

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

Whole Effluent Toxicity Test (WETT) at SCISTW

Report for the Month of Apr 2019

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.	:	LV021027(1)
Report No.	:	AY0024951(0)
Report Issued Date	:	17 May 2019

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. <u>Background</u>

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

1.2. <u>Testing laboratory and investigator</u>

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. <u>Sample</u>

A 24-hour flow-weighted composite effluent sample was collected from Stonecutters Island Sewage Treatment Works (SCISTW) on Apr 27th, 2019. 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. <u>Test species</u>

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. <u>Test protocols</u>

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. <u>Samples storage and pretreatment</u>

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. <u>Test organism</u>

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.

Type of test:	Static
Duration:	48 h, 27/4/2019-29/4/2019
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:	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 ₂
Positive control:	48 h acute toxicity test
Salinity control:	Prepared with ocean salt adding into de-ionized water, salinity: 30‰

2.3. <u>Summary of test conditions</u>

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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	10	10	10.00	0.00		
Salinity control	0	10	10	10	10	10.00	0.00		
Concentration 1	6.5	10	10	10	10	10.00	0.00		
Concentration 2	12.5	9	8	9	10	9.00	0.82		
Concentration 3	25	6	5	6	7	6.00	0.82		
Concentration 4	50	3	2	1	3	2.25	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 (%)						
Treatment		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	100	100.00	0.00	
Concentration 1	6.5	100	100	100	100	100.00	0.00	
Concentration 2	12.5	90	80	90	100	90.00	8.16	
Concentration 3	25	60	50	60	70	60.00	8.16	
Concentration 4	50	30	20	10	30	22.50	9.57	
Concentration 5	100	0	0	0	0	0.00	0.00	

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2.5 <u>Summary of water quality parameters monitoring during test.</u>

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

	Effluent concentration (%)								
Water quality parameters	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)	6.8-7.2	6.7-7.1	6.8-7.2	6.8-7.2	6.7-7.3	6.8-7.2	6.6-7.2		
Temperature (°C)	22.0	22.0	22.0	22.0	22.0	22.0	22.0		
pН	7.8-8.0	7.8-8.0	7.7-8.0	7.7-8.1	7.7-8.1	7.6-8.0	7.3-7.8		
Total ammonia (start/end, mg L ⁻¹)	0.05/0.05	0.06/0.06	1.31/1.40	2.49/2.58	4.50/4.57	9.26/9.30	18.4/19.1		
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)	<2	<2	<2	<2	<2	<2	<2		

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2.5. <u>LC₅₀ for the amphipod *Melita longidactyla* and test acceptability</u>

Table 4. LC₅₀ for the amphipods and test acceptability.

Parameter	Value	Control limit
Calculated LC ₅₀	29.1 %	NA
Negative control survival	100.00%	>90%
Reference toxicant 48-h acute test	1.32 mg L ⁻¹	$1.25\pm0.15 \text{ mg } \text{L}^{-1}$
95% of confidence range of reference toxicant test	1.05-1.46 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

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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. <u>Test organism</u>

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.

-	
Type of test:	Static
Duration:	48 h, 27/4/2019-29/4/2019
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 ₂
Positive control:	48 h acute toxicity test
Salinity control:	Prepared with ocean salt adding into de-ionized water, salinity: 30%

3.3. <u>Summary of test conditions</u>

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3.4. <u>Test results</u>

Table 1. Survival of fish after 48 hours.

Treastreamt	Effluent concentration (%)	Number of living fish after 48 hour (individuals)						
Treatment		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	20	20.00	0.00	
Concentration 1	6.5	20	20	20	20	20.00	0.00	
Concentration 2	12.5	20	20	20	20	20.00	0.00	
Concentration 3	25	17	18	19	18	18.00	0.81	
Concentration 4	50	12	15	14	13	13.5	1.29	
Concentration 5	100	0	0	0	0	0.00	0.00	

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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	100	100	100.00	0.00	
Salinity control	0	100	100	100	100	100.00	0.00	
Concentration 1	6.5	100	100	100	100	100.00	0.00	
Concentration 2	12.5	100	100	100	100	100.00	0.00	
Concentration 3	25	85	90	95	90	90.00	4.08	
Concentration 4	50	60	75	70	65	67.5	6.45	
Concentration 5	100	0	0	0	0	0.00	0.00	

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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.

	Effluent concentration (%)							
Water quality parameters	Negative control	Salinity control	6.5	12.5	25	50	100	
Salinity (‰)	30	30	30	30	30	30	30	
Dissolved oxygen (mg L-1)	6.7-7.2	6.7-7.3	6.7-7.0	6.6-7.0	6.8-7.4	6.8-7.1	6.7-6.9	
Temperature (°C)	22	22	22	22	22	22	22	
pH	7.9-8.1	7.8-8.1	7.8-8.0	7.8-8.0	7.7-8.1	7.6-8.0	7.6-7.9	
Total ammonia (start/end, mg L ⁻¹)	0.15/0.16	0.17/0.17	1.66/1.70	3.15/3.26	5.27/5.41	11.8/13.0	24.4/28.7	
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)	10/9	10/10	14/17	17/19	30/32	46/50	81/84	

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3.6. <u>LC₅₀ for the fish *Lutjanus malabaricus* and test acceptability</u>

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	51.3 %	NA
Negative survival	100.0 %	>90%
Reference toxicant 48-h acute test	13.87 mg L-1	14.6±1.78 mg L ⁻¹
95% of confidence range of reference toxicant test	$11.51-15.69 \text{ mg } \text{L}^{-1}$	NA
Daily temperature variation	<0.5 °C	Average daily temperature variation: $\pm 1 {}^{\circ}\text{C}$
Dissolved oxygen concentration	>6.1 mg L ⁻¹	>4 mg L-1

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 μ m 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. <u>Test organism</u>

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 <i>Chaetoceros gracilis</i> .

· · · · · ·	
Type of test:	Static
Duration:	48 h, 27/4/2019-29/4/2019
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:	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 ₂
Positive control:	48 h acute toxicity test
Salinity control:	Prepared with ocean salt adding into de-ionized water, salinity: 30‰

4.3. <u>Summary of test conditions</u>

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4.4. <u>Test results</u>

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	19	20	20	20	19.75	0.50		
Salinity control	0	20	19	19	19	19.25	0.50		
Concentration 1	6.5	19	16	19	18	18.00	1.41		
Concentration 2	12.5	12	16	17	16	15.25	2.22		
Concentration 3	25	15	7	12	10	11.00	3.37		
Concentration 4	50	2	4	4	0	2.50	1.91		
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	95	100	100	100	98.75	2.50		
Salinity control	0	100	95	95	95	96.25	2.50		
Concentration 1	6.5	95	80	95	90	90.00	7.07		
Concentration 2	12.5	60	80	85	80	76.25	11.09		
Concentration 3	25	75	35	60	50	55.00	16.83		
Concentration 4	50	10	20	20	0	12.50	9.57		
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

	Effluent concentration (%)							
Water quality parameters	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)	7.9-8.8	7.5-8.0	7.4-8.0	7.4-7.9	7.4-7.6	7.6-7.9	7.0-7.9	
Temperature (°C)	22.0	22.0	22.0	22.0	22.0	22.0	22.0	
pH	7.9-8.1	7.9-8.0	7.9-8.1	7.7-7.9	7.9-8.1	7.9-8.1	7.8-8.1	
Total ammonia (start/end, mg L-1)	0.17/0.15	0.17/0.17	1.24/1.30	2.83/2.92	5.08/5.18	12.3/13.4	22.9/24.5	
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	

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4.6. <u>LC₅₀ for the barnacle larvae Balanus amphitrite and test acceptability</u>

Table 4. LC₅₀ for the barnacle larvae and test acceptability

Parameter	Value	Control limit
Calculated LC ₅₀	23.2 %	NA
Negative survival	98.75 %	>90%
Reference toxicant 48-h acute test	1.08 mg L-1	1.04±0.11 mg L ⁻¹
95% of confidence range of reference toxicant test:	0.94-1.09 mg L-1	NA
Daily temperature variation	<0.5°C	Average daily temperature variation: ± 1 °C
Dissolved oxygen concentration	>7.2 mg L-1	>4 mg L-1

NA: Not applicable



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Application No.: LV021027(1)

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

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Report No. : AY0024951(0)

Application No.: LV021027(1)

Test report

5.1. Samples storage and pretreatment

Effluent sample was thoroughly mixed and passed through 5 μ m 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.	<u>Test organism</u>					
Speci	es	Diatom (Skeletonema costatum)				
Sourc	re:	Grown from laboratory culture obtained from Coastal Marine Lab, Hong Kong University of Science and Technology				
Size/	age:	Log growth phase				
Accli	matization:	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					
Туре	of test:	Static				
Dura	tion:	7 days, 27/4/2019-4/5/2019				
Dilution seawater source:		Seawater collected from a pristine site in Clear Water Bay, Sai Kung, Hong Kong				
Dilut	ion seawater pretreatment:	Filtered through 0.22 µm membrane				
Testir	ng temperature:	22±1 °C				
Light	ing:	12 h light/12 h dark cycle, 3000±500 lux				
Salini	ity:	30‰				
Testir	ng chamber:	Pre-cleaned 100 mL glass beaker				
Initia	l cell density:	$(5.0\pm0.4)\times10^4$ cell mL ⁻¹				
Repli	cate number:	4				
Volui	me of test medium:	25 mL				
Aerat	ion:	None				
Refer	ence toxicant:	CdCl ₂				
Positi	ve control:	7-day IC ₅₀ toxicity test				
Salini	ity control:	Prepared with ocean salt adding into de-ionized water, salinity: 30%				

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Application No.: LV021027(1)

5.4. Test results

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Table 1. Cell density of diatom *Skeletonema costatum* at the beginning and end of growth inhibition test. Initial cell density: (5.0±0.4)×10⁴ cell mL⁻¹.

Treatment	Effluent concentration (%)	Cell density after 7-day growth (×10 ⁶ cell mL ⁻¹)						
		Replicate 1	Replicate 2	Replicate 3	Replicate 4	Mean	SD	
Negative control	0	1.08	1.07	1.10	1.05	1.08	0.02	
Salinity control	0	1.07	1.10	1.10	1.09	1.09	0.01	
Concentration 1	2.5	1.12	1.16	1.12	1.15	1.14	0.02	
Concentration 2	5	1.40	1.34	1.34	1.37	1.36	0.03	
Concentration 3	10	1.67	1.63	1.61	1.64	1.64	0.02	
Concentration 4	25	1.05	0.99	1.02	1.06	1.03	0.03	
Concentration 5	50	0.84	0.86	0.82	0.83	0.84	0.02	
Concentration 6	100	0.00	0.00	0.00	0.00	0.00	0.00	

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T	$\Gamma(0)$	7-day average growth rate (d-1)						
Treatment	Effluent concentration (%)	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Mean	SD	
Negative control	0	0.44	0.44	0.44	0.43	0.44	0.00	
Salinity control	0	0.44	0.44	0.44	0.44	0.44	0.00	
Concentration 1	2.5	0.44	0.45	0.44	0.45	0.45	0.00	
Concentration 2	5	0.48	0.47	0.47	0.47	0.47	0.00	
Concentration 3	10	0.50	0.50	0.50	0.50	0.50	0.00	
Concentration 4	25	0.43	0.43	0.43	0.44	0.43	0.00	
Concentration 5	50	0.40	0.41	0.40	0.40	0.40	0.00	
Concentration 6	100	0.00	0.00	0.00	0.00	0.00	0.00	

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

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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

	Effluent concentration (%)								
Water quality parameters	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)	6.7-8.2	6.7-8.2	6.7-8.3	6.8-8.6	6.7-9.3	6.7-8.0	6.4-8.0	6.4-6.8	
Temperature (°C)	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0	
pH	7.8-8.2	7.9-8.2	7.7-8.2	7.8-8.2	7.8-8.1	7.8-8.1	7.4-8.1	7.2-8.0	
Total ammonia (start/end, mg L ⁻¹)	0.13/0.15	0.13/0.14	1.26/1.31	2.55/2.68	3.84/3.95	5.41/6.20	11.3/11.9	22.7/24.3	
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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5.6. <u>IC₅₀ for the diatom Skeletonema costatum and test acceptability</u>

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

	1 5	
Parameter	Value	Control limit
Calculated IC ₅₀	61 %	NA
None observed effect concentration (NOEC)	10 %	-
Reference toxicant 7-day test:	0.14 mg L-1	0.13±0.02 mg L ⁻¹
95% of confidence range of reference toxicant test	0.12-0.18 mg L-1	NA
Temperature variation	<0.5 °C	Average daily temperature variation: ± 1 °C
and a second		

NA: Not applicable

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Application No.: LV021027(1)

6. Report on Shrimp Acute Toxicity Test

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Report No. : AY0024951(0)

Application No.: LV021027(1)

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.	<u>Test organism</u>	
Speci	es	Shrimp (Metapenaeus ensis).
Sourc	ce:	Purchased from contracted fish dealer
Size/	age:	5-7 cm
Accli	matization:	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	
Туре	of test:	Static
Dura	tion:	48 h, 27/4/2019-29/4/2019
Dilution seawater source:		Seawater collected from a pristine site in Clear Water Bay, Sai Kung,
		Hong Kong
Dilut	ion seawater pretreatment:	Filtered through 0.22 µm membrane
Testi	ng temperature:	22±1 °C
Light	ing:	Continuous
Salin	ity:	25‰
Testi	ng chamber:	Pre-cleaned 20 L tank
Feedi	ing:	None
Num	ber of organisms per replicate:	10
Repli	cate number:	4
Volu	me of test medium:	10 L
Aera	tion:	Moderate, with air stone
Refer	ence toxicant:	CdCl ₂
Posit	ive control:	48 h acute toxicity test
Salin	ity control:	Prepared with ocean salt adding into de-ionized water, salinity: 25‰

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6.4. Test results

	1									
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	10	8	9	10	9.25	0.96			
Concentration 1	6.5	9	8	8	7	8.00	0.82			
Concentration 2	12.5	8	8	9	8	8.25	0.50			
Concentration 3	25	7	7	8	7	7.25	0.50			
Concentration 4	50	5	6	6	7	6.00	0.82			
Concentration 5	100	0	2	1	1	1.00	0.82			

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Table 2. Survival per	rcentage of shrimps	s after 48 hours.
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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	100	80	90	100	92.50	9.57		
Concentration 1	6.5	90	80	80	70	80.00	8.16		
Concentration 2	12.5	80	80	90	80	82.50	5.00		
Concentration 3	25	70	70	80	70	72.50	5.00		
Concentration 4	50	50	60	60	70	60.00	8.16		
Concentration 5	100	0	20	10	10	10.00	8.16		

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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.

	Effluent concentration (%)								
Water quality parameters	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)	7.5-7.7	7.3-7.8	7.6-7.9	7.3-7.8	7.3-7.8	7.4-8.0	7.5-8.2		
Temperature (°C)	22.0	22.0	22.0	22.0	22.0	22.0	22.0		
pH	8.0-8.1	7.9-8.1	7.8-8.1	7.7-8.0	7.8-8.2	7.6-8.1	7.7-8.0		
Total ammonia (start/end, mg L ⁻¹)	0.10/0.10	0.11/0.12	1.26/1.43	2.63/2.85	5.08/5.17	9.25/10.5	17.5/18.7		
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)	9/10	9/9	11/13	17/19	32/35	51/55	63/67		

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6.6 <u>LC₅₀ for the shrimp *Metapenaeus ensis* and test acceptability</u>

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

Parameter	Value	Control limit
Calculated I C=0	58.1 %	NA
Negative control survival	97 50 %	>90%
Reference toxicant 48-h acute test	$5.32 \text{ mg } \text{L}_{-1}$	510+0.51 mg I -1
95% of confidence range of reference toxicant test	4.87-5.83 mg L -1	NA
Daily temperature variation	4.07-5.05 mg L-1	Average deily temperature veriation +1 °C
Disastra d surger contration	<0.5 C	Average daily temperature variation. ±1 C
Dissolved oxygen concentration	>7.3 mg L-1	>4 mg L-1

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 ₅₀ /IC ₅₀ /NOEC	Target toxicity level
Amphipod Melita longidactyla	29.1 %	≥7.1%
Fish Lutjanus malabaricus	51.3 %	≥7.1%
Barnacle larvae Balanus amphitrite	23.2 %	≥7.1%
Diatom Skeletonema costatum	61 %	-
Diatom Skeletonema costatum	10 % (NOEC)	≥0.51%
Shrimp Metapenaeus ensis	58.1 %	≥7.1%

*The survivals in all concentration groups were >50%, and thus LC_{50} cannot be calculated.

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

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Application No.: LV021027(1)

Appendix A

Monitoring Data for Amphipod Acute Toxicity Test

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Table 1. Dissolved oxygen concentration and pri in each concentration treatment in amphipod acute toxicity test.										
Concentration	Dissolved oxygen (mg L-1)					pH				
deatherit (70)	0 h	12 h	24 h	36 h	48 h	0 h	12 h	24 h	36 h	48 h
Negative control	6.8	7.0	6.9	7.1	7.2	7.8	7.8	8.0	8.0	7.9
Salinity control	6.7	6.9	7.0	7.0	7.1	7.8	7.8	8.0	7.9	7.9
6.5	6.8	7.0	7.2	7.1	7.1	7.7	7.9	8.0	7.9	8.0
12.5	6.8	6.9	7.1	7.2	7.2	7.7	7.8	8.0	8.0	8.1
25	6.7	7.1	7.3	7.1	7.1	7.7	7.9	8.1	8.0	8.1
50	6.8	7.0	6.9	7.1	7.2	7.6	7.7	8.0	7.9	8.0
100	6.6	6.8	7.1	7.2	7.2	7.3	7.5	7.7	7.8	7.8

Table 1. Dissolved	oxygen concentration and	pH in eacl	h concentration treatment	in amphi	pod acute toxicit	v test.
		P				,



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Application No.: LV021027(1)

Table 2. Samily and temp	<i>Alatui</i> I	n cach co.	nceman	лисани		pinpou	acuic ioz	ticity its	ι.	
Concentration treatment (%)		Sa	alinity (%	o)			Tem	perature	(°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

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



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Table 3.	Ammonia-N	, sulphide,	total sus	pended solid	ls, total	residual	chlorine	conce	ntration	n at the	beginni	ng	and en	ding
of the an	nphipod acut	te toxicity f	test.								-			-

- the the Feer Feer He								
Concentration	Ammo	onia-N	Sulp	hide	Total suspe	nded solids	Total residu	al chlorine
treatment	(mg	L-1)	(mg	L-1)	(mg	L-1)	(mg	L-1)
(%)	Initial	End	Initial	End	Initial	End	Initial	End
Negative control	0.05	0.05	<0.1	<0.1	<2	<2	< 0.02	<0.02
Salinity control	0.06	0.06	< 0.1	< 0.1	<2	<2	< 0.02	< 0.02
6.5	1.31	1.40	< 0.1	< 0.1	<2	<2	< 0.02	< 0.02
12.5	2.49	2.58	< 0.1	< 0.1	<2	<2	< 0.02	< 0.02
25	4.50	4.57	< 0.1	< 0.1	<2	<2	< 0.02	< 0.02
50	9.26	9.30	< 0.1	< 0.1	<2	<2	< 0.02	< 0.02
100	18.4	19.1	< 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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1000 1. 01000100	u oxygen	concentitu	fion and p	11 m cuen	concentitu	non neu	mem m	1011 ucute	toxicity	
Concentration		Dissolve	ed oxygen	(mg L-1)				рН		
treatment (70)	0 h	12 h	24 h	36 h	48 h	0 h	12 h	24 h	36 h	48 h
Negative control	6.8	6.7	6.8	6.9	7.2	8.0	8.1	8.1	8.1	7.9
Salinity control	6.7	7.0	6.8	6.8	7.3	8.0	7.9	8.1	7.8	7.8
6.5	6.8	6.9	6.7	6.9	7.0	8.0	8.0	7.9	7.8	7.8
12.5	7.0	6.6	6.9	7.0	7.0	7.9	8.0	8.0	7.9	7.8
25	6.9	6.9	7.2	7.1	7.4	8.1	8.1	7.8	7.8	7.7
50	6.8	7.1	7.0	6.9	7.1	7.9	8.0	7.8	7.8	7.6
100	6.7	6.9	6.9	6.9	6.8	7.9	7.6	7.7	7.9	7.7

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

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Concentration		S	Salinity (‰)			Tem	perature	(°C)	
deatherit (70)	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

Table 2. Salinity	and temperature in each	concentration treatment	in fisł	n acute toxicity	y test.



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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	Ammo (mg	Ammonia-N (mg L ⁻¹)		Sulphide (mg L ⁻¹)		nded solids L ⁻¹)	Total residual chlorine (mg L ⁻¹)		
(%)	Initial	End	Initial	End	Initial	End	Initial	End	
Negative control	0.15	0.16	<0.1	< 0.1	10	9	< 0.02	< 0.02	
Salinity control	0.17	0.17	< 0.1	< 0.1	10	10	< 0.02	< 0.02	
6.5	1.66	1.70	< 0.1	< 0.1	14	17	< 0.02	< 0.02	
12.5	3.15	3.26	< 0.1	< 0.1	17	19	< 0.02	< 0.02	
25	5.27	5.41	< 0.1	< 0.1	30	32	< 0.02	< 0.02	
50	11.8	13.0	< 0.1	< 0.1	46	50	< 0.02	< 0.02	
100	24.4	28.7	< 0.1	< 0.1	81	84	< 0.02	< 0.02	

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Appendix C

Monitoring Data for Barnacle Larvae Acute Toxicity Test

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Report No. : AY0024951(0)

Application No.: LV021027(1)

Table 1. Dissolved	oxygen co	ncentratior	n and pH ir	n each conc	entration t	reatment	in barnac	le larvae a	acute toxi	city test.
Concentration		Dissolv	ed oxygen	(mg L-1)		рН				
ficultient (70)	0 h	12 h	24 h	36 h	48 h	0 h	12 h	24 h	36 h	48 h
Negative control	8.8	8.5	8.3	8.5	7.9	8.0	8.1	8.1	8.0	7.9
Salinity control	7.5	7.9	7.8	7.6	8.0	7.9	8.0	7.9	7.9	7.9
6.5	7.8	7.8	7.4	7.5	7.9	7.9	8.0	8.1	8.1	8.0
12.5	7.9	7.4	7.7	7.8	7.8	7.9	7.9	7.8	7.7	7.7
25	7.4	7.4	7.4	7.6	7.5	8.0	8.1	8.0	7.9	7.9
50	7.6	7.8	7.9	7.8	7.6	8.0	7.9	8.0	8.0	8.1
100	7.7	7.5	7.9	7.5	7.0	8.0	7.8	7.9	8.1	8.0

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Report No. : AY0024951(0)

Application No.: LV021027(1)

Table 2. Salinity and	d temperat	ure in each	n concentra	ition treatn	nent in bar	nacle larv	vae acute	toxicity te	est.			
Concentration Salinity (%) treatment (%)						Temperature (°C)						
treatment (70)	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		

Table 2 Calinita 1 1. . 1 - Lus L'aus Lu . 1 . 1 -1 - 1 to to distant and

Application No.: LV021027(1)

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	Ammo	Ammonia-N		Sulphide		nded solids	Total residual chlorine		
treatment	(mg	L-1)	(mg	L-1)	(mg	L-1)	(mg	L-1)	
(%)	Initial	End	Initial	End	Initial	End	Initial	End	
Negative control	0.17	0.15	< 0.1	< 0.1	<2	<2	< 0.02	< 0.02	
Salinity control	0.17	0.17	< 0.1	< 0.1	<2	<2	< 0.02	< 0.02	
6.5	1.24	1.30	< 0.1	< 0.1	<2	<2	< 0.02	< 0.02	
12.5	2.83	2.92	< 0.1	< 0.1	<2	<2	< 0.02	< 0.02	
25	5.08	5.18	< 0.1	< 0.1	<2	<2	< 0.02	< 0.02	
50	12.3	13.4	< 0.1	< 0.1	<2	<2	< 0.02	< 0.02	
100	22.9	24.5	< 0.1	< 0.1	<2	<2	< 0.02	< 0.02	

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Application No.: LV021027(1)

Appendix D

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

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Concentration		Dissolved oxygen (mg L ⁻¹)						pH								
treatment (%)	0	24	48	72	96	120	144	168	0	24	48	72	96	120	144	168
	h	h	h	h	h	h	h	h	h	h	h	h	h	h	h	h
Negative control	6.8	6.7	7.0	7.4	7.7	8.0	8.1	8.2	7.8	7.8	8.0	7.9	8.1	8.1	8.2	8.1
Salinity control	6.7	6.9	7.2	7.6	7.9	7.8	8.2	8.1	7.9	7.9	8.0	8.1	8.1	8.0	8.2	8.2
2.5	6.7	6.9	7.1	7.4	7.8	8.0	8.0	8.3	7.7	7.9	7.8	7.9	8.0	8.1	8.0	8.2
5	6.8	7.2	7.3	7.5	8.0	8.1	8.2	8.6	7.8	8.0	8.0	7.9	8.1	8.2	8.1	8.1
10	6.7	7.1	7.4	7.9	8.2	8.6	9.3	9.2	7.8	7.9	7.9	8.0	8.1	8.1	8.0	8.1
25	6.7	6.9	7.2	7.5	7.7	7.7	7.9	8.0	7.8	8.0	8.0	7.9	8.0	8.1	8.1	8.1
50	6.4	6.5	6.9	7.3	7.5	7.8	8.0	7.9	7.4	7.6	7.8	7.9	7.9	8.0	8.1	7.9
100	6.4	6.4	6.5	6.6	6.7	6.8	6.7	6.6	7.2	7.3	7.5	7.6	7.8	7.9	8.0	8.0

Table 1. Dissolved oxygen concentration and pH in each concentration treatment in diatom growth inhibition te	each concentration treatment in diatom growth inhibition test.
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Application No.: LV021027(1)

Table 2. Samily and temperature in each concentration treatment in diatom growth mubition test.	Table 2. Salinity and tem	perature in each co	oncentration treatment	in diatom	growth inhibition test.
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Concentration				Salini	ty (‰)						,	Гетрег	ature (°	°C)		
treatment (%)	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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Application No.: LV021027(1)

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	Ammonia-N		Sulp	hide	Total susper	nded solids	Total residual chlorine		
treatment	(mg	L-1)	(mg L-1)		(mg	L-1)	(mg L ⁻¹)		
(%)	Initial	End	Initial	End	Initial	End	Initial	End	
Negative control	0.13	0.15	< 0.1	< 0.1	<2	<2	< 0.02	< 0.02	
Salinity control	0.13	0.14	< 0.1	< 0.1	<2	<2	< 0.02	< 0.02	
2.5	1.26	1.31	< 0.1	< 0.1	<2	<2	< 0.02	< 0.02	
5	2.55	2.68	< 0.1	< 0.1	<2	<2	< 0.02	< 0.02	
10	3.84	3.95	< 0.1	< 0.1	<2	<2	< 0.02	< 0.02	
25	5.41	6.20	< 0.1	< 0.1	<2	<2	< 0.02	< 0.02	
50	11.3	11.9	< 0.1	< 0.1	<2	<2	< 0.02	< 0.02	
100	22.7	24.3	< 0.1	< 0.1	<2	<2	< 0.02	< 0.02	

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Application No.: LV021027(1)

Appendix E

Monitoring Data for Shrimp Acute Toxicity Test

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Application No.: LV021027(1)

Table 1. Dissolved oxygen concentration and pH in each concentration treatment in shrimp acute toxicity test.											
Concentration		Dissolve	ed oxygen	(mg L-1)	pH						
freditient (70)	0 h	12 h	24 h	36 h	48 h	0 h	12 h	24 h	36 h	48 h	
Negative control	7.7	7.6	7.6	7.7	7.5	8.0	8.0	8.0	8.1	8.0	
Salinity control	7.4	7.3	7.4	7.8	7.6	8.1	8.0	7.9	7.9	8.0	
6.5	7.6	7.7	7.9	7.8	7.8	8.1	8.0	7.8	7.8	7.9	
12.5	7.3	7.4	7.4	7.8	7.5	7.9	7.9	7.7	7.8	8.0	
25	7.7	7.8	7.5	7.6	7.3	7.9	7.8	7.9	7.9	8.2	
50	7.6	7.4	8.0	7.9	7.7	8.0	7.8	7.6	7.8	8.1	
100	7.6	7.9	8.2	7.9	7.5	8.0	7.9	7.7	7.8	8.0	





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Report No. : AY0024951(0)

Application No.: LV021027(1)

Table 2. Salinity and temperature in each concentration treatment in shrimp acute toxicity test.										
Concentration		S	Salinity (‰)	Temperature (°C)					
ficament (70)	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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Application No.: LV021027(1)

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

Concentration	Ammonia-N		Sulp	hide	Total susper	nded solids	Total residual chlorine		
treatment	(mg	L-1)	(mg	L-1)	(mg	L-1)	(mg L-1)		
(%)	Initial	End	Initial	End	Initial	End	Initial	End	
Negative control	0.10	0.10	< 0.1	< 0.1	9	10	< 0.02	< 0.02	
Salinity control	0.11	0.12	< 0.1	< 0.1	9	9	< 0.02	< 0.02	
6.5	1.26	1.43	< 0.1	< 0.1	11	13	< 0.02	< 0.02	
12.5	2.63	2.85	< 0.1	< 0.1	17	19	< 0.02	< 0.02	
25	5.08	5.17	< 0.1	< 0.1	32	35	< 0.02	< 0.02	
50	9.25	10.5	< 0.1	< 0.1	51	55	< 0.02	< 0.02	
100	17.5	18.7	< 0.1	< 0.1	63	67	< 0.02	< 0.02	

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