observed effect concentration (NOEC)	10%	-
Reference toxicant 7-day test:	0.13 mg L ⁻¹	0.13±0.02 mg L ⁻¹
95% of confidence range of reference toxicant test	0.11-0.17 mg L ⁻¹	NA
Temperature variation	<0.5 °C	Average daily temperature variation: ± 1 °C

NA: Not applicable



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Report No. : AZ0007329(0)

Application No.: LY041453(6)

6. Report on Shrimp Acute Toxicity Test



Report No. : AZ0007329(0)

Application No.: LY041453(6)

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, 15/1/2020-17/1/2020
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: CdCl₂
Positive control: 48 h acute toxicity test
Salinity control: Prepared with ocean salt adding into de-ionized water, salinity: 25‰



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Report No. : AZ0007329(0)

Application No.: LY041453(6)

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	9	9.50	0.58
Salinity control	0	9	10	10	10	9.75	0.50
Concentration 1	6.5	8	8	7	9	8.00	0.82
Concentration 2	12.5	6	7	6	7	6.50	0.58
Concentration 3	25	5	6	7	5	5.75	0.96
Concentration 4	50	3	5	3	4	3.75	0.96
Concentration 5	100	0	0	0	1	0.25	0.50



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Report No. : AZ0007329(0)

Application No.: LY041453(6)

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	90	95.00	5.77
Salinity control	0	90	100	100	100	97.50	5.00
Concentration 1	6.5	80	80	70	90	80.00	8.16
Concentration 2	12.5	60	70	60	70	65.00	5.77
Concentration 3	25	50	60	70	50	57.50	9.57
Concentration 4	50	30	50	30	40	37.50	9.57
Concentration 5	100	0	0	0	10	2.50	5.00



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Report No. : AZ0007329(0)

Application No.: LY041453(6)

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 ⁻¹)	7.0-7.2	7.0-7.1	7.0-7.2	7.0-7.1	7.0-7.2	7.0-7.1	6.9-7.1
Temperature (°C)	22.0	22.0	22.0	22.0	22.0	22.0	22.0
pH	7.9-8.0	7.9-8.0	7.8-8.0	7.7-7.9	7.7-7.9	7.8-8.0	7.7-8.1
Total ammonia (start/end, mg L ⁻¹)	0.07/0.07	0.09/0.11	1.18/1.20	2.43/2.50	4.06/4.27	9.06/9.12	20.4/20.9
Total sulfide (start/end, mg L ⁻¹)	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Total residual chlorine (start/end, mg L ⁻¹)	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02
Total suspended solid (start/end, mg L ⁻¹)	12/14	11/10	14/15	18/17	29/35	46/50	61/64



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Report No. : AZ0007329(0)

Application No.: LY041453(6)

6.6 LC₅₀ for the shrimp *Metapenaeus ensis* and test acceptability

Table 4. LC₅₀ for the *Metapenaeus ensis* and test acceptability.

Parameter	Value	Control limit
Calculated LC ₅₀	26.82 %	NA
Negative control survival	95.00%	>90%
Reference toxicant 48-h acute test	5.17 mg L ⁻¹	5.19±0.51 mg L ⁻¹
95% of confidence range of reference toxicant test	4.69-5.56 mg L ⁻¹	NA
Daily temperature variation	<0.5°C	Average daily temperature variation: ±1 °C
Dissolved oxygen concentration	>6.9 mg L ⁻¹	>4 mg L ⁻¹

NA: Not applicable



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Report No. : AZ0007329(0)

Application No.: LY041453(6)

7. Conclusion



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Report No. : AZ0007329(0)

Application No.: LY041453(6)

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

Test species	Measured LC ₅₀ /IC ₅₀ /NOEC	Target toxicity level
Amphipod <i>Melita longidactyla</i>	31.1 %	≥7.1%
Fish <i>Lutjanus malabaricus</i>	40.4 %	≥7.1%
Barnacle larvae <i>Balanus amphitrite</i>	19.0 %	≥7.1%
Diatom <i>Skeletonema costatum</i>	33.7 %	-
Diatom <i>Skeletonema costatum</i>	10 % (NOEC)	≥0.51%
Shrimp <i>Metapenaeus ensis</i>	26.8 %	≥7.1%

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



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Report No. : AZ0007329(0)

Application No.: LY041453(6)

Appendix A

Monitoring Data for Amphipod Acute Toxicity Test



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Report No. : AZ0007329(0)

Application No.: LY041453(6)

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

Concentration treatment (%)	Dissolved oxygen (mg L ⁻¹)					pH				
	0 h	12 h	24 h	36 h	48 h	0 h	12 h	24 h	36 h	48 h
Negative control	6.7	7.2	7.3	7.5	7.4	7.8	8.1	8.1	8.2	8.2
Salinity control	6.7	7.2	7.4	7.6	7.6	7.7	8.1	8.2	8.1	8.2
6.5	6.7	7.1	7.3	7.5	7.6	7.7	8.0	8.1	8.1	8.2
12.5	6.8	7.1	7.4	7.5	7.4	7.8	8.1	8.2	8.2	8.1
25	6.8	7.2	7.3	7.5	7.5	7.8	8.0	8.1	8.1	8.1
50	6.6	7.1	7.4	7.6	7.5	7.7	7.9	8.0	7.9	8.0
100	6.7	7.2	7.4	7.6	7.6	7.6	7.6	7.8	8.0	8.1



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Report No. : AZ0007329(0)

Application No.: LY041453(6)

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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Report No. : AZ0007329(0)

Application No.: LY041453(6)

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 ⁻¹)		Sulphide (mg L ⁻¹)		Total suspended solids (mg L ⁻¹)		Total residual chlorine (mg L ⁻¹)	
	Initial	End	Initial	End	Initial	End	Initial	End
Negative control	0.08	0.08	<1	<1	<2	<2	<0.02	<0.02
Salinity control	0.09	0.10	<1	<1	<2	<2	<0.02	<0.02
6.5	1.52	1.60	<1	<1	<2	<2	<0.02	<0.02
12.5	2.86	2.90	<1	<1	<2	<2	<0.02	<0.02
25	6.11	6.18	<1	<1	<2	<2	<0.02	<0.02
50	11.8	12.1	<1	<1	<2	<2	<0.02	<0.02
100	22.5	23.2	<1	<1	<2	<2	<0.02	<0.02



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Report No. : AZ0007329(0)

Application No.: LY041453(6)

Appendix B

Monitoring Data for Fish Acute Toxicity Test



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Report No. : AZ0007329(0)

Application No.: LY041453(6)

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

Concentration treatment (%)	Dissolved oxygen (mg L ⁻¹)					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	6.7	6.8	6.9	8.0	8.0	7.9	8.0	7.9
Salinity control	6.8	7.0	6.9	6.9	6.9	8.0	7.9	8.0	7.8	7.9
6.5	6.8	6.9	6.8	6.9	7.0	7.9	7.9	7.9	7.8	8.0
12.5	6.9	6.8	6.8	6.9	7.0	8.1	8.0	8.0	7.9	7.8
25	6.8	6.8	6.9	7.0	7.0	8.0	7.9	7.8	7.9	7.8
50	6.7	7.0	6.9	7.0	7.0	8.0	8.0	7.9	7.8	7.8
100	6.8	6.8	6.9	6.9	6.9	7.9	7.8	7.8	7.7	7.7



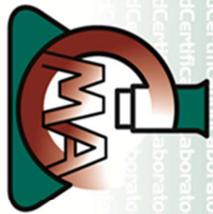
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Report No. : AZ0007329(0)

Application No.: LY041453(6)

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. : AZ0007329(0)

Application No.: LY041453(6)

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 ⁻¹)		Sulphide (mg L ⁻¹)		Total suspended solids (mg L ⁻¹)		Total residual chlorine (mg L ⁻¹)	
	Initial	End	Initial	End	Initial	End	Initial	End
Negative control	0.11	0.11	<0.1	<0.1	7	7	<0.02	<0.02
Salinity control	0.10	0.09	<0.1	<0.1	9	9	<0.02	<0.02
6.5	1.37	1.40	<0.1	<0.1	13	15	<0.02	<0.02
12.5	2.77	2.82	<0.1	<0.1	20	24	<0.02	<0.02
25	5.06	5.14	<0.1	<0.1	37	42	<0.02	<0.02
50	10.7	11.4	<0.1	<0.1	53	58	<0.02	<0.02
100	19.8	21.6	<0.1	<0.1	79	87	<0.02	<0.02



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Report No. : AZ0007329(0)

Application No.: LY041453(6)

Appendix C

Monitoring Data
for
Barnacle Larvae Acute Toxicity Test



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Report No. : AZ0007329(0)

Application No.: LY041453(6)

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

Concentration treatment (%)	Dissolved oxygen (mg L ⁻¹)					pH				
	0 h	12 h	24 h	36 h	48 h	0 h	12 h	24 h	36 h	48 h
Negative control	7.2	7.0	7.1	7.1	7.0	8.0	8.1	7.9	7.9	8.0
Salinity control	7.1	7.0	7.2	7.0	7.1	7.9	8.0	7.9	7.9	7.8
6.5	7.2	7.0	7.0	7.0	7.0	8.0	8.0	8.1	8.0	7.9
12.5	7.0	7.1	7.0	7.0	6.9	8.0	7.9	7.8	7.8	7.8
25	7.1	7.0	6.9	7.0	7.0	8.0	8.0	7.9	7.8	7.9
50	7.1	7.0	7.0	7.0	7.0	8.0	8.0	7.9	7.9	8.0
100	7.0	7.0	7.1	7.0	7.1	8.0	8.0	8.0	7.9	7.9



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Report No. : AZ0007329(0)

Application No.: LY041453(6)

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. : AZ0007329(0)

Application No.: LY041453(6)

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 ⁻¹)		Sulphide (mg L ⁻¹)		Total suspended solids (mg L ⁻¹)		Total residual chlorine (mg L ⁻¹)	
	Initial	End	Initial	End	Initial	End	Initial	End
Negative control	0.12	0.10	<0.1	<0.1	<2	<2	<0.02	<0.02
Salinity control	0.12	0.14	<0.1	<0.1	<2	<2	<0.02	<0.02
6.5	1.23	1.35	<0.1	<0.1	<2	<2	<0.02	<0.02
12.5	2.46	2.59	<0.1	<0.1	<2	<2	<0.02	<0.02
25	5.23	5.45	<0.1	<0.1	<2	<2	<0.02	<0.02
50	13.6	13.4	<0.1	<0.1	<2	<2	<0.02	<0.02
100	25.5	26.0	<0.1	<0.1	<2	<2	<0.02	<0.02



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Report No. : AZ0007329(0)

Application No.: LY041453(6)

Appendix D

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



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Report No. : AZ0007329(0)

Application No.: LY041453(6)

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

Concentration treatment (%)	Dissolved oxygen (mg L ⁻¹)								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	6.7	6.8	7.1	7.2	7.5	7.8	8.3	8.4	7.8	7.9	8.1	8.0	8.2	8.2	8.3	8.4
Salinity control	6.7	6.9	7.1	7.3	7.4	8.1	8.3	8.5	7.7	8.0	8.1	8.2	8.2	8.3	8.4	8.3
2.5	6.8	6.9	7.2	7.5	7.8	8.2	8.5	8.8	7.8	7.9	8.1	8.2	8.2	8.2	8.4	8.4
5	6.8	7.0	7.1	7.2	7.5	8.1	8.3	8.5	7.7	7.8	8.1	8.1	8.2	8.3	8.3	8.2
10	6.8	7.0	7.2	7.3	7.6	7.9	8.2	8.5	7.8	7.9	8.0	8.1	8.1	8.2	8.3	8.3
25	6.7	6.9	7.0	7.2	7.3	8.0	8.3	8.4	7.7	7.8	7.9	8.1	8.1	8.2	8.3	8.2
50	6.7	6.9	6.9	7.2	7.4	7.8	8.1	8.3	7.6	7.6	7.7	7.9	8.0	8.1	8.1	8.3
100	6.8	6.8	6.9	6.8	7.0	6.9	7.2	7.1	7.5	7.5	7.6	7.8	7.9	8.0	8.1	8.2



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Report No. : AZ0007329(0)

Application No.: LY041453(6)

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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Laboratories**
廠商會檢定中心

Report No. : AZ0007329(0)

Application No.: LY041453(6)

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 ⁻¹)		Sulphide (mg L ⁻¹)		Total suspended solids (mg L ⁻¹)		Total residual chlorine (mg L ⁻¹)	
	Initial	End	Initial	End	Initial	End	Initial	End
Negative control	0.16	0.15	<0.1	<0.1	<2	<2	<0.02	<0.02
Salinity control	0.17	0.18	<0.1	<0.1	<2	<2	<0.02	<0.02
2.5	1.41	1.44	<0.1	<0.1	<2	<2	<0.02	<0.02
5	2.61	2.65	<0.1	<0.1	<2	<2	<0.02	<0.02
10	4.26	4.35	<0.1	<0.1	<2	<2	<0.02	<0.02
25	6.82	7.03	<0.1	<0.1	<2	<2	<0.02	<0.02
50	12.4	13.0	<0.1	<0.1	<2	<2	<0.02	<0.02
100	23.7	25.2	<0.1	<0.1	<2	<2	<0.02	<0.02



CMA Testing and Certification Laboratories

廠商會檢定中心

Report No. : AZ0007329(0)

Application No.: LY041453(6)

Appendix E

Monitoring Data for Shrimp Acute Toxicity Test



Report No. : AZ0007329(0)

Application No.: LY041453(6)

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

Concentration treatment (%)	Dissolved oxygen (mg L ⁻¹)					pH				
	0 h	12 h	24 h	36 h	48 h	0 h	12 h	24 h	36 h	48 h
Negative control	7.2	7.1	7.1	7.1	7.0	8.0	7.9	8.0	7.9	7.9
Salinity control	7.1	7.1	7.0	7.0	7.1	8.0	8.0	7.9	7.8	7.9
6.5	7.2	7.1	7.1	7.0	7.0	8.0	8.0	7.8	7.9	7.8
12.5	7.1	7.0	7.0	7.1	7.1	7.9	7.9	7.8	7.7	7.8
25	7.0	7.1	7.2	7.0	7.0	7.9	7.8	7.8	7.7	7.8
50	7.1	7.1	7.0	7.1	7.0	8.0	7.9	7.8	7.8	7.9
100	7.1	7.0	7.0	6.9	6.9	8.1	7.9	7.9	7.8	7.9



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Report No. : AZ0007329(0)

Application No.: LY041453(6)

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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廠商會檢定中心

Report No. : AZ0007329(0)

Application No.: LY041453(6)

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 ⁻¹)		Sulphide (mg L ⁻¹)		Total suspended solids (mg L ⁻¹)		Total residual chlorine (mg L ⁻¹)	
	Initial	End	Initial	End	Initial	End	Initial	End
Negative control	0.07	0.07	<0.1	<0.1	12	14	<0.02	<0.02
Salinity control	0.09	0.11	<0.1	<0.1	11	10	<0.02	<0.02
6.5	1.18	1.20	<0.1	<0.1	14	15	<0.02	<0.02
12.5	2.43	2.50	<0.1	<0.1	18	17	<0.02	<0.02
25	4.06	4.27	<0.1	<0.1	29	35	<0.02	<0.02
50	9.06	9.12	<0.1	<0.1	46	50	<0.02	<0.02
100	20.4	20.9	<0.1	<0.1	61	64	<0.02	<0.02