Report

Testing of the Toxicity of Copper to Barramundi

For

Ok Tedi Mining Limited

February 02

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Executive Summary

As a supporting study for an ecological risk assessment to determine the risks to aquatic life from mine waste management strategies in the Fly River system, toxicity tests were conducted to determine the toxicity of copper to juvenile barramundi (mean length 310 mm) via copper LC_{50} values. Two sets of tests, a 48 hour range finder and a 96 hour definitive test were undertaken during this study. The results of the range finder test were used to determine the copper concentrations to be used for the definitive test.

The 96 hour LC_{50} values calculated for the definitive test based on the nominal copper concentrations was 0.5 mg/L, while the 96 hour LC_{50} values calculated based on measured copper concentrations ranged from 0.27 to 0.41 mg/L. These 96 hour LC_{50} values were higher than the value of 0.079 mg/L which has previously been determined for 14 day old post-larval barramundi .

Contents

1 Introduction

Ok Tedi Mining Limited (OTML) is conducting an ecological risk assessment as part of its environmental monitoring program for the Ok Tedi copper mine in Papua New Guinea. The aim of the risk assessment is to assess the risks to aquatic life in the Fly River system from several mine waste management strategies. A variety of fish species including barramundi occur in this system and are potentially at risk from these mine discharges.

As a supporting study for the ecological risk assessment program, AWT Environment, Science and Technology (ES&T) were contracted by OTML to determine the toxicity of copper to juvenile barramundi *Lates calcarifer* using standard copper LC_{50} values. The results of these tests are provided in this report and will be incorporated into the risk assessment.

1.1 Rationale

It was agreed at the recent OTML Ecological Risk Assessment workshop in Brisbane that toxicity testing of fish occurring in the Fly River system was required to provide essential input data for the baseline risk assessment process. Essentially, this testing, in combination with previous testing carried out by and on behalf of OTML, will permit calibration of existing literature-based species sensitivity curves or possibly the derivation of site specific species sensitivity curves for the chronic and acute effects of dissolved copper on Fly River biota. In particular, this testing will focus on the ecologically important barramundi, which are a key, high trophic level predator in river channel habitats in the Fly River system.

2 Methods

2.1 Facility

Testing was conducted in the aquarium laboratory at the Marine and Freshwater Resource Institute (MAFRI), 123 Brown Street, Heidelberg Victoria from December 7 to December 19, 1998. The time table for activities conducted while undertaking the tests is presented in Appendix A. The ambient room temperature and humidity were controlled via the aquarium air conditioning system. During testing, the room temperature and humidity were maintained at 25.6 ± 4.0° C and 65.2 ± 6.1%, respectively (Appendix C). The aquarium automated light system was set on a 12:12 photoperiod with a 12 hour 70 % maximum illumination and 12 hour 30% illumination. Each photoperiod commenced with a 45 minute twilight period.

2.2 Source of dilution water and test organisms

Carbon filtered Melbourne mains water was used for holding the fish prior to the tests and preparing test solutions. The dilution water was sampled prior to the initiation of each test. The following water quality parameters were measured in the dilution water: pH, conductivity, ammonia, alkalinity, hardness, TSS, DOC, TOC, chlorine and total copper concentrations. Conductivity and pH were measured throughout the tests using portable probes, ammonia was measured using a Merck® test kit, while analysis for the other water parameters was conducted at the AWT ES&T laboratories in Sydney. Table 1 gives the results of these analyses.

Table 1. Dilution water quality parameters

Sample Date	Alkalinity $mgCaCO_3/L$	Hardness $mgCaCO_3/L$	Copper (mg/L)	Total Chlorine (mg/L)	DOC (mg/L)	TOC (mg/L)	TSS (mg/L)
9/12/98	11.5	20.5	<2	<0.05	1.4	1.4	<2
15/12/98	11.0	20.3	<2	<0.05	7.7	8.2	<2

2.3 Fish husbandry

A commercial aquaculturist in Victoria supplied the test fish acclimated to freshwater. The fish had originally been obtained from Cairns in North Queensland. A total of 120 fish were purchased. Thirty fish were purchased on December 5 1998 and held on site at MAFRI in a 6,500 L holding tank for 4 days in preparation for the range finder test. A further 90 fish were purchased on December 10 1998 for the definitive test and held in two 6,500 L holding tanks for 5 days. MAFRI staff transported the fish to the laboratory holding tanks and AWT staff maintained the fish in the holding tanks until testing commenced. The pH in the holding tank was maintained between 7.1 and 7.6 by continual adjustment with hydrochloric acid. Temperature remained at 25.2 ± 2.8° C and the ammonia levels were less than 4.0 mg/L. A partial water change was performed on December 7, 1998 as ammonia levels were increasing in the holding tank because the filtration was not yet operating. The biofilter system was operational during the holding period from December 10 to December 15, 1998.

2.4 Preparation of test chambers

Testing was conducted in semi-transparent 600 L polyethylene chambers. The test chambers were positioned on fixed aquarium tables located approximately 1.2 metres above the floor and 1.6 metres below the light source. The chambers were each lined with polyethylene bags to prevent contaminating the chambers. Five hundred litres of test solution was added to each chamber. The pH in each individual chamber was adjusted to between 7.1 to 7.6 using sodium bicarbonate / 1.0M NaOH and the

hardness to between 140 to 200 ppm using gypsum and Epsom salts (Appendix D Tables D1 and D2).

2.5 Preparation of test solutions

Three different stock solutions were prepared using analytical grade $CuSO_4$ and were used to prepare the range finder and definitive concentrations. The stock solutions were prepared immediately before preparing the test solutions. Sub-samples were taken from each of the stock solutions and analysed for copper concentration. All samples for copper analysis were preserved by the addition of concentrated nitric acid.

Hydrous $CuSO_4$ was used to prepare the stock solutions (refer to Appendix B for calculations). This powder contains five water molecules per $CuSO_4$ molecule. When calculating the amounts of $CuSO_4$ to add to the water to prepare the stock solutions, the water associated with the $CuSO_4$ salts was not taken into account. Therefore, the actual final concentrations in the prepared stock solutions were lower than required by a factor of 0.64. As such, all the nominal test concentrations for both the range finder and the definitive test were reduced by this factor. The required and actual concentrations of the copper stock solutions are shown in Table 2.

Table 2 Required and actual concentrations of copper stock solutions

Required concentration (mg/L)	Actual concentrations (mg/L)
100	64
1,000	640
10,000	6,400

A 6,400 mg/L Cu stock solution was used to prepare the 64 and 6.4 mg/L test solutions. Fifteen litres of stock solution was required, therefore, 376.7 g of copper sulphate was weighed out and dissolved in 15 L of dilution water. To prepare the 0.64, 0.064 and 0.0064 mg/L test solutions a 64 mg/L Cu stock solution was used. Fifteen litres of stock

was required, therefore, 150 mL of the 6,400 mg/L stock solution was diluted in 14.85 L of dilution water.

To prepare the test concentrations for the definitive test a 640 mg/L Cu stock solution was used. To make the stock solution 37.7 g of copper sulphate was dissolved in 15 L of dilution water. This stock solution was prepared twice to give enough stock to add to all the test chambers.

Test solutions were prepared by filling the chambers with dilution water to half capacity and then adding the calculated volume of copper solution to achieve a selected nominal concentration. Tables 3 and 4 outline the concentrations of the copper stock solutions and the volumes used to prepare each test solution.

Table 3. Copper stock solutions and volumes used to prepare test solutions for range finder test

Test concentration (mg/L)	Volume of stock solution added to each replicate (L)
64.0	5
6.4	0.5
0.64	5
0.064	0.5
0.0064	0.05

Table 4. Copper stock solutions and volumes used to prepare test solutions for the definitive test

Test concentration (mg/L)	Volume of stock solution added to each replicate (L)
6.4	5
3.2	2.5
1.6	1.25
0.8	0.63
0.4	0.313

2.6 Water chemistry monitoring

Water temperature, conductivity, dissolved oxygen and pH were routinely measured during the tests using portable probes, while ammonia was measured using a Merck® test kit. Measurements of alkalinity and hardness to determine the need for adjustment were made using Merck® test kits. All other analyses for water parameters were conducted at the AWT ES&T laboratories in Sydney. Sub-samples of the dilution water, collected prior to conducting the range finding and definitive tests were analysed for the following parameters: alkalinity, hardness, TSS, DOC, TOC, chlorine and copper concentrations. Sub-samples from each test chamber in the range finder and definitive test were taken for analysis of alkalinity, hardness and total copper concentrations. These samples were taken just prior to the placement of fish in the tanks. At the completion of the definitive test, a second sub-sample for copper analysis was taken from each test chamber. Samples for copper analysis were preserved by the addition of concentrated nitric acid. Prior to analysis, each solution was digested in nitric acid in a microwave. The stock and test copper solutions were then analysed by ICP AES.

2.7 Range finder test

A 48-hour range finder test was conducted from December 9 to December 11, 1998 to establish the concentration range to be used for the definitive test. Test solutions were prepared on December 8, 1998 and the test commenced on December 9, 1998. A total of 24 fish were exposed to the following copper concentration range: control, 0.0064, 0.064, 0.64, 6.4, and 64.0 mg/L. Each concentration was replicated and each chamber contained two fish. Sub-samples were taken from each test chamber for copper concentration, alkalinity and hardness analyses prior to test initiation.

The fish were carefully introduced into the test chambers using nets and all chambers were then covered with shade cloth to reduce the glare created in the chambers from the ceiling lighting. Fish were not fed prior to or during testing. The pH, DO, conductivity, temperature and ammonia were measured and recorded in each test chamber before test

initiation and every 24 hours for the duration of the test. The chambers were monitored periodically throughout the day for fish survival. Mortalities were recorded and immediately removed from the chambers. At the end of the test, five fish were randomly selected and measured to determine the average weight and length of the fish used for testing. The position of the chambers was randomised within the laboratory to account for natural light and temperature variation (for details see Fig. 1).

Figure 1 Range finder test chamber randomisation design

	6.4 B		Bench space occupied
	0.0064 A		
	0.64 A		**CON. B**
0.064 B	**CON. A**		**6.4 A**
			0.64 B
64.0 B	**64.0 A**		**0.064 A**
			0.0064 B

Grey = Floor

White = Table space (numbers corresponds to the position of test chamber and concentration)

2.8 Definitive test

The definitive test was conducted between December 15 and December 19, 1998. The copper concentration range that was tested was based upon the concentrations that bracketed the 48-hour range finder LC_{50}. The test concentrations selected were control, 0.4, 0.8, 1.6, 3.2 and 6.4 mg/L. Test solutions were prepared on December 13, 1998 and the test commenced on December 15, 1998. The delay in commencing the test was due to a widespread power failure caused by a severe electrical storm that affected the boiler room which maintained the water temperatures in the test chambers. A total of 90 fish were exposed with each test concentration had three replicates, each containing five fish. Sub-samples of water were taken from each test chamber prior to test initiation for copper concentration, alkalinity and hardness analysis. When complete mortality occurred in the chamber or at test

termination, a second sub-sample for copper analysis was collected.

The fish were carefully introduced into the test chambers using nets and shade cloth was placed over the test chambers to reduce the glare within the chambers. Fish were not fed prior to or during testing. The pH, DO, conductivity, temperature and ammonia were measured in each test chamber before test initiation and then every 24 hours for the duration of the test. Test chambers were monitored periodically during the day for fish survival and mortalities were immediately removed from the test chambers. At the end of the test, ten fish were randomly selected to determine the average weight and length of the test fish.

The position of the chambers in the laboratory was randomised to account for light and temperature variation (for details see Fig. 2). Due to limited bench space, two chambers were set up on the floor. A small aquarium heater was used to assist with temperature control for these two chambers.

Figure 2 Definitive test chamber randomisation design

0.4 A		Bench space occupied
CON. A		
CON. B		**0.8 A**
3.2 A		**6.4 A**
0.4 B		**6.4 A**
1.6 A		**3.2 B**
1.6 C		**CON. C**
6.4 C		**0.8 B**
0.8 C		**1.6 B**
0.4 C		**3.2 C**

Grey = Floor

White = Table space (numbers correspond to the position of test chamber and copper concentration (mg/L). The concentrations are based on a 640 mg/L stock solution being used.

2.9 Data analysis

The statistical package Toxcalc was used to determine the LC_{50} value for each test. This package uses probit analysis or trimmed Spearman-Karber method to determine the LC_{50} value.

2.10 Disposal of test solutions and test organisms

To comply with the Guidelines for Farming Barramundi in Victoria, any test solutions or dilution water (including the water and biofilter from the holding tanks) that had been exposed to Barramundi were treated with chlorine before disposal via the sewer. This reduced the risk of transmitting the Barramundi picorna – like virus (BPLV). Whole fish specimens were gutted and soaked in a chlorine solution for 24 hours and disposed in sealed plastic bags via the waste services provided by MAFRI.

3 Results and Discussion

3.1 Observations

3.1.1 Holding Tank

The fish used for the range finder test (30) were kept in the holding tank for four days prior to testing. Fish used for the definitive test (90) were held in the holding tank for five days prior to testing. There was one mortality recorded in the holding tank. The death was attributed to possible injuries obtained during transportation to MAFRI. The fish were subdued and tended to cluster together around the edge on the bottom of the tank. There was no observed aggressive behaviour. Each fish, once placed in the holding tank at MAFRI, developed the distinctive juvenile colouration of a white strip along the pre-dorsal margin of the head region. This colouration typically fades with age, but as with most fish, colouration intensifies when the individuals are stressed or sexually active. This stress mark was absent on the fish while at the aquaculture farm. As barramundi are ambush predators, preferring to hide under snags or other cover in the wild, the lack of cover in the holding tanks might have induced this colour intensification.

3.1.2 During Testing

The activity of the fish during the test period was minimal. The fish generally remained on the bottom of the chambers unless disturbed. However, occasional observation were made of fish swimming in the water column, particularly in the lower copper concentrations. Movement tended to be sluggish and there were no visible signs of aggression. The white marking on the head was evident on all fish throughout the testing period. Mortalities from the 64, 6.4 and 3.2 mg/L copper concentrations had a mucus secretion on the body and some loss of scales. When the fish were being collected from the tanks and gutted, a copper precipitate was observed in the gills of these fish.

For the range finding test, the 64 mg/L copper test solution at test initiation was extremely cloudy. After 24 hours, it had cleared and a precipitate was present on the bottom of the chamber. The 6.4 mg/L

copper solutions were initially clear but after 24 hours a precipitate had formed on the bottom of the chambers. This also occurred in the 3.2 mg/L chambers but to a lesser extent than was evident in the higher concentration chambers.

3.2 Fish lengths and weights

The lengths and weights of the fish used in the range finder and definitive tests are presented in Tables 5 and 6. For the range finding test, the average weight was 481 ± 109 g and length was 312 ± 13 mm. For the definitive test, the average weight was 443.0 ± 110 g and length was 306 ± 21 mm. These sizes correspond well with the typical size range of 320 to 450 mm attained by barramundi prior to dispersing from coastal areas (Reynolds 1978).

Table 5. Length and weight of fish used in the range finding test

	Weight (g)	Length (mm)
	460	320
	579	311
	459	315
	587	290
	321	324
Mean	481.2	312
SD	108.8	13.3

Table 6. Lengths and weights of fish used in the definitive test

	Weight (g)	Length (mm)
	564	335
	315	276
	285	270
	520	310
	400	292
	452	314
	636	325
	477	313
	361	306
	420	318
Mean	443	305.9
SD	110.3	20.7

3.3 Water quality

Water quality was measured daily in the test chambers during the range finder and definitive tests and the results are recorded on the laboratory test sheets which are included in Appendix D.

Data for the analysis of the copper stock solutions is presented in Table 7. Each stock solution was analysed in duplicate. The measured concentrations of the 640 and 6,400 mg/L stock solutions were very similar (less than 5% variation) to the nominal concentrations. The measured concentration of the 64 mg/L stock solution was about 30% above the nominal concentrations.

Table 7 Results of analysis of stock solutions

Nominal	Copper concentration	Copper concentration
64	101.2	103.4
640	662.1	610.9
6,400	6,474	6,272

Data for the laboratory analysis of water from the test chambers for alkalinity, hardness and copper concentrations for all the test chambers are presented in Appendix D (Tables D3 and D4). For all test chambers, the measured copper concentrations were lower than the nominal concentrations. This was especially so for the higher concentrations in the definitive test. This is probably due to the precipitation of copper out of solution onto the bottom of the test chamber. Over the course of the test, precipitates of copper crystals were observed on the body of the fish and on the floor of the test chambers.

3.4 Test LC_{50} results

The results for both the range finding and definitive tests showed a consistent dose response that was related to the nominal test concentrations to which the fish were exposed. There were no deaths in the control tanks in either test. For both tests, the first fish to die were consistently those in the highest concentrations. Fish at lower concentrations only showed signs of stress and died over longer time intervals compared with the higher concentrations.

For both sets of tests, LC_{50} values were calculated for both the nominal concentrations and the measured concentrations in the test chambers. For the definitive test, copper concentrations were measured at the initiation and completion of the tests. An LC_{50} value was calculated for both the initial and final concentrations as well as for the average of the two values. To calculate LC_{50} values for the measured concentrations, the average of the two (range finder) or three (definitive) chambers for each test concentration was used.

The outputs from the ToxCalc analyses are presented in Appendix E and are summarised in Tables 8 and 9. The LC_{50} values for the measured concentrations were lower than the nominal concentrations. This is due to the lower concentrations measured in the test chambers, which resulted from copper precipitating out of solution.

Table 8 LC_{50} values for the range finder test

Time	LC_{50} values (mg/L) (95% confidence limits)	
	Nominal copper concentrations	Measured concentrations
48 hours	3.5 (3.5-3.5)	2.3 (2.3-2.3)

Table 9 LC_{50} values for the definitive test

Time	LC_{50} values (mg/L) (95% confidence limits)			
	Nominal copper concentrations	Original measured concentrations	Final measured concentrations	Average measured concentration
24 hours	1.8 (1.5-2.2)	-	-	-
48 hours	0.73 (0.61-1.23)	-	-	-
72 hours	0.65 (0.61-0.70)	-	-	-
96 hours	0.50 (0.47-0.53)	0.41 (0.38-0.45)	0.27 (0.25-0.29)	0.34 (0.31-0.38)

Ranger Uranium Mines Pty Ltd (1991) tested the toxicity of dissolved copper to post-larval barramundi (14 day old fry, mean length 24.2±1.9 mm length) in simulated Fly River water. The 96 h LC_{50} for that test was 79 (26 – 241) µg Cu /L. The order of magnitude difference between that result and the results of this testing might have been caused by the age differences of the test specimens, poor acclimation to freshwater in

the Ranger Uranium Mines testing, or differing water quality. However, as barramundi do not enter the freshwater reaches of the Fly River until they are approximately 300 mm or greater in length, the testing reported here with fish of mean length of 310 mm is more directly relevant to the potential for mine related effects on barramundi in that system.

4 References

Ranger Uranium Mines Pty Ltd (1991) "Report on the Toxicity of Dissolved Copper and Particulate Copper in Synthetic Fly River Water." Report to Ok Tedi Mining Limited, Tabubil.

Reynolds L.F. (1978) "The Population Dynamics of Barramundi *Lates calcarifer* (Pisces: Centropomidae) in Papua New Guinea." MSc Thesis, University of Papua New Guinea.

Appendix A
Testing Timetable

Day & Date	Activities	Problems & Solutions
Day 1. Saturday 05/12/98	MAFRI received the 30 fish required for the range finder test. Fish were placed in a 6,500L holding tank.	Heaters placed in tank to keep water temperature at approximately 25 °C. pH adjusted to within 7.0 – 7.6 with acid.
Day 2. Sunday 06/12/98	Fish observed in holding tank. Water temperature and pH were measured. pH was adjusted with acid. Temperature and pH measured in holding tank. pH adjustments made.	One ill fish observed.
Day 3. Monday 07/12/98	AWT staff arrive A trial test chamber was set up to investigate the requirements for pH and hardness adjustments. Water temperature, pH and ammonia in holding tank were measured. Partial water change conducted on the holding tank due to rising ammonia levels.	
Day 4. Tuesday 08/12/98	The 12 range finder chambers were set up. Chambers were filled to half capacity. The pH and hardness adjustments were made and then the copper stock solutions were added. Tanks were filled to 500 L capacity. pH and hardness were measured and additional adjustments were made until hardness was between 140 – 200 ppm and pH was between 7.0 – 7.6. Dilution water sample taken for Lk., hard, copper, total chlorine, TOC, DOC and TSS analysis.	One fish mortality observed in the holding tank.

Day 5. Wednesday 09/12/98	Water parameters measured in test chambers (pH, DO, conductivity, temperature, ammonia, alkalinity and hardness) Sub-samples were taken from each test chamber for alkalinity, hardness and copper analysis. Test was loaded at 5 pm.	The hardness in the 6.4 and 64 ppm copper concentrations was measured above 200 ppm. Did not make adjustments, conducted the test at the measured hardness. The pH was <7.0 in the 6.4 and 64 ppm copper concentrations. Further adjustments were made with sodium bicarbonate and 1.0 M NaOH. The pH in the 64 mg/L chambers remained < 7.0 despite efforts to adjust it above 7.0.
Day 6. Thursday 10/12/98	Test monitored for fish mortalities and behavioural characteristics. Water parameters measured (pH, DO, conductivity, temperature and ammonia). The 90 fish required for the definitive test were collected and placed in two 6,500L holding tanks. Biological filter system activated. Test observed for fish mortalities and behaviour. Fish in holding tank were monitored in the PM. Temperature, pH and ammonia	
Day 7. Friday 11/12/98	Test monitored for fish mortalities and behaviour. Water parameters measured (pH, DO, conductivity, temperature and ammonia). Test terminated 4:30pm Fish in holding tank were monitored in the AM and PM. Temperature, pH and ammonia were measured. pH adjustments made.	
Day 8. Saturday 12/12/98	Fish in the holding tank were monitored in the AM and PM. pH adjustments made.	

Day 9. Sunday 13/12/98	Set up definitive test chambers. Chambers were filled to half capacity and the required volume of copper stock solution added. The chambers were then filled to 500 L. Initial hardness measurements were made before calculating the required amount of gypsum and Epsom salts to be added to achieve a final hardness of between 140 – 200 ppm. Dilution water sub-samples were taken for alkalinity, hardness, copper, total chlorine, TOC, DOC and TSS analysis. Fish in the holding tank were monitored in the AM and PM. pH adjustments made.	Due to power failure the boiler had shut down. As a consequence room and water temperature were below testing requirements.
Day 10. Monday 14/12/98	Water parameters were measured (pH, DO, conductivity, temperature, ammonia, alkalinity and hardness) in each test chamber. Fish in the holding tank were monitored in the AM and PM. pH adjustments made.	Water and room temperature were too low to begin test. Late AM - Boiler fixed and room and water temp rising.
Day 11. Tuesday 15/12/98	Water temp measured in test chambers. Temperature OK to start test. Water parameters measured (pH, DO, conductivity, ammonia, alkalinity and hardness. Sub-samples were taken from each test chamber for alkalinity, hardness and copper analysis. Test loaded at 10:30 am.	The temperature in the test chambers on the floor was lower than the elevated chambers. Small heaters were placed in the chambers to boost temperature.

Day 12. Wednesday 16/12/98	Test observed for fish mortalities and behaviour. Water parameters measured (pH, DO, conductivity, temperature, ammonia). Final copper analysis samples taken from 6.4 C & B, 3.2 A & B.	The pH in some test chambers was measured below 7.0. These chambers were adjusted to 7.0 – 7.4 with 1.0M NaOH.
Day 13. Thursday 17/12/98	Test observed for fish mortalities and behaviour. Water parameters measured (pH, DO, conductivity, temperature, ammonia). Final copper analysis samples taken from 6.4A, 3.2 C, 1.6 B & A, 0.8 C.	
Day 14. Friday 18/12/98	Test observed for fish mortalities and behaviour. Water parameters measured (pH, DO, conductivity, temperature, ammonia). Final copper analysis samples taken from 1.6 C.	
Day.15 Saturday 19/12/98	Final test mortality and behavioural observations were made. Final Water parameters were measured (pH, DO, conductivity, temperature, ammonia). Final copper analysis samples taken from Control A, B & C, 0.4 A, B & C, 0.8 A Test terminated at 9:30 am	

Appendix B
Procedure for Making up Stock Solutions

Calculations for preparation of stock solutions

Range finder test

MW CuSO4 =	159.56 g / mol
MW Cu =	63.54 g / mol
the % Cu in CuSO4 = 39.82 %	
Require 100mg/L solution	Weigh out 100mg/L / 0.3982 Therefore, weigh out 251.13 mg of CuSO4
Actual 64 mg/L solution	OR 0.25 g of CuSO4 in 1 L
	To prepare 15 L of stock weigh out 3.77g of CuSO4 and dissolve in 15 L of dilution water.
Require 10,000mg/L solution	10,000 mg/L / 0.3982 Therefore, weigh out 25113.0mg
Actual 6,400 mg/L solution	OR 25.11 g in 1 L
	To prepare 15 L weigh out 376.69 g of CuS04 and dissolve in 15 L of dilution water
To prepare solutions	Require 100 mg Cu in 500 L using 10,000 mg/L Cu stock C1 X V1 = C2 X V2
	Therefore, 100 mg/L X 500 L / 10,000 mg/L = 5 L
To prepare:	100 mg/L solution = 5 L into 495.0 L dilution water 10 mg/L solution = 0.5 into 499.5 L dilution water
	Require 1.0 mg Cu in 500 L using 100 mg/L Cu stock therefore, 1.0 mg/L X 500 L / 100 mg/L = 5L
To prepare:	1.0 mg/L solution = 5 L into 495 L of dilution water 0.1 mg/L solution = 0.5 L into 499.5 L dilution water 0.01 mg/L solution = 0.05 L into 499.95 L dilution water

Definitive test

Require a 1,000 mg/L Cu stock	1000 / 0.3982 = 2511.30 mg OR 2.5 g of CuSO4 in 1 L
Actual 640 mg/L solution	Therefore to prepare a 15 L solution weigh out 37.67 g And dissolve in 15 L of dilution water
To prepare:	10 mg/L Cu solution in 500 L using a 10000 mg/L Cu stock solution C1 X V1 = C2 X V2 therefore, 10 mg/L X 500 L / 1000 mg/L = 5 L
To prepare:	10.0 mg/L solution - 5 L into 495.0 L of dilution water 5.0 mg/L solution - 2.5 L into 497.5 L of dilution water 2.5 mg/L solution - 1.25 L into 498.75 L of dilution water 1.25 mg/L solution - 0.625 L into 499.37 L of dilution water 0.625 mg/L solution - 0.3125 L into 499.68 L of dilution water

Appendix C
Test Temperature Data

Table C1 Room temperature and humidity data

Date	Temperature ^{0}C	Humidity %
8/12/98	23	63
9/12/98	23	67
10/12/98	24	64
11/12/98	26	59
12/12/98	No testing	
13/12/98	No testing and boiler not working	
14/12/98	No testing waiting for room temp to rise (boiler fixed)	
15/12/98	28.6	66
16/12/98	27	64
17/12/98	28	67
18/12/98	26	68
19/12/98	25	69
Mean	25.6	65.2
SD	2.0	3.1

Table C2 Temperature of the fish holding tank

Date	Temperature ^{0}C
5/12/98	N/R
6/12/98	N/R
712/98	N/R
08/12/1998 am	24
08/12/1998 pm	25
09/12/1998 am	24.9
09/12/98 pm	25
10/12/1998 T1	25.2
10/12/98 T2	24.2
11/12/1998 am	25
11/12/1998 pm	26
12/12/98 am	27
12/12/98 pm	28
13/12/98 am	27
13/12/98 pm	24
14/12/98 T1	23.7
14/12/98 T2	23.2
Mean	25.2
SD	1.4

T1 = Tank 1
T2 = Tank 2

Table C3 Chamber temperatures (^{0}C) in the range finder test

Concentration	Dav 0 9/12/98	Dav 1 10/12/98	Dav 2 11/12/98
Control A	22.2	22.7	24.2
Control B	23.5	24	25.2
0.0064 mg/L A	21.9	22.7	23.9
0.0064 mg/L B	23.4	23.9	25.1
0.064 mg/L A	23.1	23.6	24.9
0.064 mg/L B	22.8	23.4	24.3
0.64 mg/L A	23.2	23.8	24.9
0.64 mg/L B	23.1	23.5	24.7
6.4 mg/L A	22.5	23.2	24.3
6.4 mg/L B	22.9	23.3	
64.0 mg/L A	23.5	23.7	
64.0 mg/L B	22.1	22.6	
Mean	22.9	23.7	24.6
SD	0.56	0.48	0.45

Table C4 Chamber temperatures (^{o}C) in the definitive test

Concentration	Day 0 15/12/98	Day 1 16/12/98	Day 2 17/12/98	Day 3 18/12/98	Day 4 19/12/98
Control A	22.6	24.6	24.9	25.1	25.1
Control B	21.2	24.8	25.3	24.2	23.7
Control C	22.4	24.5	24.1	25.6	25.7
0.4 mg/L A	22.3	24.3	24.6	24.8	24.8
0.4 mg/L B	21.9	24.1	24.8	25.2	25.4
0.4 mg/L C	22.4	24.5	25	25.3	25.5
0.8 mg/L A	21.6	24.7	25	25.1	25
0.8 mg/L B	22.7	24.6	24.8	24.9	24.9
0.8 mg/L C	22	24.1	24.8		
1.6 mg/L A	22.5	24.3	24.6		
1.6 mg/L B	22.4	24.6	24.1		
1.6 mg/L C	22.6	24.3	24.4	24.6	
3.2 mg/L A	22.5	24.6			
3.2 mg/L B	22.3	24.3			
3.2 mg/L C	22.7	24.9	25.3		
6.4 mg/L A	22.9	24.9	25.4		
6.4 mg/L B	22.6	24.5			
6.4 mg/L C	22.5	24.2			
Mean	22.3	24.5	24.8	25.0	25.0
SD	0.3	0.3	0.4	0.3	0.1

Note: Blank spaces indicate that all fish in that chamber have died

Appendix D
Water Chemistry Data

Table D1 Alkalinity and hardness results for the range finder test measured using Merck® test kits before test initiation

Concentration	Alkalinity	Hardness mg/L
Control A	0.7	175
Control B	0.7	155
0.0064 mg/L A	0.7	170
0.0064 mg/L B	0.8	170
0.064 mg/L A	0.8	150
0.064 mg/L B	0.8	150
0.64 mg/L A	0.7	165
0.64 mg/L B	0.9	140
6.4 mg/L A	1.1	250
6.4 mg/L B	1.1	250
64.0 mg/L A	1	290
64.0 mg/L B	1	300

Table D2 Alkalinity and hardness results for the definitive test measured using Merck® test kits before test initiation

Concentration	Alkalinity	Hardness mg/L
Control A	0.7	185
Control B	0.7	150
Control C	0.6	140
0.4 mg/L A	0.7	185
0.4 mg/L B	0.6	185
0.4 mg/L C	0.6	170
0.8 mg/L A	0.7	180
0.8 mg/L B	0.7	185
0.8 mg/L C	0.7	145
1.6 mg/L A	0.7	170
1.6 mg/L B	0.7	165
1.6 mg/L C	0.7	200
3.2 mg/L A	0.8	195
3.2 mg/L B	0.7	170
3.2 mg/L C	0.7	160
6.4 mg/L A	0.8	150
6.4 mg/L B	0.7	150
6.4 mg/L C	0.8	245

Table D3 Alkalinity, hardness and copper results for the range finder test measured in the AWT Laboratories

Description	Control A	Control B	0.0064 mg/L A	0.00064 mg/L B	0.064 mg/L A	0.064 mg/L B	0.64mg/L A	0.64 mg/L B	6.4 mg/L A	6.4 mg/L B	64 mg/L A	64 mg/L B
Alkalinity mgCaCO3/L	18	19.5	19.5	18.5	21	18.5	22.5	23.5	34	31	9	12.5
HardnessmgCaCO3/L	155	150	152	148	148	151	140	127	160	164	162	155
Copper ug/L Final	<2	<2	3	2	50	46	573	424	4128	4061	42222	41647

Table D4 Alkalinity, hardness and copper results for the definitive test measured in the AWT Laboratories

Description	Control A	Control B	Control C	0.4 mg/L A	0.4 mg/L B	0.4 mg/L C	0.8 mg/L A	0.8 mg/L B	0.8 mg/L C
Alkalinity mgCaCO3/L	19	18.5	19	18.5	18.5	18	18	17.5	18
HardnessmgCaCO3/L	141	150	151	151	199	154	187	146	159
Copper ug/L Initial	<2	<2	<2	320	304	338	642	623	722
Copper ug/L Final	<2	<2	<2	187	182	236	478	378	564
Description	1.6 mg/L A	1.6 mg/L B	1.6 mg/L C	3.2 mg/L A	3.2 mg/L B	3.2 mg/L C	6.4 mg/L A	6.4 mg/L B	6.4 mg/L C
Alkalinity mgCaCO3/L	18	19	21	22	21	21.5	18	18.5	17
HardnessmgCaCO3/L	142	172	193	152	158	150	146	151	150
Copper ug/L Initial	1226	1208	1154	1490	1364	2189	910	979	1674
Copper ug/L Final	1058	1101	741	1078	1144	1208	656	875	1178

Appendix E
ToxCalc Sheets