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 July 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.	:	LY027200(0)
Report No.	:	AY0046497(8)
Report Issued Date	:	21 Aug 2019

For and on behalf of
CMA Industrial Development Foundation Limited

Authorized Signature : _____

Lau Yan Kin
Senior Manager
Environmental Division

CMA Industrial Development Foundation Limited
Room 1302, Yan Hing Centre, 9-13 Wong Chuk Yeung St., Fo Tan, Shatin, N.T., Hong Kong.
Tel : (852) 2698 8198 Fax : (852) 2695 4177 E-mail : info@cmatesting.org Web Site : http://www.cmatesting.org

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CMA Industrial Development Foundation Limited
Room 1302, Yan Hing Centre, 9-13 Wong Chuk Yeung St., Fo Tan, Shatin, N.T., Hong Kong.
Tel : (852) 2698 8198 Fax : (852) 2695 4177 E-mail : info@cmatesting.org Web Site : http://www.cmatesting.org

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Room 1302, Yan Hing Centre, 9-13 Wong Chuk Yeung St., Fo Tan, Shatin, N.T., Hong Kong.
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Report No. : AY0046497(8)

Application No.: LY027200(0)

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 27th, 2019. Effluent sample was shipped immediately to the testing laboratory on the same day of collection and stored at 4 ^{o}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).

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2. Report on Amphipod Acute Toxicity Test

CMA Industrial Development Foundation Limited
Room 1302, Yan Hing Centre, 9-13 Wong Chuk Yeung St., Fo Tan, Shatin, N.T., Hong Kong.
Tel : (852) 2698 8198 Fax : (852) 2695 4177 E-mail : info@cmatesting.org Web Site : http://www.cmatesting.org

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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, 27/7/2019-29/7/2019
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‰

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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	10	10	9.25	0.96
Concentration 3	25	5	7	5	6	5.75	0.96
Concentration 4	50	2	3	3	1	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 (%)					
		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	100	100	92.50	9.57
Concentration 3	25	50	70	50	60	57.50	9.57
Concentration 4	50	20	30	30	10	22.50	9.57
Concentration 5	100	0	0	0	0	0.00	0.00

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Room 1302, Yan Hing Centre, 9-13 Wong Chuk Yeung St., Fo Tan, Shatin, N.T., Hong Kong.
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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^{-1})	7.0-7.5	6.9-7.3	6.8-7.5	6.7-7.5	6.8-7.6	6.7-7.4	6.7-7.2
Temperature (^oC)	22.0	22.0	22.0	22.0	22.0	22.0	22.0
pH	7.9-8.2	7.9-8.2	7.8-8.2	7.6-8.2	7.9-8.2	7.7-8.0	7.4-8.1
Total ammonia (start/end, mg L^{-1})	0.06/0.06	0.05/0.06	1.63/1.67	2.91/3.12	5.84/5.96	10.3/10.8	19.5/20.1
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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2.6 LC_{50} for the amphipod *Melita longidactyla* and test acceptability

Table 4. LC_{50} for the amphipods and test acceptability.

Parameter	Value	Control limit
Calculated LC_{50}	29.2 %	NA
Negative control survival	100.00%	>90%
Reference toxicant 48-h acute test	1.33 mg L^{-1}	1.25±0.15 mg L^{-1}
95% of confidence range of reference toxicant test	1.08-1.47 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}

NA: Not applicable

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3. Report on Fish Acute Toxicity Test

CMA Industrial Development Foundation Limited
Room 1302, Yan Hing Centre, 9-13 Wong Chuk Yeung St., Fo Tan, Shatin, N.T., Hong Kong.
Tel : (852) 2698 8198 Fax : (852) 2695 4177 E-mail : info@cmatesting.org Web Site : http://www.cmatesting.org

Report No. : AY0046497(8)

Application No.: LY027200(0)

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, 27/7/2019-29/7/2019
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‰

CMA Industrial Development Foundation Limited
Room 1302, Yan Hing Centre, 9-13 Wong Chuk Yeung St., Fo Tan, Shatin, N.T., Hong Kong.
Tel : (852) 2698 8198 Fax : (852) 2695 4177 E-mail : info@cmatesting.org Web Site : http://www.cmatesting.org

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	20	20.00	0.00
Concentration 1	6.5	20	20	20	20	20.00	0.00
Concentration 2	12.5	20	19	20	19	19.50	0.58
Concentration 3	25	18	18	19	18	18.25	0.50
Concentration 4	50	13	14	15	10	13.00	2.16
Concentration 5	100	0	0	0	0	0.00	0.00

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Room 1302, Yan Hing Centre, 9-13 Wong Chuk Yeung St., Fo Tan, Shatin, N.T., Hong Kong.
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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	95	95	100	100	97.50	2.89
Concentration 3	25	90	90	90	95	91.25	2.50
Concentration 4	50	65	70	75	50	65.00	10.80
Concentration 5	100	0	0	0	0	0.00	0.00

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Room 1302, Yan Hing Centre, 9-13 Wong Chuk Yeung St., Fo Tan, Shatin, N.T., Hong Kong.
Tel : (852) 2698 8198 Fax : (852) 2695 4177 E-mail : info@cmatesting.org Web Site : http://www.cmatesting.org

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})	6.7-7.1	6.7-7.2	6.8-7.1	6.8-7.1	6.8-7.3	6.7-7.2	6.7-6.9
Temperature (^{o}C)	22	22	22	22	22	22	22
pH	7.9-8.1	7.8-8.1	7.8-8.0	7.8-8.1	7.7-8.1	7.7-8.1	7.7-7.9
Total ammonia (start/end, mg L^{-1})	0.12/0.12	0.14/0.14	1.40/1.37	2.92/2.94	5.31/5.35	10.1/10.1	22.6/23.5
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})	7/7	8/7	15/17	22/20	37/33	48/45	75/79

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3.6. LC_{50} for the fish *Lutjanus malabaricus* and test acceptability

Table 4. LC_{50} 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_{50} cannot be calculated.

Parameter	Value	Control limit
Calculated LC_{50}	49.8 %	NA
Negative survival	100.0 %	>90%
Reference toxicant 48-h acute test	13.62 mg L^{-1}	14.6±1.78 mg L^{-1}
95% of confidence range of reference toxicant test	11.44-15.69 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

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4. Report on Barnacle Larvae Acute Toxicity Test

CMA Industrial Development Foundation Limited
Room 1302, Yan Hing Centre, 9-13 Wong Chuk Yeung St., Fo Tan, Shatin, N.T., Hong Kong.
Tel : (852) 2698 8198 Fax : (852) 2695 4177 E-mail : info@cmatesting.org Web Site : http://www.cmatesting.org

Report No. : AY0046497(8)

Application No.: LY027200(0)

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, 27/7/2019-29/7/2019
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‰

CMA Industrial Development Foundation Limited
Room 1302, Yan Hing Centre, 9-13 Wong Chuk Yeung St., Fo Tan, Shatin, N.T., Hong Kong.
Tel : (852) 2698 8198 Fax : (852) 2695 4177 E-mail : info@cmatesting.org Web Site : http://www.cmatesting.org

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	18	18	19	18.75	0.96
Salinity control	0	20	18	19	19	19.00	0.82
Concentration 1	6.5	17	16	18	19	17.50	1.29
Concentration 2	12.5	16	12	15	15	14.50	1.73
Concentration 3	25	10	12	10	8	10.00	1.63
Concentration 4	50	0	2	5	2	2.25	2.06
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	90	90	95	93.75	4.79
Salinity control	0	100	90	95	95	95.00	4.08
Concentration 1	6.5	85	80	90	95	87.50	6.45
Concentration 2	12.5	80	60	75	75	72.50	8.66
Concentration 3	25	50	60	50	40	50.00	8.16
Concentration 4	50	0	10	25	10	11.25	10.31
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^{-1})	7.8-8.7	7.6-7.9	7.5-7.9	7.4-7.8	7.5-7.6	7.5-7.8	7.1-7.7
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	8.0-8.1	7.8-7.9	7.8-8.1	7.9-8.1	7.9-8.1
Total ammonia (start/end, mg L^{-1})	0.24/0.28	0.23/0.24	0.98/1.07	2.16/2.24	4.05/4.13	8.76/8.92	15.3/16.5
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

CMA Industrial Development Foundation Limited
Room 1302, Yan Hing Centre, 9-13 Wong Chuk Yeung St., Fo Tan, Shatin, N.T., Hong Kong.
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4.6. LC_{50} for the barnacle larvae *Balanus amphitrite* and test acceptability

Table 4. LC_{50} for the barnacle larvae and test acceptability

Parameter	Value	Control limit
Calculated LC_{50}	22.7 %	NA
Negative survival	93.75 %	>90%
Reference toxicant 48-h acute test	1.04 mg L-1	1.04±0.11 mg L^{-1}
95% of confidence range of reference toxicant test:	0.94-1.06 mg L-1	NA
Daily temperature variation	<0.5℃	Average daily temperature variation: ± 1 °C
Dissolved oxygen concentration	>7.1 mg L-1	>4 mg L^{-1}

NA: Not applicable

CMA Industrial Development Foundation Limited
Room 1302, Yan Hing Centre, 9-13 Wong Chuk Yeung St., Fo Tan, Shatin, N.T., Hong Kong.
Tel : (852) 2698 8198 Fax : (852) 2695 4177 E-mail : info@cmatesting.org Web Site : http://www.cmatesting.org

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

CMA Industrial Development Foundation Limited
Room 1302, Yan Hing Centre, 9-13 Wong Chuk Yeung St., Fo Tan, Shatin, N.T., Hong Kong.
Tel : (852) 2698 8198 Fax : (852) 2695 4177 E-mail : info@cmatesting.org Web Site : http://www.cmatesting.org

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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, 27/7/2019-3/8/2019
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:	$(5.0\pm0.4)\times10^4$ cell mL^{-1}
Replicate number:	4
Volume of test medium:	25 mL
Aeration:	None
Reference toxicant:	$CdCl_2$
Positive control:	7-day IC_{50} toxicity test
Salinity control:	Prepared with ocean salt adding into de-ionized water, salinity: 30‰

CMA Industrial Development Foundation Limited
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Tel : (852) 2698 8198 Fax : (852) 2695 4177 E-mail : info@cmatesting.org Web Site : http://www.cmatesting.org

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±0.4)×10^4 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	1.13	1.12	1.15	1.15	1.14	0.01
Salinity control	0	1.12	1.13	1.15	1.15	1.14	0.01
Concentration 1	2.5	1.17	1.18	1.13	1.18	1.17	0.02
Concentration 2	5	1.44	1.47	1.42	1.45	1.45	0.02
Concentration 3	10	1.72	1.72	1.73	1.71	1.72	0.01
Concentration 4	25	1.80	1.83	1.75	1.77	1.79	0.04
Concentration 5	50	1.01	1.08	1.07	1.03	1.05	0.03
Concentration 6	100	0.00	0.00	0.00	0.00	0.00	0.00

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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	0.45	0.44	0.45	0.45	0.45	0.00
Salinity control	0	0.44	0.45	0.45	0.45	0.45	0.00
Concentration 1	2.5	0.45	0.45	0.45	0.45	0.45	0.00
Concentration 2	5	0.48	0.48	0.48	0.48	0.48	0.00
Concentration 3	10	0.51	0.51	0.51	0.50	0.51	0.00
Concentration 4	25	0.51	0.51	0.51	0.51	0.51	0.00
Concentration 5	50	0.43	0.44	0.44	0.43	0.43	0.00
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^{-1})	6.9-8.4	6.9-8.5	6.8-8.6	6.8-8.4	6.9-8.8	6.9-9.3	6.7-8.1	6.6-6.9
Temperature (°C)	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0
pH	7.9-8.3	7.9-8.4	7.9-8.3	7.8-8.3	7.8-8.3	7.7-8.3	7.4-8.3	7.3-7.9
Total ammonia (start/end, mg L^{-1})	0.19/0.17	0.19/0.20	1.32/1.36	2.47/2.48	4.61/4.65	5.86/5.91	12.4/12.7	23.0/24.2
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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5.6. IC_{50} for the diatom *Skeletonema costatum* and test acceptability

Table 4. IC_{50}, none observed effect concentration (NOEC) for the diatom and test acceptability

Parameter	Value	Control limit
Calculated IC_{50}	65.4 %	NA
None observed effect concentration (NOEC)	25 %	-
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	0.13-0.18 mg L-1	NA
Temperature variation	<0.5 °C	Average daily temperature variation: ± 1 °C

NA: Not applicable

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Report No. : AY0046497(8)

Application No.: LY027200(0)

6. Report on Shrimp Acute Toxicity Test

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Room 1302, Yan Hing Centre, 9-13 Wong Chuk Yeung St., Fo Tan, Shatin, N.T., Hong Kong.
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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, 27/7/2019-29/7/2019
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:	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:	$CdCl_2$
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	10	9	9.75	0.50
Salinity control	0	9	9	9	10	9.25	0.50
Concentration 1	6.5	7	7	9	9	8.00	1.15
Concentration 2	12.5	8	7	9	8	8.00	0.82
Concentration 3	25	6	7	6	5	6.00	0.82
Concentration 4	50	4	4	5	5	4.50	0.58
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	100	90	97.50	5.00
Salinity control	0	90	90	90	100	92.50	5.00
Concentration 1	6.5	70	70	90	90	80.00	11.55
Concentration 2	12.5	80	70	90	80	80.00	8.16
Concentration 3	25	60	70	60	50	60.00	8.16
Concentration 4	50	40	40	50	50	45.00	5.77
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^{-1})	7.5-7.7	7.4-7.7	7.6-7.8	7.5-7.7	7.4-7.6	7.5-7.9	7.7-8.1
Temperature (°C)	22.0	22.0	22.0	22.0	22.0	22.0	22.0
pH	7.9-8.1	7.9-8.1	7.7-8.1	7.8-8.0	7.8-8.1	7.7-8.0	7.7-8.1
Total ammonia (start/end, mg L^{-1})	0.14/0.14	0.14/0.15	1.44/1.48	2.67/2.72	5.13/5.18	10.6/11.2	19.4/20.3
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})	8/6	9/9	14/12	16/18	28/27	43/46	67/72

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6.6 LC_{50} for the shrimp *Metapenaeus ensis* and test acceptability

Table 4. LC_{50} for the *Metapenaeus ensis* and test acceptability.

Parameter	Value	Control limit
Calculated LC_{50}	39.0 %	NA
Negative control survival	97.50%	>90%
Reference toxicant 48-h acute test	5.21 mg L-1	5.19±0.51 mg L^{-1}
95% of confidence range of reference toxicant test	4.68-5.76 mg L-1	NA
Daily temperature variation	<0.5°C	Average daily temperature variation: ±1 °C
Dissolved oxygen concentration	>7.4 mg L-1	>4 mg L^{-1}

NA: Not applicable

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7. Conclusion

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Room 1302, Yan Hing Centre, 9-13 Wong Chuk Yeung St., Fo Tan, Shatin, N.T., Hong Kong.
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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*	29.2 %	≥7.1%
Fish *Lutjanus malabaricus*	49.8 %	≥7.1%
Barnacle larvae *Balanus amphitrite*	22.7 %	≥7.1%
Diatom *Skeletonema costatum*	65.4 %	-
Diatom *Skeletonema costatum*	25 % (NOEC)	≥0.51%
Shrimp *Metapenaeus ensis*	39.0 %	≥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

CMA Industrial Development Foundation Limited
Room 1302, Yan Hing Centre, 9-13 Wong Chuk Yeung St., Fo Tan, Shatin, N.T., Hong Kong.
Tel : (852) 2698 8198 Fax : (852) 2695 4177 E-mail : info@cmatesting.org Web Site : http://www.cmatesting.org

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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^{-1})					pH				
	0 h	12 h	24 h	36 h	48 h	0 h	12 h	24 h	36 h	48 h
Negative control	7.0	7.2	7.4	7.5	7.5	8.0	7.9	8.0	8.2	8.1
Salinity control	6.9	7.0	7.3	7.2	7.3	7.9	8.0	8.0	8.2	8.0
6.5	6.8	7.0	7.4	7.5	7.5	7.9	7.8	8.2	8.1	8.1
12.5	6.7	7.1	7.4	7.5	7.4	7.6	7.8	8.1	8.1	8.2
25	6.8	7.0	7.2	7.5	7.6	7.9	8.0	7.9	8.2	8.1
50	6.7	7.2	7.1	7.2	7.4	7.7	7.7	7.9	7.9	8.0
100	6.7	6.9	7.0	7.2	7.2	7.4	7.5	7.9	8.1	8.0

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Tel : (852) 2698 8198 Fax : (852) 2695 4177 E-mail : info@cmatesting.org Web Site : http://www.cmatesting.org

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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^{-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	0.06	0.06	<0.1	<0.1	<2	<2	<0.02	<0.02
Salinity control	0.05	0.06	<0.1	<0.1	<2	<2	<0.02	<0.02
6.5	1.63	1.67	<0.1	<0.1	<2	<2	<0.02	<0.02
12.5	2.91	3.12	<0.1	<0.1	<2	<2	<0.02	<0.02
25	5.84	5.96	<0.1	<0.1	<2	<2	<0.02	<0.02
50	10.3	10.8	<0.1	<0.1	<2	<2	<0.02	<0.02
100	19.5	20.1	<0.1	<0.1	<2	<2	<0.02	<0.02

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Tel : (852) 2698 8198 Fax : (852) 2695 4177 E-mail : info@cmatesting.org Web Site : http://www.cmatesting.org

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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.

Concenration 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	6.9	6.8	6.7	6.8	7.1	8.1	8.0	8.1	8.0	7.9
Salinity control	6.7	7.1	6.8	6.8	7.2	8.0	8.0	8.1	7.8	7.9
6.5	6.9	6.8	6.7	6.9	7.1	7.9	8.0	7.9	7.8	7.9
12.5	7.0	6.8	6.9	7.1	6.9	8.1	8.0	8.1	7.9	7.8
25	6.8	6.8	7.1	7.1	7.3	8.1	8.0	7.8	7.8	7.8
50	6.7	7.1	7.1	7.0	7.2	8.0	8.1	7.9	7.8	7.7
100	6.8	6.8	6.9	6.8	6.7	7.9	7.7	7.7	7.8	7.7

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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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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	0.12	0.12	<0.1	<0.1	7	7	<0.02	<0.02
Salinity control	0.14	0.14	<0.1	<0.1	8	7	<0.02	<0.02
6.5	1.40	1.37	<0.1	<0.1	15	17	<0.02	<0.02
12.5	2.92	2.94	<0.1	<0.1	22	20	<0.02	<0.02
25	5.31	5.35	<0.1	<0.1	37	33	<0.02	<0.02
50	10.1	10.1	<0.1	<0.1	48	45	<0.02	<0.02
100	22.6	23.5	<0.1	<0.1	75	79	<0.02	<0.02

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Tel : (852) 2698 8198 Fax : (852) 2695 4177 E-mail : info@cmatesting.org Web Site : http://www.cmatesting.org

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Appendix C

Monitoring Data for Barnacle Larvae Acute Toxicity Test

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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	8.7	8.3	8.3	8.4	7.8	8.1	8.0	8.0	7.9	7.9
Salinity control	7.6	7.8	7.8	7.6	7.9	7.9	8.0	7.9	7.8	7.9
6.5	7.9	7.8	7.7	7.5	7.8	8.0	8.1	8.1	8.0	8.1
12.5	7.8	7.4	7.6	7.7	7.8	7.9	7.7	7.8	7.8	7.8
25	7.6	7.5	7.5	7.6	7.6	8.0	8.0	8.1	7.8	7.9
50	7.5	7.7	7.8	7.7	7.5	8.1	8.0	8.1	7.9	8.0
100	7.5	7.6	7.7	7.3	7.1	8.1	7.9	7.9	8.0	8.0

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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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Room 1302, Yan Hing Centre, 9-13 Wong Chuk Yeung St., Fo Tan, Shatin, N.T., Hong Kong.
Tel : (852) 2698 8198 Fax : (852) 2695 4177 E-mail : info@cmatesting.org Web Site : http://www.cmatesting.org

Report No. : AY0046497(8)

Application No.: LY027200(0)

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	0.24	0.28	<0.1	<0.1	<2	<2	<0.02	<0.02
Salinity control	0.23	0.24	<0.1	<0.1	<2	<2	<0.02	<0.02
6.5	0.98	1.07	<0.1	<0.1	<2	<2	<0.02	<0.02
12.5	2.16	2.24	<0.1	<0.1	<2	<2	<0.02	<0.02
25	4.05	4.13	<0.1	<0.1	<2	<2	<0.02	<0.02
50	8.76	8.92	<0.1	<0.1	<2	<2	<0.02	<0.02
100	15.3	16.5	<0.1	<0.1	<2	<2	<0.02	<0.02

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Tel : (852) 2698 8198 Fax : (852) 2695 4177 E-mail : info@cmatesting.org Web Site : http://www.cmatesting.org

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Appendix D

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

CMA Industrial Development Foundation Limited
Room 1302, Yan Hing Centre, 9-13 Wong Chuk Yeung St., Fo Tan, Shatin, N.T., Hong Kong.
Tel : (852) 2698 8198 Fax : (852) 2695 4177 E-mail : info@cmatesting.org Web Site : http://www.cmatesting.org

CMA Industrial Development Foundation Limited
Room 1302, Yan Hing Centre, 9-13 Wong Chuk Yeung St., Fo Tan, Shatin, N.T., Hong Kong.
Tel : (852) 2698 8198 Fax : (852) 2695 4177 E-mail : info@cmatesting.org Web Site : http://www.cmatesting.org

Report No. : AY0046497(8)

Application No.: LY027200(0)

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	6.9	6.9	7.2	7.2	7.7	8.0	8.2	8.4	7.9	7.9	8.0	8.0	8.2	8.3	8.2	8.2
Salinity control	6.9	7.0	7.1	7.2	7.9	8.0	8.4	8.5	7.9	8.0	7.9	8.2	8.3	8.3	8.2	8.4
2.5	6.8	7.0	7.2	7.4	7.9	8.2	8.3	8.6	7.9	7.9	7.9	8.0	8.2	8.1	8.3	8.2
5	6.8	6.9	7.2	7.4	7.8	8.2	8.4	8.4	7.8	7.9	8.1	8.0	8.0	8.2	8.3	8.3
10	6.9	7.2	7.4	7.6	8.0	8.5	8.7	8.8	7.8	7.9	7.9	8.1	8.2	8.2	8.3	8.2
25	6.9	7.2	7.3	7.7	8.2	8.6	8.9	9.3	7.7	7.8	8.1	8.2	8.3	8.3	8.1	8.2
50	6.7	6.7	6.9	7.2	7.6	7.9	8.1	8.0	7.4	7.7	7.7	8.0	8.0	8.1	8.3	8.1
100	6.6	6.7	6.8	6.9	6.7	6.7	6.8	6.7	7.3	7.4	7.3	7.6	7.6	7.9	7.7	7.8

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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

CMA Industrial Development Foundation Limited
Room 1302, Yan Hing Centre, 9-13 Wong Chuk Yeung St., Fo Tan, Shatin, N.T., Hong Kong.
Tel : (852) 2698 8198 Fax : (852) 2695 4177 E-mail : info@cmatesting.org Web Site : http://www.cmatesting.org

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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	0.19	0.17	<0.1	<0.1	<2	<2	<0.02	<0.02
Salinity control	0.19	0.20	<0.1	<0.1	<2	<2	<0.02	<0.02
2.5	1.32	1.36	<0.1	<0.1	<2	<2	<0.02	<0.02
5	2.47	2.48	<0.1	<0.1	<2	<2	<0.02	<0.02
10	4.61	4.65	<0.1	<0.1	<2	<2	<0.02	<0.02
25	5.86	5.91	<0.1	<0.1	<2	<2	<0.02	<0.02
50	12.4	12.7	<0.1	<0.1	<2	<2	<0.02	<0.02
100	23.0	24.2	<0.1	<0.1	<2	<2	<0.02	<0.02

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Room 1302, Yan Hing Centre, 9-13 Wong Chuk Yeung St., Fo Tan, Shatin, N.T., Hong Kong.
Tel : (852) 2698 8198 Fax : (852) 2695 4177 E-mail : info@cmatesting.org Web Site : http://www.cmatesting.org

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Appendix E

Monitoring Data for Shrimp Acute Toxicity Test

CMA Industrial Development Foundation Limited
Room 1302, Yan Hing Centre, 9-13 Wong Chuk Yeung St., Fo Tan, Shatin, N.T., Hong Kong.
Tel : (852) 2698 8198 Fax : (852) 2695 4177 E-mail : info@cmatesting.org Web Site : http://www.cmatesting.org

CMA Industrial Development Foundation Limited
Room 1302, Yan Hing Centre, 9-13 Wong Chuk Yeung St., Fo Tan, Shatin, N.T., Hong Kong.
Tel : (852) 2698 8198 Fax : (852) 2695 4177 E-mail : info@cmatesting.org Web Site : http://www.cmatesting.org

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Application No.: LY027200(0)

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	7.7	7.6	7.7	7.5	7.5	8.1	7.9	8.1	8.0	8.0
Salinity control	7.4	7.5	7.5	7.7	7.7	8.1	8.1	7.9	7.9	7.9
6.5	7.7	7.6	7.8	7.7	7.7	8.0	8.1	7.7	7.9	8.0
12.5	7.5	7.6	7.6	7.7	7.6	7.8	7.8	7.8	7.9	8.0
25	7.6	7.6	7.4	7.6	7.5	8.0	7.9	7.8	7.8	8.1
50	7.5	7.7	7.9	7.8	7.6	8.0	7.9	7.7	7.9	8.0
100	7.7	7.8	8.1	7.8	7.7	8.1	7.9	7.8	7.7	7.9

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Room 1302, Yan Hing Centre, 9-13 Wong Chuk Yeung St., Fo Tan, Shatin, N.T., Hong Kong.
Tel : (852) 2698 8198 Fax : (852) 2695 4177 E-mail : info@cmatesting.org Web Site : http://www.cmatesting.org

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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

Report No. : AY0046497(8)

Application No.: LY027200(0)

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	0.14	0.14	<0.1	<0.1	8	6	<0.02	<0.02
Salinity control	0.14	0.15	<0.1	<0.1	9	9	<0.02	<0.02
6.5	1.44	1.48	<0.1	<0.1	14	12	<0.02	<0.02
12.5	2.67	2.72	<0.1	<0.1	16	18	<0.02	<0.02
25	5.13	5.18	<0.1	<0.1	28	27	<0.02	<0.02
50	10.6	11.2	<0.1	<0.1	43	46	<0.02	<0.02
100	19.4	20.3	<0.1	<0.1	67	72	<0.02	<0.02

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