Chapter 4

RESULTS

4. RESULTS

4.1 USE OF O.D. MEASUREMENTS TO DETERMINE CELL COUNTS

4.1.1 Correlation and regression between O.D. and cell counts

The results of the initial experiment carried out to determine the correlation and regression between the optical density (O.D.) measured by the Multiskan MCC/340 MK II and the UV-VIS Spectrophotometer, and the cell counts of the four species of marine phytoplankton to be used as test organisms, shows that O.D. measurements correlated very well with cell counts, with r values of 0.99 and regressions as in Table 4.1 (see Appendix 7). Due to the good correlation, the cell counts of all toxicity test samples in this study were determined from the O.D measured by the Multiskan, by calculation via the regression equations (Table 4.1). A good correlation was also observed between the O.D measured by the Multiskan MCC/340 MK II and by the UV-VIS Spectrophotometer (Table 4.2).

4.1.2 Correlation between O.D. and actual cell counts on random test samples

The quality control tests done by subjecting random samples of each treatment in each test to actual cell counts showed that there were also good correlations (see r values in Tables 4.11 to 4.18) between the O.D.measurements and direct cell counts, in all range-finding and definitive tests.

4.2 RANGE-FINDING TESTS

The results of the range-finding tests with cadmium, copper, manganese and arsenic, in the presence and absence of EDTA, with the four test species are summarised

Table 4.1 : Correlations, r, ($P<0.05$) and regressions between the culture optical density (OD_{620}) measured using the Multiskan MCC/340 MKII (x_1) and the UV-VIS Spectrophotometer (x_2) and cell counts (y; cells X 10^4 mL^{-1}), determined using an improved Neubauer brightline haemocytometer.

Microalgae	Correlation and Regression between OD and Direct Cell Count			
	Multiskan		**Spectrophotometer**	
	r	$y=ax_1+b$	r	$y=ax_2+b$
Chaetoceros calcitrans	0.9988	$\mathbf{y=1735.0x_1-6.9}$	0.9997	$y=824.9x_2-7.6$
Isochrysis galbana	0.9997	$\mathbf{y=3989.4x_1-22.0}$	0.9998	$y=1764.3x_2-25.4$
Tetraselmis tetrahele	0.9998	$\mathbf{y=647.4x_1-3.8}$	0.9999	$y=312.7x_2-0.6$
Tetraselmis sp.	0.9992	$\mathbf{y=761.0x_1-9.2}$	0.9995	$y=390.3x_2-6.8$

Note : See Appendix 7 for raw data

Table 4.2 : Correlations, r, ($p<0.05$) and regressions between OD measurements done with the Multiskan MCC/340 MKII (x) and the UV-VIS Spectrophotometer (y).

Microalgae	r	$y=ax+b$
Chaetoceros calcitrans	0.9991	$y=2.1x$
Isochrysis galbana	0.9996	$y=2.3x$
Tetraselmis tetrahele	0.9994	$y=2.1x$
Tetraselmis sp.	0.9999	$y=2x$

Note : See Appendix 7 for raw data

in Tables 4.3 to 4.10 (See Appendices 8(1) - 8(8) for raw data). Some stimulation were observed in tests with manganese and arsenic, both in the presence and absence of EDTA. Based on the percentages of inhibition obtained in the range tests, the suitable test series was chosen for the definitive tests of each heavy metal with each test species.

4.3 DEFINITIVE TESTS

The NOEC, LOEC, IC_{25} and IC_{50} values derived from definitive tests of cadmium, copper, manganese and arsenic conducted in the multiwell plates and flasks, in the presence and absence of EDTA, with the four test species are summarised in Tables 4.11 to 4.18.

4.3.1 Cadmium

Toxicity data from cadmium toxicity tests with the four test species in the presence and absence of EDTA are presented in Tables 4.11 and 4.12 respectively.

4.3.2 Copper

Toxicity data from copper toxicity tests with the four test species in the presence and absence of EDTA are presented in Tables 4.13 and 4.14 respectively.

4.3.3 Manganese

Toxicity data from manganese toxicity tests with the four test species in the presence and absence of EDTA are presented in Tables 4.15 and 4.16 respectively.

4.3.4 Arsenic

Toxicity data from arsenic toxicity tests with the four test species in the presence and absence of EDTA are presented in Tables 4.17 and 4.18 respectively.

Table 4.3 : Range-finding tests results of cadmium (Cd) with four selected marine phytoplankton in the presence of EDTA

Test algae	Exp No.	Percentage of growth inhibition (%)					Selected test series in definitive tests (mgL^{-1})	r
		0.01 mgL^{-1}	0.1 mgL^{-1}	1.0 mgL^{-1}	10.0 mgL^{-1}	100.0 mgL^{-1}		
Chaetoceros calcitrans	A1	7.7%	15.5%	18.1%	25.8%	96.5%	0, 6.25, 12.5, 25.0, 50.0, 100.0	0.9983
Isochrysis galbana	A2	3.8%	5.7%	9.6%	74.3%	98.5%	0, 6.25, 12.5, 25.0, 50.0, 100.0	0.9972
Tetraselmis tetrahele	A3	3.0%	6.1%	9.1%	22.7%	97.9%	0, 6.25, 12.5, 25.0, 50.0, 100.0	0.9967
Tetraselmis sp.	A4	3.6%	4.8%	7.3%	27.7%	97.6%	0, 6.25, 12.5, 25.0, 50.0, 100.0	0.9968

Note : See Appendix 8 (1a-1d) for raw data
r =correlation ($P<0.05$) between O.D. readings of randomly selected test samples and actual cell counts

Table 4.4 : Range-finding tests results of cadmium (Cd) with four selected marine phytoplankton in the absence of EDTA

Test algae	Exp No.	Percentage of growth inhibition (%)					Selected test series in definitive tests (mgL^{-1})	r
		0.01 mgL^{-1}	0.1 mgL^{-1}	1.0 mgL^{-1}	10.0 mgL^{-1}	100.0 mgL^{-1}		
Chaetoceros calcitrans	B1	20.0%	69.8%	79.9%	95.3%	94.4%	0, 0.10, 0.18, 0.32, 0.56, 1.0	1.0000
Isochrysis galbana	B2	26.1%	87.0%	94.8%	97.7%	97.7%	0, 0.10, 0.18, 0.32, 0.56, 1.0	0.9980
Tetraselmis tetrahele	B3	7.1%	9.1%	29.4%	68.3%	96.9%	0, 6.25, 12.5, 25.0, 50.0, 100.0	0.9961
Tetraselmis sp.	B4	5.7%	7.8%	19.6%	70.8%	94.8%	0, 6.25, 12.5, 25.0, 50.0, 100.0	0.9971

Note : See Appendix 8 (2a-2d) for raw data
r =correlation ($P<0.05$) between O.D. readings of randomly selected test samples and actual cell counts

Table 4.5 : Range-finding tests results of copper (Cu) with four selected marine phytoplankton in the presence of EDTA

Test algae	Exp No.	Percentage of growth inhibition (%)					Selected test series in definitive tests (mgL^{-1})	r
		0.01 mgL^{-1}	0.1 mgL^{-1}	1.0 mgL^{-1}	10.0 mgL^{-1}	100.0 mgL^{-1}		
Chaetoceros calcitrans	C1	4.1%	8.3%	11.6%	97.5%	99.1%	0, 1.0, 1.8, 3.2, 5.6, 10.0	1.0000
Isochrysis galbana	C2	2.0%	6.1%	8.1%	95.6%	98.4%	0, 1.0, 1.8, 3.2, 5.6, 10.0	1.0000
Tetraselmis tetrahele	C3	6.52%	10.9%	17.0%	90.6%	98.9%	0, 1.0, 1.8, 3.2, 5.6, 10.0	0.9980
Tetraselmis sp.	C4	7.8%	9.4%	34.3%	90.7%	98.9%	0, 1.0, 1.8, 3.2, 5.6, 10.0	0.9997

Note : See Appendix 8 (3a-3d) for raw data
r =correlation ($P<0.05$) between O.D. readings of randomly selected test samples and actual cell counts

Table 4.6 : Range-finding tests results of copper (Cu) with four selected marine phytoplankton in the absence of EDTA

Test algae	Exp . No.	Percentage of growth inhibition (%)					Selected test series in definitive tests (mgL^{-1})	r
		0.01 mgL^{-1}	0.1 mgL^{-1}	1.0 mgL^{-1}	10.0 mgL^{-1}	100.0 mgL^{-1}		
Chaetoceros calcitrans	D1	31.7%	63.2%	93.7%	98.7%	98.7%	0, 0.10, 0.18, 0.32, 0.56, 1.0	0.9991
Isochrysis galbana	D2	30.3%	64.8%	94.5%	98.0%	98.0%	0, 0.10, 0.18, 0.32, 0.56, 1.0	0.9997
Tetraselmis tetrahele	D3	20.3%	40.6%	88.6%	94.3%	96.0%	0, 0.10, 0.18, 0.32, 0.56, 1.0	0.9997
Tetraselmis sp.	D4	8.9%	19.2%	84.7%	96.3%	97.5%	0, 0.10, 0.18, 0.32, 0.56, 1.0	0.9996

Note : See Appendix 8 (4a-4d) for raw data
r =correlation ($P<0.05$) between O.D. readings of randomly selected test samples and actual cell counts

Table 4.7 : Range-finding tests results of manganese (Mn) with four selected marine phytoplankton in the presence of EDTA

Test algae	Exp No.	Percentage of growth inhibition[I] or stimulation[S] (%)					Selected test series in definitive tests (mgL^{-1})	r
		0.01 mgL^{-1}	0.1 mgL^{-1}	1.0 mgL^{-1}	10.0 mgL^{-1}	100.0 mgL^{-1}		
Chaetoceros calcitrans	E1	7.8%^{S}	18.0%^{S}	1.2%^{S}	12.0%^{I}	69.9%^{I}	0, 6.25, 12.5, 25.0, 50.0, 100.0	0.9992
Isochrysis galbana	E2	5.3%^{S}	11.9%^{S}	2.6%^{I}	22.5%^{I}	82.1%^{I}	0, 6.25, 12.5, 25.0, 50.0, 100.0	0.9993
Tetraselmis tetrahele	E3	5.4%^{S}	14.3%^{S}	1.0%^{I}	9.5%^{I}	61.6%^{I}	0, 6.25, 12.5, 25.0, 50.0, 100.0	0.9957
Tetraselmis sp.	E4	7.9%^{S}	20.1%^{S}	1.7%^{I}	3.4%^{I}	68.8%^{I}	0, 6.25, 12.5, 25.0, 50.0, 100.0	0.9990

Note : See Appendix 8 (5a-5d) for raw data
r =correlation ($P<0.05$) between O.D. readings of randomly selected test samples and actual cell counts

Table 4.8 : Range-finding tests results of manganese (Mn) with four selected marine phytoplankton in the absence of EDTA

Test algae	Exp No.	Percentage of growth inhibition[I] or stimulation[S] (%)					Selected test series in definitive tests (mgL^{-1})	r
		0.01 mgL^{-1}	0.1 mgL^{-1}	1.0 mgL^{-1}	10.0 mgL^{-1}	100.0 mgL^{-1}		
Chaetoceros calcitrans	F1	5.1%^{S}	10.1%^{S}	4.1%^{I}	29.3%^{I}	78.8%^{I}	0, 6.25, 12.5, 25.0, 50.0, 100.0	0.9993
Isochrysis galbana	F2	3.9%^{S}	7.9%^{S}	7.9%^{I}	62.8%^{I}	91.5%^{I}	0, 6.25, 12.5, 25.0, 50.0, 100.0	0.9968
Tetraselmis tetrahele	F3	4.2%^{S}	8.6%^{S}	2.9%^{I}	18.5%^{I}	81.3%^{I}	0, 6.25, 12.5, 25.0, 50.0, 100.0	0.9979
Tetraselmis sp.	F4	5.3%^{S}	12.2%^{S}	3.5%^{I}	10.6%^{I}	87.8%^{I}	0, 6.25, 12.5, 25.0, 50.0, 100.0	0.9992

Note : See Appendix 8 (6a-6d) for raw data
r =correlation ($P<0.05$) between O.D. readings of randomly selected test samples and actual cell counts

Table 4.9 : Range-finding tests results of arsenic (As) with four selected marine phytoplankton in the presence of EDTA

Test algae	Exp No.	Percentage of growth inhibition[I] or stimulation[S] (%)					Selected test series in definitive tests (mgL^{-1})	r
		0.01 mgL^{-1}	0.1 mgL^{-1}	1.0 mgL^{-1}	10.0 mgL^{-1}	100.0 mgL^{-1}		
Chaetoceros calcitrans	G1	5.2%[S]	12.5%[S]	65.6%[S]	79.2%[S]	30.2%[S]	0, 100, 200, 400, 800, 1600	0.9984
Isochrysis galbana	G2	2.6%[S]	7.8%[S]	57.2%[S]	70.2%[S]	13.0%[I]	0, 100, 200, 400, 800, 1600	0.9993
Tetraselmis tetrahele	G3	3.6%[S]	7.1%[S]	5.1%[I]	23.0%[I]	61.8%[I]	0, 6.25, 12.5, 25.0, 50.0, 100.0	0.9917
Tetraselmis sp.	G4	5.4%[S]	8.2%[S]	1.8%[I]	16.5%[I]	66.1%[I]	0, 6.25, 12.5, 25.0, 50.0, 100.0	0.9939

Note : See Appendix 8 (7a-7d) for raw data
r =correlation ($P<0.05$) between O.D. readings of randomly selected test samples and actual cell counts

Table 4.10 : Range-finding tests results of arsenic (As) with four selected marine phytoplankton in the absence of EDTA

Test algae	Exp No.	Percentage of growth inhibition[I] or stimulation[S] (%)					Selected test series in definitive tests (mgL^{-1})	r
		0.01 mgL^{-1}	0.1 mgL^{-1}	1.0 mgL^{-1}	10.0 mgL^{-1}	100.0 mgL^{-1}		
Chaetoceros calcitrans	H1	7.0%[S]	9.9%[S]	45.0%[S]	50.7%[S]	9.8%[I]	0, 100, 200, 400, 800, 1600	0.9970
Isochrysis galbana	H2	5.4%[S]	8.2%[S]	41.1%[S]	57.5%[S]	13.7%[I]	0, 100, 200, 400, 800, 1600	0.9981
Tetraselmis tetrahele	H3	3.8%[S]	7.6%[S]	4.7%[I]	39.6%[I]	65.9%[I]	0, 6.25, 12.5, 25.0, 50.0, 100.0	0.9940
Tetraselmis sp.	H4	2.6%[S]	6.7%[S]	3.5%[I]	28.1%[I]	88.1%[I]	0, 6.25, 12.5, 25.0, 50.0, 100.0	0.9985

Note : See Appendix 8 (8a-8d) for raw data
r =correlation ($P<0.05$) between O.D. readings of randomly selected test samples and actual cell counts

Table 4.11 : 96h chronic toxicity data from cadmium toxicity tests with 4 selected marine phytoplankton, in the presence of EDTA

Test Algae	Exp. No.	Test Vessel	Cd (with EDTA) mgL^{-1}				r
			NOEC	LOEC	IC_{25} #	IC_{50} #	
Chaetoceros calcitrans	A1.1	Multiwell	5.80	11.80	6.46 (3.83-8.64)	12.30 (9.90-14.37)	0.9995
	A1.2	Multiwell	5.00	12.50	12.70 (-3.18-14.02)	16.34 (15.53-17.13)	0.9995
	A1.3	Flask	12.50	22.60	13.24 (12.25-14.36)	16.97 (16.02-18.05)	0.9996
	A1.4	Flask	6.25	12.50	5.78 (4.10-7.99)	9.95 (8.39-11.99)	0.9947
Isochrysis galbana	A2.1	Multiwell	<5.80	5.80	2.01 (1.85-2.28)	4.02 (3.73-4.49)	0.9992
	A2.2	Flask	5.00	12.50	2.65 (2.35-2.93)	5.46 (4.39-6.25)	0.9989
	A2.3*	Flask	6.25	12.50	5.70 (2.66-7.83)	8.37 (7.22-9.48)	0.9996
Tetraselmis tetrahele	A3.1*	Multiwell	<6.20	6.20	3.32 (1.91-6.46)	8.12 (2.53-20.90)	0.9997
	A3.2	Multiwell	11.80	23.70	13.72 (10.94-16.08)	18.69 (17.16-19.93)	0.9937
	A3.3	Flask	12.50	25.00	11.57 (10.46-12.76)	17.58 (16.31-18.72)	0.9975
***Tetraselmis* sp.**	A4.1	Multiwell	6.20	12.50	4.00 (2.34-7.00)	11.58 (3.75-16.08)	0.9960
	A4.2	Multiwell	11.80	23.70	12.46 (6.20-16.14)	16.51 (13.55-18.97)	0.9927
	A4.3	Flask	12.50	25.00	14.62 (12.15-16.05)	18.34 (16.52-19.54)	0.9975

Note : See Appendices 9 (1a - 4b) for corresponding raw data.

* : Rejected experiment (s);

\# : numbers in parentheses are the 95% expanded confidence limits

r = correlation (P<0.05) between O.D. readings of randomly selected test samples and actual cell counts

Table 4.12 : 96h chronic toxicity data from cadmium toxicity tests with 4 selected marine phytoplankton, in the absence of EDTA.

Test Algae	Exp. No.	Test Vessel	Cd (without EDTA) mgL^{-1}				r
			NOEC	LOEC	IC_{25} #	IC_{50} #	
Chaetoceros calcitrans	B1.1	Multiwell	<0.09	0.09	0.027 (0.025-0.028)	0.053 (0.050-0.058)	0.9999
	B1.2*	Multiwell	<0.10	0.10	0.059 (0.040-0.117)	0.126 (0.079-0.521)	0.9984
	B1.3	Multiwell	<0.08	0.08	0.037 (0.033-0.044)	0.074 (0.068-0.232)	0.9990
Isochrysis galbana	B2.1*	Multiwell	<0.09	0.09	0.051 (0.033-0.083)	0.148 (0.015-0.261)	0.9998
	B2.2*	Multiwell	0.10	0.15	0.051 (0.045-0.063)	0.104 (0.084-0.124)	0.9992
	B2.3	Multiwell	<0.08	0.08	0.025 (0.022-0.029)	0.051 (0.045-0.059)	0.9987
	B2.4	Multiwell	<0.12	0.12	0.034 (0.033-0.036)	0.069 (0.066-0.072)	0.9992
	B2.5	Flask	<0.12	0.12	0.032 (0.031-0.033)	0.064 (0.062-0.065)	0.9998
Tetraselmis tetrahele	B3.1	Multiwell	<6.10	6.10	2.90 (2.16-3.87)	5.80 (4.40-8.56)	0.9976
	B3.2*	Multiwell	<6.70	6.70	3.73 (2.62-6.28)	16.53 (-4.79-22.21)	0.9984
	B3.3	Multiwell	<5.80	5.80	2.18 (1.99-2.33)	4.36 (3.99-4.66)	0.9970
	B3.4	Flask	<6.11	6.11	3.27 (2.34-5.19)	6.88 (3.60-10.11)	0.9979
***Tetraselmis* sp.**	B4.1	Multiwell	<6.10	6.10	2.25 (1.55-4.64)	4.51 (3.18-6.76)	0.9997
	B4.2	Multiwell	<6.70	6.70	3.50 (2.42-5.08)	7.46 (4.43-11.95)	0.9980
	B4.3	Multiwell	<5.80	5.80	1.94 (1.83-2.10)	3.89 (3.46-4.74)	0.9996
	B4.4	Multiwell	<6.11	6.11	3.42 (2.44-4.82)	7.16 (4.52-9.43)	0.9941
	B4.5	Flask	<6.11	6.11	2.61 (2.28-3.06)	5.23 (4.55-6.44)	0.9989

Note : See Appendices 10 (1a - 4b) for corresponding raw data.

* : Rejected experiment (s);

\# : numbers in parentheses are the 95% expanded confidence limits

r = correlation ($P<0.05$) between O.D. readings of randomly selected test samples and actual cell counts

Table 4.13 : 96h chronic toxicity data from copper toxicity tests with 4 selected marine phytoplankton, in the presence of EDTA.

Test Algae	Exp. No.	Test Vessel	Cu (with EDTA) mgL^{-1}				r
			NOEC	LOEC	IC_{25} #	IC_{50} #	
Chaetoceros calcitrans	C1.1	Multiwell	0.90	1.60	2.44 (0.86-3.54)	6.66 (6.44-6.98)	0.9988
	C1.2	Multiwell	4.50	8.10	5.29 (4.80-5.57)	6.26 (5.84-6.46)	0.9985
	C1.3	Flask	4.50	8.10	4.60 (-0.11-5.15)	5.81 (5.05-6.12)	0.9975
Isochrysis galbana	C2.1	Multiwell	5.40	9.90	5.56 (-0.05-5.92)	7.03 (6.73-7.24)	0.9994
	C2.2	Multiwell	4.50	8.10	5.45 (5.24-5.47)	6.41 (6.31-6.44)	0.9967
	C2.3	Flask	4.50	8.10	5.25 (4.93-5.64)	6.25 (5.97-6.48)	0.9989
Tetraselmis tetrahele	C3.1	Multiwell	4.50	8.10	5.00 (4.08-5.52)	6.30 (5.66-6.58)	0.9968
	C3.2	Flask	1.80	3.20	3.35 (2.30-4.50)	6.01 (4.93-6.91)	0.9959
***Tetraselmis* sp.**	C4.1	Multiwell	<0.90	0.90	0.57 (0.46-0.85)	5.35 (1.50-6.20)	0.9934
	C4.2	Multiwell	1.60	3.00	0.75 (0.58-1.56)	6.38 (5.86-6.76)	0.9964
	C4.3	Multiwell	<0.80	0.80	0.58 (0.35-7.42)	5.74 (4.63-7.01)	0.9992
	C4.4	Flask	3.20	5.60	3.66 (1.26-4.94)	6.38 (5.91-6.95)	0.9941

Note : See Appendices 11 (1a - 4b) for corresponding raw data.

* : Rejected experiment (s);

: numbers in parentheses are the 95% expanded confidence limits

r = correlation (P<0.05) between O.D. readings of randomly selected test samples and actual cell counts

Table 4.14 : 96h chronic toxicity data from copper toxicity tests with 4 selected marine phytoplankton, in the absence of EDTA.

Test Algae	Exp. No.	Test Vessel	Cu (without EDTA) mgL^{-1}				r
			NOEC	LOEC	IC_{25} #	IC_{50} #	
Chaetoceros calcitrans	D1.1	Multiwell	0.02	0.10	0.016 (0.014-0.020)	0.055 (0.048-0.065)	0.9958
	D1.2	Multiwell	<0.13	0.13	0.038 (0.037-0.039)	0.077 (0.075-0.079)	0.9996
	D1.3	Flask	<0.13	0.13	0.033 (0.033-0.034)	0.067 (0.066-0.068)	0.9999
Isochrysis galbana	D2.1	Multiwell	0.02	0.10	0.012 (0.007-0.021)	0.034 (0.005-0.065)	0.9999
	D2.2	Multiwell	<0.07	0.07	0.018 (0.018-0.019)	0.037 (0.036-0.037)	0.9999
Tetraselmis tetrahele	D3.1	Multiwell	<0.02	0.10	0.017 (0.009-0.064)	0.122 (0.042-0.126)	0.9938
	D3.2	Flask	<0.13	0.13	0.063 (0.050-0.088)	0.126 (0.103-0.314)	0.9972
Tetraselmis sp.	D4.1	Multiwell	0.20	0.30	0.183 (0.180-0.187)	0.366 (0.360-0.373)	0.9973
	D4.2	Multiwell	0.07	0.15	0.124 (0.010-0.152)	0.342 (0.219-0.439)	0.9987
	D4.3	Multiwell	<0.13	0.13	0.077 (0.067-0.104)	0.405 (0.240-0.509)	0.9996

Note : See Appendices 12 (1a - 4) for corresponding raw data.

* : Rejected experiment (s);

\# : numbers in parentheses are the 95% expanded confidence limits

r = correlation ($P<0.05$) between O.D. readings of randomly selected test samples and actual cell counts

Table 4.15 : 96h chronic toxicity data from manganese toxicity tests with 4 selected marine phytoplankton, in the presence of EDTA.

Test Algae	Exp. No.	Test Vessel	Mn (with EDTA) mgL^{-1}				r
			NOEC	**LOEC**	**IC_{25} #**	**IC_{50} #**	
Chaetoceros calcitrans	E1.1	Multiwell	<5.70	5.70	31.52 (22.90-39.58)	62.19 (53.25-71.36)	0.9991
	E1.2	Multiwell *	23.00	45.90	34.05 (17.72-56.46)	65.93 (50.23-81.39)	0.9971
	E1.3	Multiwell	4.50	12.20	41.04 (26.77-52.82)	72.62 (63.80-78.94)	0.9978
Isochrysis galbana	E2.1	Multiwell	12.00	23.70	15.02 (-6.18-20.73)	31.36 (19.86-53.27)	0.9990
	E2.2	Multiwell	5.80	11.50	24.73 (-4.92-29.31)	44.86 (43.26-47.99)	0.9985
Tetraselmis tetrahele	E3.1	Multiwell	4.50	12.20	9.93 (5.40-9.54)	73.65 (65.59-83.84)	0.9892
	E3.2	Flask	4.50	12.20	53.68 (-8.84-67.55)	86.50 (NA)	0.9978
***Tetraselmis* sp.**	E4.1	Multiwell	45.90	89.20	51.92 (-44.50-69.16)	82.01 (NA)	0.9940
	E4.2	Multiwell	<4.50	4.50	8.76 (7.33-10.10)	55.31 (50.94-60.66)	0.9989
	E4.3	Flask	4.50	12.20	27.43 (-4.63-45.57)	70.31 (64.46-75.99)	0.9918

Note : See Appendices 13 (1- 4b) for corresponding raw data

* : Rejected experiment (s)

\# : numbers in parentheses are the 95% expanded confidence limits

r = correlation ($P<0.05$) between O.D. readings of randomly selected test samples and actual cell counts

NA : Not available; No confidence limits could be produced by ICPIN

Table 4.16 : 96h chronic toxicity data from manganese toxicity tests with 4 selected marine phytoplankton, in the absence of EDTA.

Test Algae	Exp. No.	Test Vessel	Mn (without EDTA) mgL^{-1}				r
			NOEC	**LOEC**	**IC_{25} #**	**IC_{50} #**	
Chaetoceros calcitrans	F1.1	Multiwell	<5.70	5.70	4.59 (3.59-6.20)	18.59 (14.67-21.56)	0.9986
	F1.2	Flasks	4.90	11.90	6.97 (2.07-10.30)	19.21 (15.91-22.92)	0.9959
Isochrysis galbana	F2.1	Multiwell	<5.90	5.90	2.77 (2.25-3.40)	5.53 (4.64-6.98)	0.9988
	F2.2	Multiwell	<4.90	4.90	2.54 (1.92-3.47)	9.14 (-0.47-29.06)	0.9991
	F2.3	Flask	<4.90	4.90	2.65 (1.35-4.04)	6.87 (0.83-11.62)	0.9985
Tetraselmis tetrahele	F3.1	Multiwell	5.90	11.80	6.01 (1.75-20.16)	17.81 (5.62-24.32)	0.9988
	F3.2	Multiwell	5.20	14.90	16.75 (15.44-18.08)	22.04 (21.34-22.75)	0.9964
	F3.3	Flask	<5.20	5.20	4.70 (3.08-27.90)	20.77 (17.07-23.04)	0.9933
Tetraselmis sp.	F4.1	Multiwell	<5.20	5.20	6.36 (1.72-23.85)	19.13 (17.78-20.21)	0.9989
	F4.2	Flask	14.90	25.60	19.26 (18.91-19.67)	23.61 (23.14-24.38)	0.9958

Note : See Appendices 14 (1a -4a) for corresponding raw data

* : Rejected experiment (s)

\# : numbers in parentheses are the 95% expanded confidence limits

r = correlation ($P<0.05$) between O.D. readings of randomly selected test samples and actual cell counts

Table 4.17 : 96h chronic toxicity data from arsenic toxicity tests with 4 selected marine phytoplankton, in the presence of EDTA.

Test Algae	Exp. No.	Test Vessel	As (with EDTA) mgL^{-1}				r
			NOEC	**LOEC**	**IC_{25}** #	**IC_{50}** #	
Chaetoceros calcitrans	G1.1	Multiwell	<100.00	100.00	110.38 (65.73-189.45)	300.21 (248.36-354.99)	0.9970
	G1.2	Multiwell	<100.00	100.00	133.29 (110.02-178.46)	327.86 (291.46-373.76)	0.9993
	G1.3	Multiwell	200.00	400.00	168.27 (149.08-185.06)	272.75 (236.04-297.90)	0.9980
	G1.4	Multiwell	200.00	400.00	200.15 (179.24-231.93)	325.72 (294.74-344.71)	0.9997
	G1.5	Multiwell	200.00	400.00	184.11 (140.55-225.69)	306.53 (268.57-327.21)	0.9990
	G1.6	Flask	100.00	200.00	206.40 (170.26-236.46)	309.38 (289.79-326.27)	0.9972
Isochrysis galbana	G2.1	Multiwell	200.00	400.00	146.25 (107.53-189.73)	309.88 (221.43-345.59)	0.9972
	G2.2	Multiwell	100.00	200.00	205.59 (178.00-243.26)	320.63 (294.85-345.43)	0.9988
	G2.3	Multiwell	133.50	200.00	160.26 (137.82-190.53)	258.01 (194.61-301.87)	0.9994
Tetraselmis tetrahele	G3.1	Multiwell	<6.25	6.25	22.89 (10.64-34.69)	78.48 (57.03-99.82)	0.9993
	G3.2	Multiwell	25.00	50.00	39.44 (33.05-48.94)	68.86 (59.93-77.13)	0.9993
	G3.3	Multiwell	<6.25	6.25	5.89 (3.71-15.96)	53.69 (44.84-62.66)	0.9975
	G3.4	Flask	6.25	12.50	9.89 (5.28-14.11)	96.57 (NA)	0.9973
Tetraselmis sp.	G4.1	Multiwell	25.00	50.00	44.70 (41.19-49.67)	90.24 (78.06-105.48)	0.9955
	G4.2	Multiwell	6.25	12.50	50.37 (-8.52-56.88)	76.45 (73.94-79.56)	0.9977
	G4.3	Flask	<6.25	6.25	9.12 (4.16-13.79)	60.65 (50.98-70.09)	0.9922

Note : See Appendices 15 (1a - 4b) for corresponding raw data

* : Rejected experiment (s)

\# : numbers in parentheses are the 95% expanded confidence limits

r = correlation (P<0.05) between O.D. readings of randomly selected test samples and actual cell counts

NA : Not available; No confidence limits could be produced by ICPIN

Table 4.18 : 96h chronic toxicity data from arsenic toxicity tests with 4 selected marine phytoplankton, in the absence of EDTA.

Test Algae	Exp. No.	Test Vessel	As (without EDTA) mgL^{-1}				r
			NOEC	LOEC	IC_{25} #	IC_{50} #	
Chaetoceros calcitrans	H1.1	Multiwell	<100.00	100.00	69.55 (47.99-110.59)	274.09 (189.02-355.69)	0.9926
	H1.2	Multiwell	150.00	225.00	219.60 (201.23-243.15)	324.28 (287.70-409.96)	0.9953
	H1.3	Flask	<100.00	100.00	228.95 (214.36-243.90)	302.86 (291.63-314.20)	0.9994
Isochrysis galbana	H2.1	Multiwell	100.00	200.00	168.99 (56.74-271.45)	312.80 (262.93-370.05)	0.9990
	H2.2	Multiwell	225.00	337.50	220.83 (185.27-247.31)	336.46 (308.13-381.76)	0.9902
	H2.3	Multiwell	200.00	300.00	211.34 (120.75-265.91)	292.30 (252.62-358.81)	0.9986
	H2.4	Flask	200.00	400.00	96.55 (63.93-355.29)	335.60 (292.87-373.59)	0.9993
Tetraselmis tetrahele	H3.1	Multiwell	<6.25	6.25	5.2 (3.60-15.65)	46.43 (5.80-111.51)	0.9905
	H3.2	Multiwell	<6.25	6.25	7.59 (2.88-13.89)	44.75 (28.89-67.95)	0.9994
	H3.3	Multiwell	<6.25	6.25	6.96 (3.40-10.42)	28.26 (19.16-40.45)	0.9993
	H3.4	Multiwell	<6.25	6.25	4.61 (3.24-6.62)	29.12 (13.04-48.34)	0.9984
	H3.5	Multiwell	6.25	12.50	9.93 (9.34-10.86)	36.50 (19.93-46.25)	0.9976
	H3.6	Flask	6.25	12.50	9.70 (9.46-9.93)	40.28 (25.55-101.17)	0.9967
Tetraselmis sp.	H4.1	Multiwell	12.50	25.00	11.01 (-1.93-25.36)	26.20 (1.84-39.92)	0.9952
	H4.2	Multiwell	<6.25	6.25	3.54 (2.69-5.18)	31.70 (-22.79-42.84)	0.9985
	H4.3	Multiwell	6.25	12.50	11.61 (1.89-38.51)	49.02 (26.21-71.12)	0.9997
	H4.4	Multiwell	12.50	25.00	13.52 (9.03-25.27)	33.41 (22.90-44.51)	0.9981
	H4.5	Flask	6.25	12.50	9.57 (8.96-10.77)	29.33 (-6.30-41.98)	0.9987

Note : See Appendices 16 (1a - 4b) for corresponding raw data

* : Rejected experiment (s)

\# : numbers in parentheses are the 95% expanded confidence limits

r = correlation ($P<0.05$) between O.D. readings of randomly selected test samples and actual cell counts

NA : Not available; No confidence limits could be produced by ICPIN

4.4 MULTIWELL EXPERIMENTS VS FLASK EXPERIMENTS

Results obtained from toxicity tests conducted in multiwell plates were similar to those carried out in shake-flasks (Tables 4.11- 4.18), where the IC_{50} values were also reproducible. The similarity between results of multiwell tests and flask tests is further shown in the summarised results in Tables 4.19 (with EDTA) and 4.20 (without EDTA).

4.5 HEAVY METAL TOXICITY

Generally, in the presence of EDTA, Cu was the most toxic metal for all test species with the exception of *Isochrysis galbana* where Cd was the most toxic. Mn was relatively less toxic than these two metals. Meanwhile As was the least toxic metal for all phytoplankton tested except *Tetraselmis tetrahele* where As was slightly more toxic than Mn.

In experiments without EDTA, Cu was also the most toxic metal to all test species but it was as toxic as Cd to *Chaetoceros calcitrans*. Mn was relatively less toxic than Cd and Cu while As was the least toxic to all test species. Tables 4.21 (a-d) summarise the toxicity of each heavy metal, in the presence and absence of EDTA, to each test species.

4.5.1 Cadmium

4.5.1.1 Cadmium with EDTA

Among the test species, cadmium was most toxic to *I. galbana* (Table 4.21b) at an average IC_{50} value of 4.7 mgL^{-1} in the presence of EDTA. Cd was about three to four times less toxic to *C. calcitrans, T. tetrahele* and *T.* sp. (Tables 4.21a, c and d respectively).

Table 4.19 : Comparison of **IC$_{50}$ (mgL^{-1})** values between experiments conducted in multiwells and shake-flasks, in the presence of EDTA

Heavy metal-Test vessel **(with EDTA)**	*Chaetoceros calcitrans*	*Isochrysis galbana*	*Tetraselmis tetrahele*	*Tetraselmis* sp.
Cd-Multiwell	14.3 ± 2.9 (2)	4.0 (1)	18.7 (1)	14.0 ± 3.5 (2)
Cd-Flask	13.5 ± 5.0 (2)	5.5(1)	17.6 (1)	18.3 (1)
Cu-Multiwell	6.5 ± 0.3 (2)	6.7 ± 0.4 (2)	6.3 (1)	5.8 ± 0.5 (3)
Cu-Flask	5.8 (1)	6.3 (1)	6.0 (1)	6.4 (1)
Mn-Multiwell	66.9 ± 5.3 (3)	38.1 ± 9.6 (2)	73.7 (1)	68.7 ± 18.9 (2)
Mn-Flask	NA	NA	86.5 (1)	70.3 (1)
As-Multiwell	306.6±22.4(5)	296.2 ± 33.5 (3)	67.0 ± 12.5 (3)	83.4 ± 9.8 (2)
As-Flask	309.4 (1)	NA	96.6 (1)	60.7 (1)

Note : NA : not available for comparison
( ) : number of experiments

Table 4.20 : Comparison of **IC$_{50}$ (mgL^{-1})** values between experiments conducted in multiwells and shake-flasks, in the absence of EDTA

Heavy metal -Test vessel **(without EDTA)**	*Chaetoceros calcitrans*	*Isochrysis galbana*	*Tetraselmis tetrahele*	*Tetraselmis* sp.
Cd-Multiwell	0.06 ± 0.01 (2)	0.06 ± 0.01 (2)	5.1 ± 1.0 (2)	5.8 ± 1.8 (5)
Cd-Flask	NA	0.06 (1)	6.9 (1)	5.2 (1)
Cu-Multiwell	0.07 ± 0.01 (2)	0.04 ± 0.01 (2)	0.12 (1)	0.37 ± 0.04(3)
Cu-Flask	0.07 (1)	NA	0.13 (1)	NA
Mn-Multiwell	18.6 (1)	7.3 ± 2.6 (2)	19.9 ± 3.0 (2)	19.1 (1)
Mn-Flask	19.2 (1)	6.9 (1)	20.8 (1)	23.6 (1)
As-Multiwell	299.2±35.5 (2)	319.3 ± 21.1 (4)	37.0 ± 8.5 (5)	35.1 ± 9.8 (4)
As-Flask	302.9 (1)	NA	40.3 (1)	29.3 (1)

Note : NA : not available for comparison
( ) : number of experiments

Table 4.21 : Summary of IC_{50} values (mgL^{-1}) from the 96h chronic toxicity tests with the four selected marine phytoplankton in the presence and absence of EDTA.

(a) ***Chaetoceros calcitrans***

	Cd	**Cu**	**Mn**	**As**
+ EDTA	13.9 ± 3.3 (4)	6.2 ± 0.4 (3)	66.9 ± 5.3 (3)	307.1 ± 20.1 (6)
- EDTA	0.06 ± 0.01 (2)	0.07 ± 0.01 (3)	18.9 ± 0.4 (2)	300.4 ± 25.8 (3)

(b) ***Isochrysis galbana***

	Cd	**Cu**	**Mn**	**As**
+ EDTA	4.7 ± 1.0 (2)	6.6 ± 0.4 (3)	38.1 ± 9.6 (2)	296.2 ± 33.5 (6)
- EDTA	0.06 ± 0.01 (3)	0.04 ± 0.01 (2)	7.2 ± 1.8 (3)	319.3 ± 21.1 (4)

(c) ***Tetraselmis tetrahele***

	Cd	**Cu**	**Mn**	**As**
+ EDTA	18.1 ± 0.8 (2)	6.2 ± 0.2 (2)	80.1 ± 9.1 (2)	74.4 ± 18.0 (4)
- EDTA	5.4 ± 1.3 (3)	0.13 ± 0.01 (2)	20.2 ± 2.2 (3)	35.6 ± 7.7 (6)

(d) ***Tetraselmis* sp.**

	Cd	**Cu**	**Mn**	**As**
+ EDTA	15.5 ± 3.5 (3)	6.0 ± 0.5 (4)	69.2 ± 13.4 (3)	75.8 ± 14.8 (3)
- EDTA	5.7 ± 1.6 (5)	0.37 ± 0.04 (3)	21.4 ± 3.2 (2)	33.9 ± 8.9 (5)

Note :
( )= number of experiments; + EDTA : with EDTA; -EDTA : without EDTA

4.5.1.2 Cadmium without EDTA

Cd in the absence of EDTA which was as toxic to *C. calcitrans* as it was to *I. galbana*, was also most toxic to both *C. calcitrans* and *I. galbana*, each with IC_{50} values of 0.06 mgL^{-1} (Tables 4.21 a and b respectively). Meanwhile, Cd was less toxic to *T. tetrahele* and *T.* sp. with IC_{50} values 2 degrees of magnitude higher (Tables 4.21c and d respectively) than the two former species.

In the absence of EDTA, Cd was approximately 200, 100, four and three times more toxic to *C. calcitrans*, *I. galbana*, *T. tetrahele* and *T.* sp. respectively, than in the presence of EDTA.

4.5.2 Copper

4.5.2.1 Copper with EDTA

In the presence of EDTA, copper was similarly toxic to *C. calcitrans*, *I. galbana*, *T. tetrahele* and *T.* sp. with an average IC_{50} value of 6.3 mgL^{-1} (Tables 4.21a,b, c and d).

4.5.2.2 Copper without EDTA

Copper was most toxic to *I. galbana* at the average IC_{50} value of 0.04 mgL^{-1} (Table 4.21b) in the absence of EDTA. It was also quite toxic to *C. calcitrans* with IC_{50} value of 0.07 mgL^{-1} (Table 4.21a). However Cu was a degree of magnitude less toxic to *T. tetrahele* and *T.* sp. at IC_{50} values 0.13 and 0.37 mgL^{-1} respectively (Tables 4.21c and d).

Generally, Cu in the absence of EDTA was more toxic to the four species at concentrations one to two degrees of magnitude lower than Cu in tests with EDTA.

4.5.3 Manganese

4.5.3.1 Manganese with EDTA

Among the test algae, Mn was most toxic to *I. galbana* at an average IC_{50} value of 38.1 mgL^{-1} (Table 4.21b) and least toxic to *T. tetrahele* at IC_{50} 80.1 mgL^{-1} (Table 4.21c) in tests with EDTA. It was moderately and almost similarly toxic to *C. calcitrans* and *T.* sp. at an average IC_{50} of 68.1 mgL^{-1}.

4.5.3.2 Manganese without EDTA

Manganese was approximately three to five times more toxic to the four test species in the absence than in the presence of EDTA.

As in the tests with EDTA, Mn was also most toxic to *I. galbana*, with an average IC_{50} value of 7.2 mgL^{-1} (Table 4.21b) in the absence of EDTA. Meanwhile it was relatively less toxic to the three other test species at an average IC_{50} value of 20.2 mgL^{-1}.

4.5.4 Arsenic

4.5.4.1 Arsenic with EDTA

Among the four microalgae tested, arsenic was least toxic to *C. calcitrans* and *I. galbana* in the presence of EDTA at the average IC_{50} value of 301.7 mgL^{-1} (Tables 4.21a and 4.21b). It was approximately four times more toxic to *T. tetrahele* and *T.* sp than the two former species, at an average IC_{50} of 75.1 mgL^{-1} (Tables 4.21c and d).

4.5.4.2 Arsenic without EDTA

Toxicity of arsenic to *C. calcitrans* and *I. galbana* in the absence of EDTA was similar as in the presence of EDTA at an average IC_{50} of 309.9 mgL^{-1} (Tables 4.21a and

4.21b). However, arsenic in the absence of EDTA was two times more toxic to *T. tetrahele* and *T.* sp. (Tables 4.21c and 4.21d) than in the presence of EDTA with an average IC_{50} of 34.8 mgL^{-1}.

4.6 MICROALGAL SENSITIVITY AND TOLERANCE TO HEAVY METALS

The toxicity data obtained may also be discussed in terms of the sensitivity and tolerance of the test species, which varied among the organisms. In general, all test species were quite sensitive to Cd and Cu, relatively tolerant to Mn and quite tolerant to As.

4.6.1 Sensitivity to heavy metals in experiments with EDTA

I. galbana was the test species most sensitive to Cd and Mn (Table 4.21b) while *T. tetrahele* was the most tolerant to both metals in the presence of EDTA (Table 4.21c). The former species was approximately four and two times more sensitive to Cd and Mn respectively, than the latter species. Meanwhile *C. calcitrans* was as sensitive as *T.* sp. to the two metals (Tables 4.21a and d respectively), where both species were four to five times more sensitive to Cd than to Mn.

In the presence of EDTA the four test species exhibited similar sensitivity levels to Cu, where with the exception of *I. galbana*, all species were more sensitive to Cu than to Cd. *T. tetrahele* and *T.* sp. were about 4 times more sensitive to As than *C. calcitrans* and *I. galbana* which were quite tolerant to the metal in experiments with EDTA.

4.6.2 Sensitivity to heavy metals in experiments without EDTA

C. calcitrans was as sensitive as *I. galbana* to Cd where the two were the most sensitive test species to Cd in experiments without EDTA, while *T. tetrahele* and *T.* sp.

were about 100 times more tolerant to the metal than the two former algae. *C. calcitrans* and *I. galbana* were also both as sensitive to Cd as they were to Cu. However *I. galbana* was approximately two times more sensitive to Cu than *C. calcitrans*, being the species most sensitive to Cu while *T.* sp was the least.

All test species were relatively more tolerant to Mn. *I. galbana* was the species most sensitive to Mn while the other three were approximately 3 times more tolerant. *T. tetrahele* and *T.* sp. were both about ten times more sensitive to As than *C. calcitrans* and *I. galbana* in the absence of EDTA.

4.7 REFERENCE TOXICANT (CADMIUM) CONTROL CHARTS

In this study, cadmium which represented one of the test metals also functioned as the reference toxicant. Based on the IC_{50} values obtained from cadmium definitive tests with the four species, in the presence and absence of EDTA, separate reference toxicant control charts were constructed for cadmium and each test species. Figures 4.1 to 4.8 present the final control charts for each marine phytoplankton (See Appendix 17 for control chart data). The means presented in the charts were based only on results which were acceptable. Eventhough the outliers were not acceptable and therefore not considered in the calculation of the mean, they were still presented in the control charts.

4.7.1 Cadmium with EDTA

4.7.1.1 *Chaetoceros calcitrans*

The reference toxicant results for *C. calcitrans* were all acceptable as they remained within the 95% Confidence limits (Figure 4.1) with the data distributed on both sides of the mean (13.9 mgL^{-1}).

Figure 4.1 : *Chaetoceros calcitrans* reference toxicant (Cd with EDTA) control chart

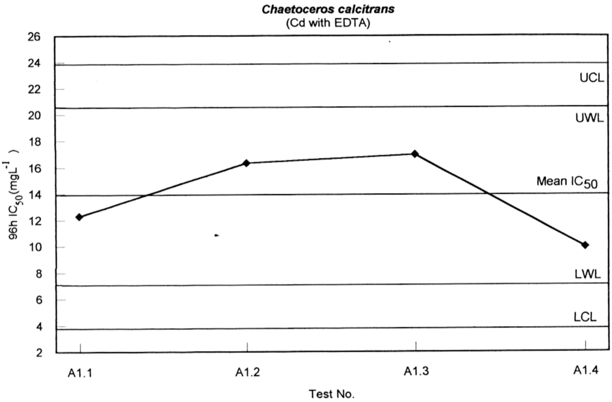
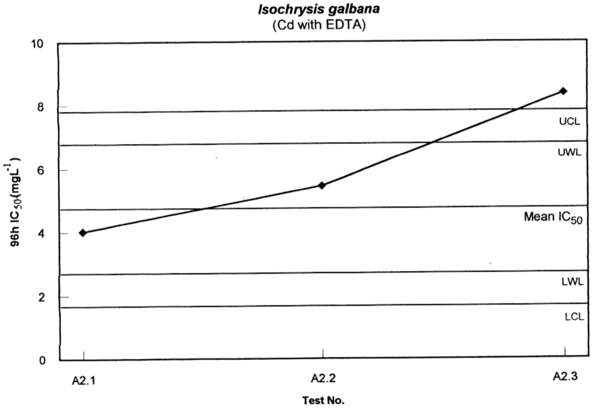


Figure 4.2 : *Isochrysis galbana* reference toxicant (Cd with EDTA) control chart



4.7.1.2 *Isochrysis galbana*

The first two reference toxicant results for *I. galbana* were within the 95% confidence limits (Figure 4.2). However the third value obtained lay out-of-range (test A2.3), above the upper confidence limit. Therefore it was not accounted for in the final mean (4.7 mgL^{-1}) of this control chart.

4.7.1.3 *Tetraselmis tetrahele*

The first reference toxicant result for *T. tetrahele* was out-of-range (A3.1), while the two last experiments were acceptable with mean 18.1 mgL^{-1} (Figure 4.3).

4.7.1.4 *Tetraselmis* sp.

All reference toxicant results for *T.* sp. were within 95% Confidence limits and therefore acceptable with mean value 15.5 mgL^{-1} (Figure 4.4).

4.7.2 Cadmium without EDTA

4.7.2.1 *Chaetoceros calcitrans*

Figure 4.5 shows that two of the reference toxicant results were within the acceptable limits (mean 0.06 mgL^{-1}) with an outlying result (test B1.2) above the upper confidence limits.

4.7.2.2 *Isochrysis galbana*

The first two reference toxicant results (tests B2.1 and B2.2) were not acceptable as they lay out-of-range (Figure 4.6). However, the results of the following experiments were all within the 95% confidence limits, with values on both sides of the mean (0.06 mgL^{-1}).

Figure 4.3 : *Tetraselmis tetrahele* reference toxicant (Cd with EDTA) control chart

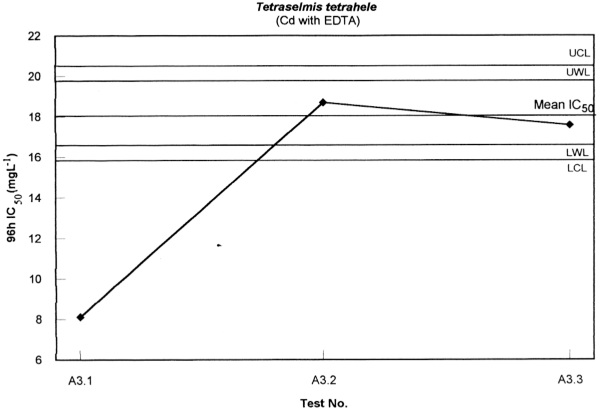


Figure 4.4 : *Tetraselmis* sp. reference toxicant (Cd with EDTA) control chart

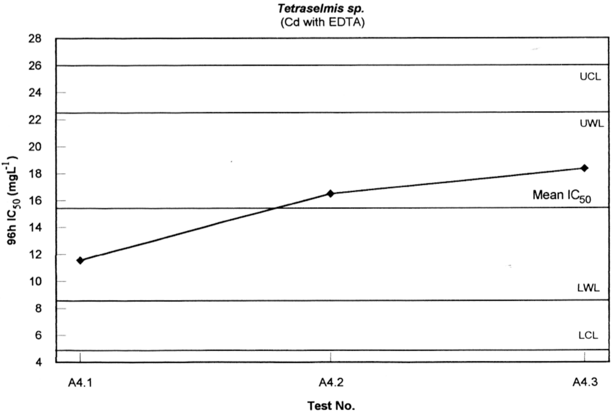


Figure 4.5 : *Chaetoceros calcitrans* reference toxicant (Cd without EDTA) control chart

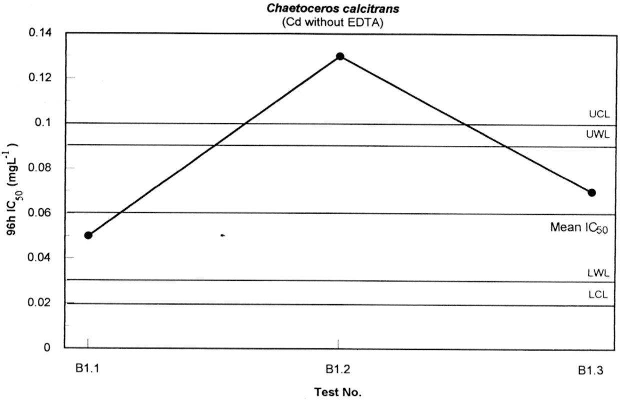
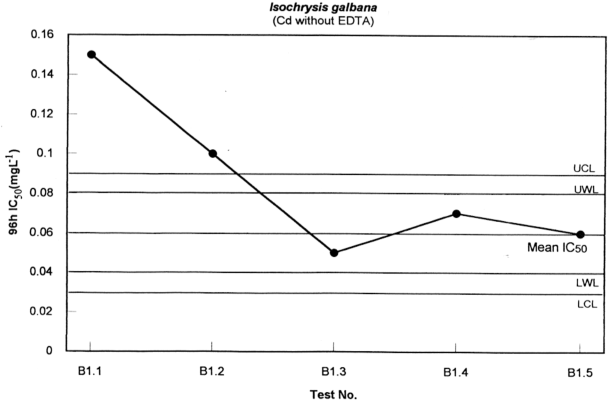


Figure 4.6 : *Isochrysis galbana* reference toxicant (Cd without EDTA) control chart



4.7.2.3 *Tetraselmis tetrahele*

All of the reference toxicant results were within the 95% confidence limits (mean 5.7 mgL^{-1}) with the exception of the second test (B3.2) which was out-of range (Figure 4.7).

4.7.2.4 *Tetraselmis* sp.

All of the *T.* sp. reference toxicant results (Figure 4.8) were acceptable with the results evenly scattered on both sides of the mean (5.7 mgL^{-1}).

Figure 4.7 : *Tetraselmis tetrahele* reference toxicant (Cd without EDTA) control chart

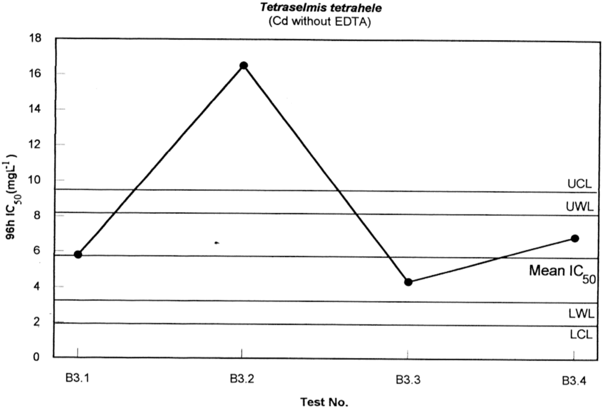


Figure 4.8 : *Tetraselmis* sp. reference toxicant (Cd without EDTA) control chart