



Testing of the Toxicity of Copper to Barramundi

Addendum Report

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, a toxicity test was conducted to determine the toxicity of copper to immature barramundi. Following comments from the peer review committee for the study, the need for additional tests to assess temporal changes in total and dissolved copper concentrations were identified.

The test was subsequently repeated using the same range of concentrations as for the original test but without the addition of fish to the chambers. The results of the repeat test were similar to the results of the original test. In both sets of tests the highest total and dissolved copper concentrations were recorded in the 3.2 mg/L test chambers. Concentrations in these chambers were higher than the concentrations in the 6.4 mg/L chambers. For all test chambers in the repeat test the concentrations of total and dissolved copper decreased over the course of the test. The highest decrease in dissolved copper concentrations compared to nominal concentrations occurred at the highest test concentration (6.4 mg/L). This decrease is considered to be due to precipitation of copper out of solution onto the floor of the test chamber.

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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 the Fly River 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 immature barramundi *Lates calcarifer* using standard copper LC₅₀ values.

Following submission of the report detailing the results of the initial study (AWT 1999), the peer review committee for the project raised a number of issues regarding changes in total and dissolved copper concentrations in the test chambers over the time period that the tests were conducted. These changes were considered to be due to precipitation of copper out of solution, which would have lowered the *in situ* concentrations of dissolved copper to which the fish were exposed. As a consequence, the test was repeated, without fish, to provide further information on changes in the concentrations of total and dissolved copper that occurred over time due to the test procedure itself. The experiments were conducted under the same test conditions as previously used including the same water source, staff and test concentrations. The main difference between the two sets of tests was that the repeat test was conducted using 20 L chambers compared with the 600 L chambers used in the original test and that the repeat test was conducted without the addition of fish to the chambers.

2 Methods

Facility

Testing was conducted in the AWT ES&T laboratory in Sydney from May 31 to June 6, 1999. The ambient room temperature was controlled via the building air conditioning system and was maintained at $22 \pm 1^\circ \text{C}$ for the duration of the tests (Appendix C).

2.2 Source of dilution water

The water for the repeat test was obtained from the same source as that used in the original tests. Stock and test solutions were prepared using carbon filtered Melbourne City mains water obtained from the Victorian Fisheries laboratory in Melbourne and transported to the AWT laboratory in Sydney where the repeat test was conducted.

Test chambers

The original test was conducted using semi-transparent 600 L polyethylene chambers. Due to logistical constraints, the repeat test was conducted in 20 L white polyethylene buckets. During the test, the chambers were positioned randomly on the laboratory floor. All chambers received gentle aeration and remained covered with a lid to prevent evaporation of the test solutions.

Preparation of test solutions

To prepare the test solutions for the repeat tests a 640 mg/L Cu stock solution was prepared using analytical grade hydrous CuSO_4 (refer to Appendix B for calculations). To make the stock solution 3.77 g of copper sulphate was dissolved in 1.5 L of dilution water. The stock solution was prepared immediately before addition to the test chambers and was prepared following the same procedure used for the original test. A sub-sample of the stock solution was analysed for total copper concentration. Table 1 outlines the concentrations of the copper stock solutions and the volumes used to prepare each test solution.

Table 1 Copper stock solutions and volumes used to prepare test solutions for the definitive test

| Nominal test concentration (mg/L) | Volume of stock solution added to each replicate (mL) ¹ |
|-----------------------------------|--|
| 6.4 | 200 |
| 3.2 | 100 |
| 1.6 | 50 |
| 0.8 | 25 |
| 0.4 | 12.5 |
| 0 | 0 |

Test solutions were prepared by filling the chambers with dilution water to half capacity and then adding the calculated volume of copper stock solution to achieve a selected nominal concentration. The remaining volume of dilution water was then added to each of the test chambers to achieve a final test volume of 20 L. The pH in each individual chamber was then 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 in a ratio of 0.8:1. The sodium bicarbonate, gypsum and Epsom salts used in the pH and hardness adjustments were added to the test chambers as a dry powder while the 1.0M NaOH was added as a solution.

Water chemistry monitoring

The dilution water was sampled prior to the initiation of the test and the following water quality parameters were measured: pH, conductivity, ammonia, alkalinity, hardness, TSS, DOC, TOC, and total copper concentrations. The pH was measured daily in the chambers using portable probes while conductivity was measured at the initiation and completion of the test. Measurements of alkalinity and hardness to determine the need for adjustment during the course of the tests were made using Merck® test kits.

At the start of the test, sub-samples from each test chamber were analysed for alkalinity, hardness, and total and dissolved copper concentrations. At the completion of the test, a second sub-sample for alkalinity, hardness, and total and dissolved copper concentrations was taken from each test chamber. The timing of the final sample was dependent on what time the fish had died in the original test (refer to Section 2.6 and Table 2). Water samples were stored at 4°C prior to

analysis. Analysis of the test samples and all the initial water parameters samples was conducted at the AWT ES&T analytical laboratory in Sydney.

Two separate water samples were taken for total and dissolved copper analysis. Samples for total copper analysis were preserved by the addition of concentrated nitric acid. Each solution was then analysed by digestion in nitric acid in a microwave and analysed using ICP AES. Samples for dissolved copper analysis were not preserved with acid. To determine dissolved copper concentrations, a subsample was filtered using a 0.45 µm filter syringe, digested in nitric acid in a microwave and analysed by ICP AES.

2.6 Test design and rationale

In the original test, the test concentrations selected were control (0), 0.4, 0.8, 1.6, 3.2 and 6.4 mg/L with three replicate chambers per concentration. The same concentrations, with three replicate chambers per concentration, were tested in the repeat test. In the original test there was a two day delay from when the test solutions were prepared to when the fish were introduced into the test chambers. This delay was due to a widespread power failure caused by an electrical storm. As a result, in the repeat test there was a two day delay from when the chambers were set up to when the first water samples were taken for analysis. Test solutions were prepared on May 31 1999 and the initial water samples were taken on June 2, 1999 (Appendix A).

In the original test, an initial sample for copper analysis was taken when the fish were introduced into the test chambers and a final sample was taken when all the fish in that test chamber had died. For a number of chambers, the final sample was taken after 24, 48 or 72 hours. Therefore, to provide comparable results between the two tests, water samples were taken from each chamber at the same time as for the original test (Table 2). For chambers in the original test in which fish remained alive for more than 48 hours, an additional water sample was taken at 48 hours in the repeat test.

Table 2 Times when water samples were taken in the original and repeat tests

| Nominal concentration (mg/L) | 0 Hrs | 24 Hrs | 48 Hrs | 72 Hrs | 96 Hrs |
|---|--------------|-------------------|-------------------|-------------------|-------------------|
| Control A | Initial | | | | Final |
| Control B | Initial | | | | Final |
| Control C | Initial | | | | Final |
| 0.4 A | Initial | | | | Final |
| 0.4 B | Initial | | | | Final |
| 0.4 C | Initial | | | | Final |
| 0.8 A | Initial | | | | Final |
| 0.8 B | Initial | | | | Final |
| 0.8 C | Initial | | | | Final |
| 1.6 A | Initial | | | | Final |
| 1.6 B | Initial | | | | Final |
| 1.6 C | Initial | | | Final | |
| 3.2 A | Initial | | Final | | |
| 3.2 B | Initial | Final | | | |
| 3.2C | Initial | Final | | | |
| 6.4 A | Initial | | Final | | |
| 6.4 B | Initial | Final | | | |
| 6.4 C | Initial | Final | | | |

Initial = Time when initial water sample was taken
 Final = Time when final water sample was taken

3 Results and Discussion

Observations during testing

Within 24 hours of addition of the copper stock solution a brown floc was present on the floor of all the test chambers. This precipitate was due to the epsom salts and gypsum that were added to the chambers to adjust the hardness of the test solution. The three chambers with the highest copper concentration (6.4 mg/L) also contained a blue coloured floc caused by the precipitation of copper out of solution. A blue coloured floc was also observed in the highest test concentration chambers in the original test (AWT 1999). A blue coloured floc was not observed in the chambers of any of the lower test concentrations over the course of the test period.

3.2 Water quality

Table 3 gives the results of the analysis of the dilution water samples. The values for the different water parameters were similar to those measured for the dilution water used in the initial test (AWT 1999).

Table 3 Dilution water quality parameters recorded prior to commencement of tests

| Sample Date | pH | Conductivity (µS/cm) | Alkalinity (mgCaCO ₃ /L) | Hardness (mgCaCO ₃ /L) | Total Copper (mg/L) | DOC (mg/L) | TOC (mg/L) | TSS (mg/L) |
|-------------|-----|----------------------|-------------------------------------|-----------------------------------|---------------------|------------|------------|------------|
| 31/5/99 | 6.7 | 62.6 | 12.0 | 15.0 | <2 | 1.5 | 1.7 | <2 |

Data for the analysis of the copper stock solution is presented in Table 4. The measured concentration of the stock solution was very similar (less than 5% variation) to the nominal concentration.

Table 4 Results of analysis of copper stock solutions

| Nominal total copper concentration (mg/L) | Measured total copper concentration (mg/L) |
|---|--|
| 640 | 625 |

The pH was measured daily in the test chambers and the results are summarised in Appendix D (Table D1). Results for conductivity measurements are summarised in Appendix D (Table D2).

Data for the laboratory analysis of water samples from all the test chambers (initial, 48 hours and final) for alkalinity, hardness and total and dissolved copper concentrations are presented in Appendix D (Table D3). The mean initial and final dissolved copper concentrations are summarised in Table 5.

Table 5 Mean total and dissolved copper concentrations

| Description | Control | 0.4 mg/L | 0.8 mg/L | 1.6 mg/L | 3.2 mg/L | 6.4 mg/L |
|-----------------------|---------|----------|----------|----------|----------|----------|
| T copper mg/L Initial | <0.002 | 0.229 | 0.452 | 0.804 | 2.007 | 1.377 |
| T copper mg/L Final | <0.002 | 0.170 | 0.342 | 0.637 | 1.597 | 1.257 |
| D Copper mg/L Initial | NS | 0.208 | 0.414 | 0.713 | 0.912 | 0.853 |
| D Copper mg/L Final | NS | 0.158 | 0.294 | 0.551 | 0.907 | 0.867 |

NS = No sample

The measured total and dissolved copper concentrations increased up to the 3.2 mg/L chambers and then decreased in the 6.4 mg/L chambers (Tables 5 and D3). The decrease in copper concentrations at the highest test concentration was due to copper precipitating out of solution. This was evident as a blue coloured floc the floor of the chambers (Section 3.1).

Over the course of the test the concentrations of total and dissolved copper decreased in all test chambers. The highest proportional difference between initial and final concentrations occurred at the lower test concentrations (0.4 to 1.6 mg/L) with a lower proportional difference for the higher test concentrations (3.2 and 6.4 mg/L) (Table 5). This decrease was probably due to precipitation of copper out of the solutions over the course of the test. However, a blue floc indicating the presence of copper precipitates was only obvious in the 6.4 mg/L chambers. In all the other chambers only a brown floc was evident (Section 3.1). This brown floc probably contained precipitated copper but not at concentrations that were visually obvious.

The greatest proportional difference between total and dissolved copper concentrations for both initial and final samples occurred in the 3.2 mg/L chambers followed by the 6.4 mg/L chambers. The lower concentration chambers had a proportionally lower difference between total and dissolved copper concentrations than the higher test concentrations.

The results of the repeat test were similar to the results of the original test (AWT 1999). In both sets of tests the highest total and dissolved copper concentrations were recorded in the 3.2 mg/L test chambers. Concentrations in these chambers were higher than the concentrations in the 6.4 mg/L chambers. In both sets of tests, this decrease was due to precipitation of copper out of solution in the highest concentration test chambers.

References

AWT (1999). Testing of the toxicity of copper to Barramundi. Report prepared by AWT ES&T for Ok Tedi Mining Limited.

Appendix A

Testing Timetable

| Day | Activity |
|--------------|--|
| 0 | <ul style="list-style-type: none">-Preparation of copper test solutions, adjustment of pH and hardness.-Aerate and cover chambers.-Measurement the alkalinity, hardness, pH, temperature and conductivity of each test solution.-Observations of test solutions ie. clarity of solutions, possible precipitates.-Record room temperature. |
| 1 | <ul style="list-style-type: none">-Monitor water parameters, pH and temperature.-Observations of test solutions ie. clarity of solutions, possible precipitates.-Record room temperature. |
| 2 (0 hours) | <ul style="list-style-type: none">-Monitor water parameters, pH, temperature and conductivity.-Test initiation (sub-samples taken from each chamber for alkalinity, hardness, total and dissolved copper concentration analysis, refer to Table 2.-Observations of test solutions ie. clarity of solutions, possible precipitates.-Record room temperature. |
| 3 (24 hours) | <ul style="list-style-type: none">-Monitor water parameters, pH and temperature.-Sub-samples from chambers terminated at 24 hours.-Observations of test solutions ie. clarity of solutions, possible precipitates.-Record room temperature. |
| 4 (48 hours) | <ul style="list-style-type: none">-Monitor water parameters, pH and temperature.-Sub-sample all remaining chambers.-Observations of test solutions ie. clarity of solutions, possible precipitates.-Record room temperature. |
| 5 (72 hours) | <ul style="list-style-type: none">-Monitor water parameters, pH and temperature.-Sub-samples from chambers terminated at 72 hours.-Observations of test solutions ie. clarity of solutions, possible precipitates.-Record room temperature. |
| 6 (96 hours) | <ul style="list-style-type: none">-Monitor water parameters, pH, temperature and conductivity.-Sub-sample all remaining chambers.-Observations of test solutions ie. clarity of solutions, possible precipitates.-Record room temperature.-Terminate test chambers |

Appendix B

Procedure for Making up Stock Solutions

Calculations for preparation of stock solutions

Copper Stock Solution

Using copper sulphate pentahydrate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$)

MW =

249.6036

% of Cu in CuSO_4

$$= 63.546/249.6036 \times$$

100

$$= 25.485\%$$

Required to prepare a 640 mg/L copper stock solution, therefore,

$640/0.25458 = 2513.94$ mg of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ dissolved in 1L of dilution water

However, total solution volume required is 1.5L

Therefore, weigh out $2513.94 \times 1.5 = 3770.91$ mg of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$

OR 3.77 g of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ dissolved in 1.5 L of dilution water

Preparation of test solutions

Required to prepare the following test series;

0.0, 0.4, 0.8, 1.6, 3.2 and 6.4 mg/L copper test solutions.

Therefore, the volume of 640 mg/L copper stock solution required to prepare each of the above concentrations;

To prepare 6.4 mg/L test solution

$$C1 \times V1 = C2 \times V2$$

$$C1 = 640 \text{ mg/L} \quad V1 = ? \quad C2 = 6.4 \text{ mg/L} \quad V2 = 20 \text{ L}$$

$$V1 = (6.4 \text{ mg/L} \times 20 \text{ L})/640 \text{ mg/L}$$

$$V1 = 0.2 \text{ L}$$

OR to achieve a 6.4 mg/L test solution in 20 L, 200 mL of a 640 mg/L copper stock solution needs to be added to 19.8 L of dilution water.

To prepare the remaining test concentration a 0.5 dilution factor is applied to calculate the volume of copper stock solution required.

| Test concentration (mg/L) | Volume of copper stock solution required (mL) to be added to each replicate |
|---------------------------|---|
| 6.4 | 200 |
| 3.2 | 100 |
| 1.6 | 50 |
| 0.8 | 25 |
| 0.4 | 12.5 |
| 0 (control) | 0 |

Appendix C

Test Temperature Data

Table C1 Room temperature data

| Date | Temperature °C |
|---------|----------------|
| 31/5/99 | 22.5 |
| 1/6/99 | 22.0 |
| 2/6/99 | 22.0 |
| 3/6/99 | 22.0 |
| 4/6/99 | 22.0 |
| 5/6/99 | 22.0 |
| 6/6/99 | 23.0 |

Table C2 Chamber temperatures (°C) in the repeat test

| Concentration | 0 hr | 24hr | 48 hr | 72 hr | 96 hr |
|---------------|------|------|-------|-------|-------|
| Control A | 21.5 | 22.1 | 22.1 | 22.0 | 22.0 |
| Control B | 21.4 | 22.0 | 22.0 | 22.1 | 22.0 |
| Control C | 21.4 | 22.1 | 22.1 | 22.0 | 22.1 |
| 0.4 mg/L A | 21.6 | 22.0 | 22.0 | 22.1 | 22.0 |
| 0.4 mg/L B | 21.3 | 22.0 | 21.9 | 22.0 | 22.0 |
| 0.4 mg/L C | 21.5 | 21.9 | 22.0 | 21.9 | 22.0 |
| 0.8 mg/L A | 21.5 | 22.0 | 22.0 | 22.0 | 21.9 |
| 0.8 mg/L B | 21.5 | 22.0 | 22.1 | 22.0 | 22.0 |
| 0.8 mg/L C | 21.6 | 22.2 | 22.0 | | |
| 1.6 mg/L A | 21.5 | 22.0 | 22.0 | | |
| 1.6 mg/L B | 21.4 | 22.1 | 22.0 | | |
| 1.6 mg/L C | 21.5 | 22.0 | 22.0 | 22.1 | |
| 3.2 mg/L A | 21.5 | 22.0 | | | |
| 3.2 mg/L B | 21.5 | 21.9 | | | |
| 3.2 mg/L C | 21.4 | 22.0 | 21.9 | | |
| 6.4 mg/L A | 21.5 | 22.0 | 22.0 | | |
| 6.4 mg/L B | 21.6 | 22.1 | | | |
| 6.4 mg/L C | 21.5 | 22.0 | | | |

Note: Blank spaces indicate that all fish in that chamber had died in the original test.

Appendix D

Water Chemistry Data

Table D1 Summary of daily measurements of pH in the test chambers during the test

| Hour | 0 hr | 24 hr | 48 hr | 72 hr | 96 hr |
|------------|------|-------|-------|-------|-------|
| Control A | 7.3 | 7.5 | 7.3 | 7.3 | 7.5 |
| Control B | 7.4 | 7.5 | 7.3 | 7.3 | 7.3 |
| Control C | 7.4 | 7.5 | 7.3 | 7.3 | 7.3 |
| 0.4 mg/L A | 7.4 | 7.5 | 7.3 | 7.4 | 7.5 |
| 0.4 mg/L B | 7.3 | 7.4 | 7.4 | 7.4 | 7.5 |
| 0.4 mg/L C | 7.4 | 7.5 | 7.4 | 7.4 | 7.5 |
| 0.8 mg/L A | 7.4 | 7.5 | 7.4 | 7.5 | 7.3 |
| 0.8 mg/L B | 7.4 | 7.5 | 7.4 | 7.5 | 7.3 |
| 0.8 mg/L C | 7.4 | 7.5 | 7.5 | | |
| 1.6 mg/L A | 7.3 | 7.5 | 7.5 | | |
| 1.6 mg/L B | 7.3 | 7.5 | 7.5 | | |
| 1.6 mg/L C | 7.3 | 7.5 | 7.5 | 7.6 | |
| 3.2 mg/L A | 7.2 | 7.4 | 7.6 | | |
| 3.2 mg/L B | 7.2 | 7.4 | | | |
| 3.2 mg/L C | 7.2 | 7.4 | | | |
| 6.4 mg/L A | 7.2 | 7.3 | 7.7 | | |
| 6.4 mg/L B | 7.2 | 7.3 | | | |
| 6.4 mg/L C | 7.2 | 7.3 | | | |
| Minimum | 7.2 | 7.3 | | | |
| Maximum | 7.4 | 7.5 | | | |
| Mean | 7.3 | 7.4 | 7.4 | 7.4 | 7.4 |
| SD | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |

Note: Blank spaces indicate that all fish in that chamber had died in the original test.

Table D2 Summary of initial and final measurements of conductivity ($\mu\text{S}/\text{cm}$) in the test chambers during the repeat test

| Hour | Initial | Final |
|------------|---------|-------|
| Control A | 480 | 484 |
| Control B | 464 | 470 |
| Control C | 472 | 476 |
| 0.4 mg/L A | 465 | 475 |
| 0.4 mg/L B | 459 | 474 |
| 0.4 mg/L C | 445 | 456 |
| 0.8 mg/L A | 472 | 481 |
| 0.8 mg/L B | 469 | 480 |
| 0.8 mg/L C | 481 | 485 |
| 1.6 mg/L A | 491 | 496 |
| 1.6 mg/L B | 485 | 491 |
| 1.6 mg/L C | 492 | 501 |
| 3.2 mg/L A | 444 | 457 |
| 3.2 mg/L B | 432 | 451 |
| 3.2 mg/L C | 444 | 455 |
| 6.4 mg/L A | 396 | 410 |
| 6.4 mg/L B | 395 | 404 |
| 6.4 mg/L C | 397 | 408 |
| Minimum | 395 | 404 |
| Maximum | 492 | 501 |
| Mean | 451.1 | 464.1 |
| SD | 32.2 | 29.6 |

Table D3 Alkalinity, hardness, total and dissolved copper results for the repeat test measured in the AWT laboratory

| | | | | | | | | | |
|--|------------|------------|------------|------------|------------|------------|------------|------------|------------|
| 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 mg CaCO ₃ /L Initial | 31.5 | 25.5 | 26.5 | 27.0 | 27.0 | 30.5 | 31.5 | 31.5 | 29.0 |
| Alkalinity mg CaCO ₃ /L Final | 32.0 | 22.5 | 26.0 | 25.0 | 26.0 | 28.5 | 32.0 | 31.5 | 32.0 |
| Hardness mg CaCO ₃ /L Initial | 230 | 232 | 236 | 238 | 230 | 222 | 230 | 230 | 208 |
| Hardness mg CaCO ₃ /L Initial | 241 | 236 | 242 | 242 | 244 | 223 | 236 | 243 | 236 |
| T copper mg/L Initial | <0.002 | <0.002 | <0.002 | 0.238 | 0.242 | 0.207 | 0.397 | 0.421 | 0.537 |
| T copper mg/L 48 hr | NS | NS | NS | 0.196 | 0.217 | 0.167 | 0.330 | 0.352 | NS |
| T copper mg/L Final | <0.002 | <0.002 | <0.002 | 0.164 | 0.199 | 0.146 | 0.300 | 0.313 | 0.413 |
| D Copper mg/L Initial | NS | NS | NS | 0.213 | 0.219 | 0.193 | 0.361 | 0.387 | 0.494 |
| D Copper mg/L 48 hr | NS | NS | NS | 0.174 | 0.204 | 0.158 | 0.287 | 0.302 | 0.151 |
| D Copper mg/L Final | NS | NS | NS | 0.151 | 0.180 | 0.142 | 0.261 | 0.276 | 0.346 |
| | | | | | | | | | |
| 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 mg CaCO ₃ /L Initial | 39.5 | 40.0 | 40.0 | 58.5 | 56.0 | 58.5 | 82.5 | 80.0 | 78.5 |
| Alkalinity mg CaCO ₃ /L Final | 38.0 | 36.0 | 37.0 | 53.5 | 55.5 | 58.5 | 69.0 | 74.5 | 73.0 |
| Hardness mg CaCO ₃ /L Initial | 236 | 235 | 233 | 184 | 182 | 195 | 128 | 126 | 125 |
| Hardness mg CaCO ₃ /L Initial | 239 | 236 | 238 | 188 | 187 | 190 | 134 | 127 | 131 |
| T copper mg/L Initial | 0.707 | 0.864 | 0.841 | 1.750 | 2.180 | 2.090 | 1.610 | 1.310 | 1.210 |
| T copper mg/L 48 hr | NS | NS | 0.697 | NS | NS | NS | NS | NS | NS |
| T copper mg/L Final | 0.561 | 0.695 | 0.656 | 1.280 | 1.940 | 1.570 | 1.420 | 1.300 | 1.050 |
| D copper mg/L Initial | 0.562 | 0.792 | 0.784 | 0.764 | 1.050 | 0.922 | 0.970 | 0.825 | 0.764 |
| D Copper mg/L 48 hr | NS | NS | 0.635 | NS | NS | NS | NS | NS | NS |
| D Copper mg/L Final | 0.455 | 0.601 | 0.597 | 0.749 | 1.030 | 0.942 | 0.950 | 0.886 | 0.766 |

NS = No sample since fish had died by this time in the original test.