Appendix 3D

Bioaccumulation Test Report

TEST REPORT

Certificate No.	:	TTR0914
Project Name	:	Relocation of Yi Lian Floating Dock No.3 Sampling and Testing Plan for Assessing the Characterisation of Marine Sediment and Mobilisation of Contaminants in the Marine Sediment During Dredging Works
Client Name	:	Hyder Consulting Limited
Client Address	:	47/F Hopewell Centre,183 Queen's Road East, Wan Chai, Hong Kong
Contract No.	:	N/A
Works Order No.	:	N/A
Lab. Job No.	:	J416
Lab. Sample Ref. No.	:	15699/1 & 15661/2
No. of Sample(s) & Description	:	2 no. of sediment (grab and vibrocore) as the following: Composite sample prepared from vibrocore samples as per client's instruction 1 reference sediment sample
Sample Receive Date	:	25-27 Jun & 2 Jul, 2005
Test Date	:	24 Aug - 21 Sept, 2005

Test Parameter

Parameter	Test Method
Bivalve Sediment Bioaccumulation Test	Inhouse Method Referencing to ASTM Guideline [4]

Note(s):
1. Results relate to samples as received.
2. NA = Not applicable.
3. Uncertainty is calculated as 2 SD.
4. ASTM Guildline: Standard Guide for Determination of the Bioaccumulation of Sediment - Associated Contaminants by Benthic Invertebrates. ASTM E1688-00a.

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1. Method

This 28-day bioaccumulation test with *Gafrarium tumidum* was conducted following an in-house developed method referencing to ASTM guideline. Adult *Gafrarium tumidum* is exposed to the test sediment overlaid with seawater for a 28-day test period. Upon completion of the exposure, test organisms are recovered in clean sand and seawater for 24 hours. Body burdens at test initiation and termination are analyzed on tissues obtained from the test organisms.

2. Sample storage and pretreatment

Composite samples were prepared as per client's instruction from the extruded vibrocore sections and homogenized thoroughly. Debris and indigenous organisms present in the sediment were removed and the sediment samples were stored at 4°C in dark until analyzed.

3. Test organism

Species:	*Gafrarium tumidum*
Source:	Collected from a clean area on the eastern coast of the New Territories. Mortality during shipping was 0%.
Size/age:	Adult.
Acclimation:	Under test conditions with feeding provided. Mortality during acclimation was 0 %
Health condition:	Healthy

4. Summary of test particulars

Type of test:	Renewal. 2 volumes per day.
Duration:	24 Aug - 21 Sept 2005
Control sediment:	mud and sand collected from a clean area on the eastern coast of the New Territories and Hong Kong Island respectively, shipped to the laboratory on the same day, sieved through 425 micrometer mesh sieve, mixed and stored at 4°C in dark dark until use
Control seawater:	collected from a clean area on the eastern coast of the Hong Kong Island, filtered through 0.45 mm filter paper, adjusted to 28 ppt, aerated for two days after preparation
Test temperature:	20±2°C
Lighting:	14h light : 10h dark cycle
Aeration:	provided (around 100 bubbles/min)
Test vessel:	20 L plastic tank
Volume of sediment:	2.5 L
Volume of overlying water:	7.5 L

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No. of replicates: 5
No. of organisms/replicate: 20
Feeding: none
Monitoring: overlying temperature and DO daily; pH, salinity and ammonia weekly; sediment sulphide at test initiation and termination
Reference toxicant test: 96 hour water only test with $CdCl_2$

5. Summary of test results

Table 1. Survival of test organisms on Day 28

Sample ID	Number of living organisms on Day 28						
	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Mean	SD
Negative Control with sediment	20	20	20	20	19	19.8	0.4
Composite sample No.1	19	19	20	18	20	19.2	0.8
Reference sediment	20	20	20	19	19	19.6	0.5

Table 2. Survival percentage of test organisms on Day 28

Sample ID	Survival percentage of test organisms on Day 28 (%)						
	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Mean	SD
Negative Control with sediment	100	100	100	100	95	99.0	2.2
Composite sample No.1	95	95	100	90	100	96.0	4.2
Reference sediment	100	100	100	95	95	98.0	2.7

Table 3. Total wet weight of organism tissue on Days 0 and 28

Sample ID	Total wet weight of organism tissue on Day 0 (g)						
	20.17						
	Total wet weight of organism tissue on Day 28 (g)						
	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Mean	SD
Composite sample No.1	20.78	18.81	20.52	19.00	17.78	19.38	1.25
Reference sediment	24.42	22.54	20.01	21.97	17.58	21.30	2.61

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Table 4. Total dry weight of organism tissue on Days 0 and 28

Sample ID	Total dry weight of organism tissue on Day 0 (g)						
	3.03						
	Total dry weight of organism tissue on Day 28 (g)						
	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Mean	SD
Composite sample No.1	3.38	3.16	3.46	3.09	2.97	3.21	0.20
Reference sediment	3.68	3.27	3.43	3.48	2.78	3.33	0.34

6. Quality Control Data

Table 5. Qquality control data in the bioaccumulation test

Parameter	Minimum during the test period	Maximum during the test period	Acceptable Range
Overlying salinity	28 ppt	29 ppt	26-30 ppt
Dissolved oxygen	6.3 mg/L	7.0 mg/L	Not defined
Overlying pH	7.9	8.2	NA [2]
Temperature	19.6 °C	21.0 °C	17.0-23.0 °C, time weighed average 18.0-22.0 °C
Total ammonia in overlying water (weekly)	0.002 mg/L	1.990 mg/L	Not defined
Acid volatile sulphide in sediment (initiation/termination)	124 ug/g	222 ug/g	Not defined
Organism survival in the negative control	95-100%, averagely 99%		≥ 90% average ≥ 80% in any individual replicate
96-h LC_{50} obtained from the reference toxicant test	820.77 mg/L		NA [3]
Remarks on test condition	None.		

1. 60% of saturation level at 20 ppt
2. pH is not adjusted or controlled
3. No empirical data available for comparsion

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7. Bioaccumulation of Contaminants

Table 6. Concentration of Cu in organism tissue at test initiation and termination

Sample ID	Concentration of Cu at test initiation (mg/kg dry weight)						
	3.20						
	Concentration of Cu at test termination (mg/kg dry weight)						
	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Mean	SD
Composite sample No.1	4.40	3.50	4.70	4.00	3.70	4.06	0.49
Reference sediment	2.90	3.10	4.00	4.60	3.50	3.62	0.69
Result of t-test (two tail, hypothesized mean difference = 0, assuming unequal variance): Insignificantly different, t critical=1.86, t stat=0.700, p=0.4583							

Table 7. Concentration of Zn in organism tissue at test initiation and termination

Sample ID	Concentration of Zn at test initiation (mg/kg dry weight)						
	9.00						
	Concentration of Zn at test termination (mg/kg dry weight)						
	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Mean	SD
Composite sample No.1	9.80	9.60	9.50	9.70	9.80	9.68	0.13
Reference sediment	7.90	9.00	9.30	9.60	9.20	9.00	0.65
Result of t-test (two tail, hypothesized mean difference = 0, assuming unequal variance): Insignificantly different, t critical=1.86, t stat=3.568, p=0.4682							

Table 8. Concentration of Ag in organism tissue at test initiation and termination

Sample ID	Concentration of Ag at test initiation (mg/kg dry weight)						
	1.30						
	Concentration of Ag at test termination (mg/kg dry weight)						
	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Mean	SD
Composite sample No.1	1.30	1.60	1.50	1.30	1.30	1.40	0.14
Reference sediment	1.50	1.10	1.60	1.40	1.30	1.38	0.19
Result of t-test (two tail, hypothesized mean difference = 0, assuming unequal variance): Insignificantly different, t critical=1.86, t stat=0.642, p=0.4496							

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Table 9. Concentration of Hg in organism tissue at test initiation and termination

Sample ID	Concentration of Hg at test initiation (mg/kg dry weight)						
	0.064						
	Concentration of Hg at test termination (mg/kg dry weight)						
	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Mean	SD
Composite sample No.1	0.07	0.07	0.07	0.08	0.07	0.07	0.01
Reference sediment	0.06	0.06	0.07	0.08	0.07	0.06	0.03
Result of t-test (two tail, hypothesized mean difference = 0, assuming unequal variance): Insignificantly different, t critical=1.86, t stat=1.361, p=0.2825							

Table 10. Bioaccumulation of contaminants

Sample ID	Increase in tissue concentration after 28 days (mg/kg)	Concentration in sediment (mg/kg)	tissue: sediment ratio
	Cu		
Composite sample No.1	0.86	160	0.01
Reference sediment	0.42	50	0.01
	Zn		
Composite sample No.1	0.68	740	0.001
Reference sediment	0.00	110	0
	Ag		
Composite sample No.1	0.10	0.8	0.125
Reference sediment	0.08	<0.05	1.6 [1]
	Hg		
Composite sample No.1	0.006	0.66	0.009
Reference sediment	0.004	0.6	0.007
1. Ag concentration in sediment was assumed as 0.05 mg/kg to give the lowest estimate of tissue:sediment ratio.			

End of report

Data entry checked by: Y.W.Yau

Approved signatory: Yi Zhang **Date:** 28-Oct-2005