

## ALGAL GROWTH INHIBITION ASSAY

1. Test substance:

2. Test method: Japanese METI (OECD #201)

Species	<i>Pseudokirchneriella subcapitata</i>
Test solution volume	300mL per group (100mL per flask)
Duration of exposure	72 hrs (without renewal of test media)
Test temperature	23.2 – 23.4°C
Test concentration	

Test solutions were taken from control and test flasks at 24, 48 and 72 hours and the cell densities measured. The estimate of cell numbers in each sample was based on the mean of three consecutive counts, corrected for background counts of uninoculated dilution media. The presence of any abnormal cells was also noted during screening of each test level.

3. Test Laboratory:

4. Testing Date:

From January 28<sup>th</sup>, 2011 to March 3<sup>rd</sup>, 2011

5. Result:

Table 1	pH of test solutions
Table 2	Culture temperature and light intensity in incubator
Table 3	Value of biomass at each time
Table 4	Growth rate and growth inhibition
Table 5	E <sub>r</sub> C <sub>50</sub> NOEC
Table 6	Result of statistical analysis
Table 7	Variation of growth rates in control
Figure 1	Concentration-response curve
Figure 2	Growth curve

## 6. Conclusion:

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Table 1 pH of test solutions

Content rate of stock solution (%)	pH	
	At the start	At the end
Control	7.9	8.0
	7.9	8.0
	7.9	8.0
	7.9	8.0
	7.9	8.0
	7.9	8.0

Table 2 Culture temperature and light intensity in incubator

Time	At the start	1-day	2-day	At the end
Culture temperature (°C)	23.4	23.2	23.2	23.2
Light intensity ( $\mu\text{E}/\text{m}^2/\text{s}$ )	90	89	93	88

Table 3 Value of biomass at each time

Content rate of stock solution (%)	No.	Cell concentration ( $\times 10^4$ cells/mL)			
		0 hour <sup>a</sup>	24 hours	48 hours	72 hours
Control	A	0.75	3.0	15	82 <sup>b</sup>
	B	0.75	3.2	16	85
	C	0.75	3.0	16	84
	D	0.75	3.3	16	83
	E	0.75	3.0	16	84
	F	0.75	2.9	15	91
	Mean	0.75	3.1	16	85
	S.D.	0	0.13	0.69	3.1

a The value based on the measured value of pre-culture

b The minimum cell growth in control (biomass at the end of exposure / biomass at the start of exposure)

$$82 / 0.75 = 109$$

Table 4 Growth rate and growth inhibition

Content rate of stock solution (%)	No.	Growth rate (0-3d)	Inhibition rate (%)
Control	A	1.56	-
	B	1.58	-
	C	1.57	-
	D	1.57	-
	E	1.57	-
	F	1.60	-
	Mean	1.58	-
	S.D.	0.0119	-

Table 5  $E_rC_{50}$ , NOEC

Table 6 Result of statistical analysis

Content rate of stock solution (%)	Statistical analysis	Statistical procedure
		Bartlett's test One-way ANOVA Dunnett's multiple comparison test

\* : Significant difference ( $p < 0.05$ )

Table 7 Variation of growth rates in control

< Variation for section-by-section specific growth rates in the controls >

Control No.	Mean	Standard deviation	Coefficient of variation (%)	
A	1.56	0.176	11	9.8 (Mean)
B	1.58	0.124	7.9	
C	1.57	0.166	11	
D	1.57	0.0923	5.9	
E	1.57	0.155	9.9	
F	1.60	0.215	13	

< Variation of average specific growth rates in replicate controls >

	0-3day
Mean	1.58
Standard deviation	0.0119
Coefficient of variation (%)	0.76

Figure 1 Concentration-response curve.

Figure 2 Growth curve.