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Determination of the acute toxicity of process chemicals in mine tailings from Nordic Mining ASA to the marine alga *Skeletonema costatum*, the marine copepod *Tisbe battagliai* and the polychaete *Arenicola marina*

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REPORT

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Abstract

The acute toxicity of process chemicals applied to mine tailings at Nordic Mining ASA have been determined. The concentrations of the process chemicals applied to the sediment ranged from an order of magnitude lower than the Environmentally Relevant Concentration (ERC) to an order of magnitude higher with 2 intermediate concentrations and appropriate controls. There were no effects on any of the organisms at the ERC of process chemicals. There was a statistically significant effect at the 3.2 times ERC on the growth of the algae but not in the copepod or polychaete worm tests. The 10 times concentration also had an effect on growth of the algae and caused a complete kill in the polychaete worms and crustacean (LC100). Therefore the Low Observed Effect Concentration (LOEC) is 3.2 times the environmentally relevant concentration of process chemicals in the mine tailings. The No Observed Effect Concentration (NOEC) for this study was at the ERC of process chemicals in the mine tailings. Therefore, the concentration of process chemicals in the mine tailings being used by Nordic Mining ASA should not cause acute toxicity in the environment.

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1. *Arenicola marina*	1. *Arenicola marina*
2. *Tisbe battagliai*	2. *Tisbe battagliai*
3. *Skeletonema costatum*	3. *Skeletonema costatum*
4. Gruveavgang	4. Mine tailings

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Determination of the acute toxicity of process chemicals in mine tailings from Nordic Mining ASA to the marine alga *Skeletonema costatum*, the marine copepod *Tisbe battagliai* and the polychaete *Arenicola marina*

Preface

This study has been conducted by staff from the Section for Ecotoxicology and Risk Assessment, Norwegian Institute for Water Research (NIVA). The author acknowledges the contribution by Steven Brooks, Harald Heiaas and Kenny Macrae for support during the study.

Oslo, 21.12.2009

Adam Lillicrap

Contents

1 Abstract

The acute toxicity of process chemicals applied to mine tailings at Nordic Mining ASA have been determined. The concentrations of the process chemicals applied to the sediment ranged from an order of magnitude lower than the Environmentally Relevant Concentration (ERC) to an order of magnitude higher with 2 intermediate concentrations and appropriate controls. There were no effects on any of the organisms at the ERC of process chemicals. There was a statistically significant effect at the 3.2 times ERC on the growth of the algae but not in the copepod or polychaete worm tests. The 10 times concentration also had an effect on growth of the algae and caused a complete kill in the polychaete worms and crustacean (LC100). Therefore the Low Observed Effect Concentration (LOEC) is 3.2 times the environmentally relevant concentration of process chemicals in the mine tailings. The No Observed Effect Concentration (NOEC) for this study was at the ERC of process chemicals in the mine tailings. Therefore, the concentration of process chemicals in the mine tailings being used by Nordic Mining ASA should not cause acute toxicity in the environment.

2 Introduction

The acute toxicity of process chemicals in mine tailings to the marine alga *Skeletonema costatum*, the marine copepod *Tisbe battagliai* and the polychaete *Arenicola marina* was carried out at NIVA Gaustadalléen 21, 0349, Oslo, Norway at the request of Nordic Mining ASA. The tests were performed according to methods detailed in OSPAR guidelines for testing of contaminated marine sediment and aimed to determine the acute toxicity to 3 different trophic levels, a sediment consumer (*A. marina*), a primary producer (*S. costatum*) and a primary consumer (*T. battagliai*). The process chemicals and mine tailings were all supplied by Nordic Mining ASA. The study number for the study was 29324 and the exposure dates were 22 October to 09 November 2009.

3 Materials and methods

3.1 Test substances

The test substances used for the investigations were supplied by Nordic mining ASA and were prepared in the form of a mixture applied to sediment to simulate the processes used in the mining industry. The mixture was prepared at NIVA and a range of concentrations of the mixture was applied to mine tailings, and the subsequent substrates were used to assess the acute toxicity to the 3 marine species.

The process chemicals used for this assessment are detailed in Table 1 in addition to the concentrations and amounts used for each test concentration. The chemicals were prepared as stock solutions in distilled water, prior to testing. The tailings (sediment) were supplied as a coarse and fine fraction, and the particle size of each fraction was indicated to be approximately < 40 µm and 40-250 µm in diameter for the fine and coarse sediments respectively. The sediments were prepared in 500 g batches for each replicate of each test concentration and consisted of 15% fine particles to 85% coarse particles. There were 2 replicates per concentration, and 5 concentrations ranging from an order of magnitude lower to an order of magnitude higher than the environmentally relevant concentration of the process chemicals relative to quantity of sediment that was used.

The sediments were prepared individually as fine and coarse fractions (Figure 1, Appendices). The fine fraction had a flocculating agent (Magnafloc) added and freshwater was used as the wetting agent at a ratio of 30:70 by weight (sediment to water). The sediment was stirred vigorously over a 30

minute period to ensure homogeneity of chemical to sediment and to simulate the process used during mineral extraction. The fine sediment mix was allowed to settle over night and then the overlying water was removed and discarded.

The coarse sediment was prepared on the same day and fresh water was added to achieve a ratio of 30:70 by weight (sediment to water) before adding the process chemicals. The sediment was also stirred vigorously over a 30 minute period to ensure homogeneity of chemical to sediment and to simulate the process used during mineral extraction. This was then allowed to settle over night before the overlying water was removed.

Subsequently, the coarse sediment was combined with the fine sediment preparation along with 5 L of reference seawater and mixed thoroughly. This resulted in each replicate containing 5 L of seawater and 500 g of sediment. The mixtures were then allowed to stand for approximately 24 hours to ensure that the sediment had settled. Following the settling period, approximately 2 L of the overlying water was decanted, using an adapted siphon tube, for the copepod and algal tests. The remaining sediment and seawater were used for the polychaete test.

There were also 4 independent controls used for the experiment: a tailings only control, a clean reference sediment control (obtained from a source known to contain sediment dwelling organisms), a tailings plus process chemicals control containing no Magnafloc (at the environmentally relevant concentration) and a tailings plus Magnafloc only control (at the environmentally relevant concentration).

Table 1. Preparation of the process chemicals for added to the sediment

Chemical		Dextrin	Sulphuric acid	Na- silikat Vannglass	SM15	FS2	Flotol B	Magnafloc
Quantity of chemical used in process (kg)*		120	800	720	360	120	32	10
Quantity (%)		0.003	0.02	0.018	0.009	0.003	0.0008	0.00025
Quantity (mg/kg)		30	200	180	90	30	8	2.5
Concentration of stock solution used (mg/ml)		10	Conc. H_2SO_4	20	20	20	10	5
Quantity of each stock solution required/500g for sediment prep (ml)	0.1	0.15	0.006#	0.45	0.225	0.0725	0.04	0.025
	0.32	0.48	0.018#	1.80	0.9	0.24	0.13	0.08
	1.0	1.50	0.055#	4.50	2.25	0.725	0.4	0.25
	3.2	4.80	0.18#	18.0	9.00	2.40	1.3	0.8
	10	15.00	0.55#	45.0	22.50	7.250	2.8¤	2.50
	C1	-	-	-	-	-	-	-
	C2	-	-	-	-	-	-	-
	C3	1.5	0.055#	4.5	2.25	0.725	0.4	-
	C4	-	-	-	-	-	-	0.25

* Relevant to 4000000 kg tailings released per year
\# Quantity determined based on conc. H_2SO_4 with a specific gravity of 184
¤ Due to lack of sample, only 7 times the concentration of Flotol B was used in the 10 times concentration
C1 Tailings only control
C2 Clean reference sediment control
C3 Tailings plus process chemicals control containing no Magnafloc
C4 Tailings plus Magnafloc only control

The process chemicals and sediments were stored in a refrigerated room in the container in which it was received until required for testing. All stock solutions were prepared on the day of the sediment preparation.

3.2 Test organisms

The test organisms used for the test was the marine algae *Skeletonema costatum*, the marine copepod *Tisbe battagliai* and the polychaete worm *Arenicola marina*. The copepods (approximately 7 days old) and algae (NIVA-strain BAC 1) were from continuous cultures, maintained at NIVA Gaustadalléen 21, 0349, Oslo, Norway. The polychaete worms were obtained from Mermaid Sustainable Resources LLP, Woodhorn Village, Ashington, Northumberland, NE63 9NW UK and each worm was approximately 1 g in weight.

3.3 Dilution water

The dilution water for the study was natural seawater taken from a depth of 60 m from within the outer Oslo fjord. Seawater taken from this source is well characterised and is used routinely for culturing and testing purposes at NIVA. The freshwater used for the initial application of the process chemicals was standard lab tap water.

3.4 Algal toxicity test

The algae growth inhibition test was performed according to International Standard ISO 10253: Water Quality – Marine algal growth inhibition test with *Skeletonema costatum* and *Phaeodactylum tricornutum*. The algal toxicity test was performed using the overlying water removed from each replicate of each concentration. The overlying water was filtered to remove any biological or particulate material and spiked with a growth medium concentrate. All batches were inoculated with *S. costatum* from an exponentially growing laboratory culture and incubated in glass flasks on a shaking table at 20 °C under continuous illumination from fluorescent tubes providing approximately 75 µM m^{-2} s^{-1} of photosynthetic active radiation (PAR). The test was performed with three replicates from each of the individual test replicates from each concentration. The cell density was determined using an electronic particle counter (Coulter Multisizer) after approximately 24, 48 and 72 hours. The growth rate of each culture was calculated and expressed as a percentage of the growth rate of control cultures in untreated overlying water. Where more than 50 % growth inhibition was observed in the treated overlying water, the EC_{50} and the EC_{10} (i.e. the concentrations causing 50 and 10 % growth inhibition respectively) were calculated using non linear regression analysis of the growth rate against concentration of process chemicals in the overlying water.

3.5 Acute toxicity to the copepod *Tisbe battagliai*

An acute toxicity test was performed on the copepod *Tisbe battagliai* according to ISO 14669: Determination of acute lethal toxicity to marine copepods (*Copepoda, Crustacea*). Copepods that were approximately 7 days old, were added to the overlying water in plastic microplates containing approximately 5 ml test solution/well. There were 2 replicates used for each test replicate of each concentration and 10 animals per replicate (40 animals per test concentration). The number of survivors were determined after 24 and 48 hours and the no and low observed effect concentrations (NOEC/LOEC) were calculated. The study was carried out in a temperature controlled room (20°C) and with a photoperiod of 16:8 (light:dark) cycle.

3.6 Acute toxicity to the polychaete worm *Arenicola marina*

An acute toxicity test was performed on the polychaete worm *Arenicola marina* in accordance with the PARCOM guideline (Thain and Bifield, 1994). Five individual worms (weighing approximately 1 g each) were removed from a holding tank and added to each replicate test vessel containing sediment and overlying water. Observations for dead animals were made on a daily basis and any dead organisms were removed. At the end of the study, the sediment was sieved and the number of live and dead worms counted in each test replicate and a NOEC and LOEC were calculated. The animals were not fed during the study and the overlying water was aerated continuously throughout. The study was carried out in a temperature controlled room (15°C) and with a photoperiod of 16:8 (light:dark) cycle.

3.7 Physico-chemical parameters

The following parameters, pH, dissolved oxygen and temperature were measured on the excess overlying test solutions at the start of the studies. At the end of the studies, the pH and dissolved oxygen were measured in the overlying waters of the copepod and polychaete worm tests and only the pH was measured in the excess solutions from the algal test. The temperature was measured daily in

the algae and copepod tests and twice in the polychaete worm test. In addition, the sediment, containing the process chemicals at the environmentally relevant concentration, was analysed for total organic carbon (TOC).

3.8 Quality Assurance

All data and subsequent reports have been subject to internal quality assurance within NIVA.

3.9 Archiving

All raw and electronic data will be archived for a minimum period of 5 years.

4 Results

4.1 Physical parameters

The physical parameter and water quality data for the study are shown in Table 2 and Table 3 of the appendices respectively. In summary, the pH and the dissolved oxygen content of the overlying water used for the algae and the copepod test ranged between 7.12 - 7.73 and between 8.75 and 13.42 (mg/L) respectively. The temperature of the algae test ranged between 20.7 and 21.2 (ºC) and in the copepod test it was 20.1 (ºC) at both 24 and 48 hours. The light intensity of the algae test was 67 µmol $m^{-2}s^{-1}$. The pH and dissolved oxygen in the polychaete worm test ranged between 7.08 - 8.82, and between 7.55 and 7.96 (mg/L) respectively. The temperature of the polychaete worm test ranged between 14.7 - 15.3 (ºC). The water quality was within the range expected for the study and none of the analytes present would have affected the outcome of the study. The TOC content of the sediment was measured to be <1.0 µg Carbon/mg.

4.2 Biological data

The effect data from the ecotoxicity studies are presented within the appendices. The data from the copepod and polychaete worm test indicate that at the highest test concentration (10 times higher than the Environmentally Relevant Concentration [ERC]) there was 100% mortality and at the next lowest concentration (3.2 times the ERC) there was no significant difference between the controls. This indicates that for these 2 species, the LOEC is 10 times higher than the ERC and the NOEC is 3.2 times higher than the ERC. With regards to the algal test, the data indicated that the algae were more sensitive to the process chemicals in the overlying water and there were significant differences between the controls and the 3.2 and 10 times the ERC. This means that the LOEC for the algae test was 3.2 times the ERC and the NOEC for the test was at the ERC. The concentration that caused 50% inhibition of growth (EC50) in the algae was also calculated, and this was determined to be 2.9 times the ERC. Furthermore, the EC10 was calculated to be 2.3 times the ERC.

5 Conclusion

There were no effects on any of the organisms at the ERC of process chemicals in the mine tailings supplied by Nordic mining ASA. There was a statistically significant effect at the 3.2 times concentration on the growth of the algae but not in the copepod or polychaete worm tests. Therefore, taking the most sensitive species for the environmental risk assessment, the LOEC is 3.2 times the environmentally relevant concentration of process chemicals in the mine tailings. Furthermore, the NOEC was at the ERC of process chemicals in the mine tailings. This indicates that the concentration

of process chemicals in the mine tailings being used by Nordic Mining ASA should not cause acute toxic effects in the environment.

6 References

International Standard ISO 10253: Water Quality – Marine algal growth inhibition test with *Skeletonema costatum* and *Phaeodactylum tricornutum*.

International Standard ISO 14669: Determination of acute lethal toxicity to marine copepods (*Copepoda, Crustacea*).

Thain, J.E. and Bifield, S. (1993). A sediment bioassay using the polychaete *Arenicola marina*. Test guideline for PARCOM sediment reworker ring-test. MAFF Fisheries Laboratory, Burnham-on-Crouch, essex, UK.

Appendices

Glossary of terms

DO	Dissolved oxygen concentration
EC50	Concentration causing a 50% effect
ERC	Environmentally relevant concentration
LOEC	Low observed effect concentration
NOEC	No observed effect concentration
TOC	Total organic carbon

Figure 1. Process for sediment preparation

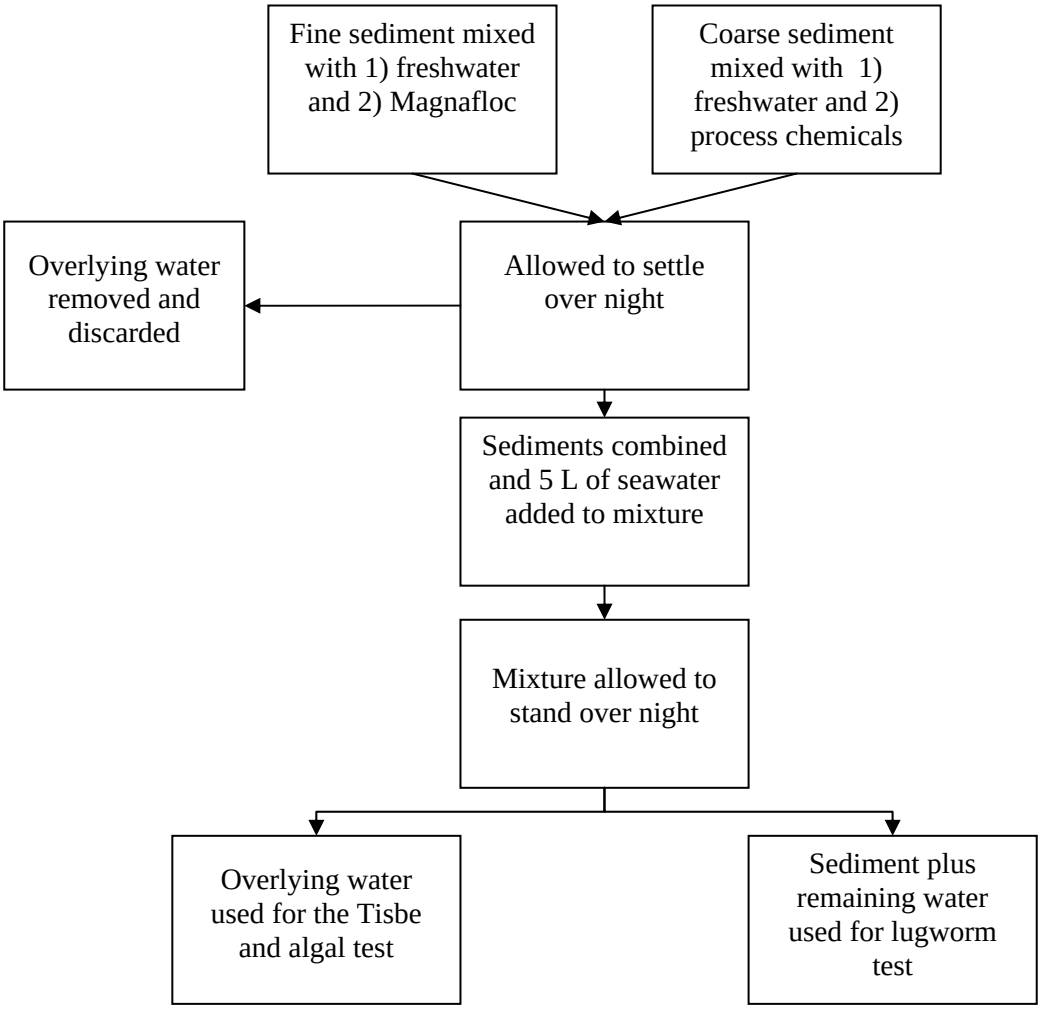

Table 2. Physical parameters of the over lying water

Conc (%)	ON		OFF		
			Algae	Tisbe[a]	
	DO (mg/L)	pH	pH	DO (mg/L)	pH
C1A	13.27	7.73	8.13	12.61	7.96
C1B	13.26	7.71	8.24		
C2A	8.75	7.30	8.26	12.62	7.97
C2B	9.26	7.29	8.25		
C3A	12.06	7.65	8.28	12.80	7.97
C3B	12.97	7.65	8.10		
C4A	13.38	7.70	8.26	12.59	8.01
C4B	13.28	7.70	8.19		
0.1A	13.42	7.69	8.21	12.69	7.99
0.1B	13.40	7.71	8.20		
0.32A	13.02	7.69	8.28	12.70	7.99
0.32B	13.08	7.69	8.23		
1.0A	13.12	7.67	8.21	12.87	8.01
1.0B	13.32	7.66	8.22		
3.2A	12.20	7.58	8.01	12.60	7.99
3.2B	12.47	7.55	8.00		
10A	12.33	7.14	7.97	12.52	7.96
10B	11.98	7.12	7.98		
Min	**8.75**	**7.12**	**7.97**	**12.52**	**7.96**
Max	**13.42**	**7.73**	**8.28**	**12.87**	**8.01**

[a] Parameters measured on pooled samples as the volume size was too small to measure for each independent replicate

Table 3. Physical parameters of the *Arenicola marina* test solutions

Conc (%)	ON			OFF		
	DO (mg/L)	pH	Temp (ºC)	DO (mg/L)	pH	Temp (ºC)
C1A	7.56	7.69	14.7	8.22	7.95	14.9
C1B	7.40	7.70	14.7	8.62	7.91	15.0
C2A	7.38	7.64	14.7	8.66	7.78	14.8
C2B	7.08	7.55	14.7	8.58	7.75	15.0
C3A	7.37	7.65	14.7	8.50	7.90	15.0
C3B	7.68	7.70	14.7	8.51	7.90	15.0
C4A	7.86	7.73	14.7	8.55	7.93	15.0
C4B	7.78	7.73	14.7	8.64	7.93	15.0
0.1A	7.96	7.73	14.7	8.64	7.95	14.8
0.1B	8.11	7.73	14.7	8.71	7.96	14.8
0.32A	7.86	7.73	14.7	8.64	7.89	14.9
0.32B	8.12	7.72	14.8	8.64	7.92	15.0
1.0A	8.10	7.70	14.8	8.78	7.94	15.1
1.0B	8.12	7.68	14.8	8.68	7.95	15.1
3.2A	8.17	7.69	14.8	8.64	7.90	15.2
3.2B	8.26	7.70	14.8	8.78	7.92	15.1
10A	7.94	7.62	14.8	8.73	7.91	15.2
10B	8.08	7.60	14.8	8.82	7.91	15.3
Min	**7.08**	**7.55**	**14.7**	**8.22**	**7.75**	**14.8**
Max	**8.26**	**7.73**	**14.8**	**8.82**	**7.96**	**15.3**

Table 4. Algae toxicity data

Hours	Day 1 24	Day 2 48	Day 3 72	G. rate 2 day	G. rate 3 day	Yield 2 day	Yield 3 day	G. rate 2 day	G. rate 3 day	Yield 2 day	Yield 3 day
Conc.	mill/l	mill/l	mill/l	day^{-1}	day^{-1}	10^6 cells/l	10^6 cells/l	%	%	%	%
C1 A	14 9 24	94 56 107	837 551 797	1.47 1.21 1.53	1.71 1.57 1.69	89 51 102	832 546 792	91 75 95	100 92 99	69 39 79	99 65 95
C1 B	15 35 15	107 84 100	630 997 635	1.53 1.41 1.50	1.61 1.77 1.61	102 79 95	625 992 630	95 87 93	95 104 95	79 61 73	75 118 75
C2 A	27 35 19	114 139 121	881 750 838	1.56 1.66 1.59	1.72 1.67 1.71	109 134 116	876 745 833	97 103 99	101 98 100	84 104 90	105 89 99
C2 B	31 35 19	124 149 119	888 945 648	1.61 1.70 1.58	1.73 1.75 1.62	119 144 114	883 940 643	100 105 98	101 103 95	92 111 88	105 112 77
C3 A	15 14 7	138 121 61	1055 1163 623	1.66 1.59 1.25	1.78 1.82 1.61	133 116 56	1050 1158 618	103 99 78	105 107 94	103 90 43	125 138 74
C3 B	4 4 3	32 34 28	313 387 320	0.93 0.96 0.86	1.38 1.45 1.39	27 29 23	308 382 315	58 59 53	81 85 81	21 22 18	37 46 38
C4 A	29 33 35	216 201 180	1024 1015 761	1.88 1.85 1.79	1.77 1.77 1.68	211 196 175	1019 1010 756	117 114 111	104 104 98	163 151 135	122 121 90
C4 B	33 41 39	170 225 197	784 935 941	1.76 1.90 1.84	1.68 1.74 1.75	165 220 192	779 930 936	109 118 114	99 102 103	127 170 148	93 111 112
0.1A	24 30 17	127 180 104	794 835 751	1.62 1.79 1.52	1.69 1.71 1.67	122 175 99	789 830 746	100 111 94	99 100 98	94 135 76	94 99 89
0.1B	19 14 18	115 97 120	706 727 749	1.57 1.48 1.59	1.65 1.66 1.67	110 92 115	701 722 744	97 92 98	97 97 98	85 71 89	84 86 89
0.32A	16 27 17	87 196 144	702 1100 913	1.43 1.83 1.68	1.65 1.80 1.74	82 191 139	697 1095 908	89 114 104	97 106 102	63 148 107	83 131 108
0.32B	9 12 14	80 106 111	805 1040 1007	1.39 1.53 1.55	1.69 1.78 1.77	75 101 106	800 1035 1002	86 95 96	99 104 104	58 78 82	95 124 120
1.0A	8 11 11	67 100 88	703 1183 1094	1.30 1.50 1.43	1.65 1.82 1.80	62 95 83	698 1178 1089	80 93 89	97 107 105	48 73 64	83 141 130
1.0B	69 11 13	65 63 76	767 761 882	1.28 1.27 1.36	1.68 1.68 1.72	60 58 71	762 756 877	79 79 84	99 98 101	46 45 55	91 90 105
3.2A	4 7 6	7 7 2	21 11 11	0.17 0.17 -0.46	0.48 0.26 0.26	2 2 -3	16 6 6	10 10 -28	28 15 15	2 2 -2	2 1 1
3.2B	4 5 4	4 5 5	24 38 35	-0.11 0.00 0.00	0.52 0.68 0.65	-1 0 0	19 33 30	-7 0 0	31 40 38	-1 0 0	2 4 4
10.0A	5 4 3	3 2 1	3 3 4	-0.26 -0.46 -0.80	-0.17 -0.17 -0.07	-2 -3 -4	-2 -2 -1	-16 -28 -50	-10 -10 -4	-2 -2 -3	0 0 0
10.0B	8 4 4	1 1 1	2 4 3	-0.80 -0.80 -0.80	-0.31 -0.07 -0.17	-4 -4 -4	-3 -1 -2	-50 -50 -50	-18 -4 -10	-3 -3 -3	0 0 0

Figure 2. Algae EC50 calculation data

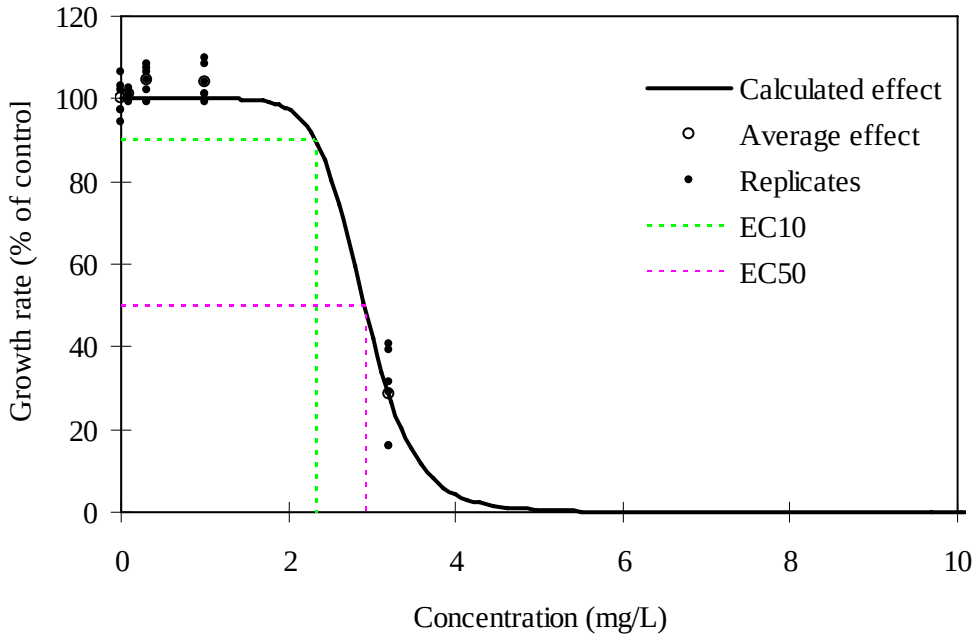

Table 4. Mortality data

Conc (%)	*Tisbe battagliai*			*Arenicola marina*		
	No. dead at 24 hours	No. dead at 48 hours	% mortality	No. alive at the end of the test	No. dead at the end of the test	% mortality
C1A	0	0	0	4	1	10
	0	0				
C1B	0	0		5	0	
	0	0				
C2A	0	0	0	5	0	10
	0	0				
C2B	0	0		4	1	
	0	0				
C3A	0	0	0	5	0	10
	0	0				
C3B	0	0		4	1	
	0	0				
C4A	0	0	0	5	0	0
	0	0				
C4B	0	0		5	0	
	0	0				
0.1A	0	0	0	0	5#	0
	0	0				
0.1B	0	0		5	0	
	0	0				
0.32A	0	0	0	4	1	10
	0	0				
0.32B	0	0		5	0	
	0	0				
1.0A	0	0	0	5	0	10
	0	0				
1.0B	0	0		4	1	
	0	0				
3.2A	0	0	0	4	1	10
	0	0				
3.2B	0	0		5	0	
	0	0				
10A	10	10	100	0	5	100
	10	10				
10B	10	10		0	5	
	10	10				

During the study there was a problem with the air pump in this replicate, therefore the data from this replicate has been discounted and the % mortality has been based on only 1 replicate

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