Ecotoxicological assessment of acid drainage plumes from the LMRIA being discharged to the Lower Murray River

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September, 2013

Final Report

For South Australia EPA

Commercial-in-Confidence

Water for a Healthy Country Flagship Report series ISSN: 9780643108981 (LMRIA Project)

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Citation

Adams MS, Kumar A, Doan H, Gonzago D, Jarolimek CV, King J, Simpson SL (2013) Ecotoxicological assessment of acid drainage plumes from the LMRIA being discharged to the Lower Murray River. Water for a Healthy Country Flagship Report. CSIRO, Australia.

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Acknowledgements

Dr. Luke Mosley and David Palmer from the South Australia Environmental Protection Authority (SA EPA) are thanked for assistance with study design and logistics for the collection of the samples. Francesca Gissi and Megan Gilmore (CSIRO Land and Water) are thanked for assistance with the toxicity tests. We thank Graeme Batley, Lisa Golding and Brad Angel for comments on the report.

Executive Summary

The Lower Murray Reclaimed Irrigation Area (LMRIA) comprises of 24 individual irrigation areas over approximately 5,200 hectares of flood irrigated agriculture protected by a levee bank system on the former floodplain of the River Murray, between the townships of Mannum and Wellington, in South Australia. The irrigation bays are typically 1.0-1.5 m below the normal river pool level, enabling gravity fed flood irrigation. The drainage from irrigation and groundwater inputs to the LMRIA is then returned to the river using large pumps. Drought conditions and long-term low inflows from 2006-2010 in the Murray-Darling catchment area led to unprecedented low water levels in the LMRIA, resulting in the water table dropping to below pre-drought levels and dry, saline and cracked soil. Since 2010, water levels have recovered and in some cases flooded back onto pasture land.

Environmental monitoring has shown that soils in much of the LMRIA continue to exhibit widespread acidity and the majority of the drainage channels contain highly acidic drainage water (pH 2-5). In many cases, the acidic drainage water also contains high concentrations of metals and metalloids with multiple exceedances of water quality guidelines (WQGs) for aquatic ecosystems (for Al, As, Cd, Cr, Co, Cu, Fe, Mn, Ni and Zn). A recent study by CSIRO investigated the behaviour and potential impacts of acid drainage water from Jervois (Wellington and Woods Point), Toora, Mobilong and Long Flat being discharged to the River Murray. This study confirmed the high concentrations of many potentially toxic metals in the drainage waters, but the dissolved concentrations of these metals would decrease rapidly upon dilution with River Murray water as a result of precipitation and adsorption to particulate phases. The dissolved concentrations of Co, Cu, Ni and Zn continued to exceed WQGs up to dilutions of 1:100. These metals, and the metal-rich colloids and precipitates that form during mixing of the waters, represent a potential risk to the aquatic environment.

The aim of this study was to;

(a) - assess the ecotoxicological risk of drainage waters following dilution and neutralisation with river water and,

(b) - assess risk posed to benthic organisms by the metal-rich precipitates that form as a result of these inputs and may deposit on sediments within the river system.

Ecotoxicological assessment of acid drainage waters from Jervois (Wellington), Jervois (Woods Point), Toora and Mobilong measured the toxicity of drainage water (collected 22 October 2012) diluted with River Murray water to freshwater biota. Test species were selected based on their sensitivity to contaminants and availability of robust test methods. Tests measuring acute (short-term) and chronic (long-term) toxicity included;

- Survival (acute, 48 h) and reproduction (chronic, 9 d) of the cladoceran *Ceriodaphnia dubia*
- Survival of the shrimp *Paratya australiensis* over 96 h (acute)
- Survival and growth of the larvae of native fish *Maccullochella peelii* (Murray Cod, aquacultured) over 7 days (chronic)

The biomarkers, glutathione reductase and glutathione-S-transferase were also measured in shrimp (after 96 h) as indicators of exposure and oxidative stress.

As part of a CSIRO investigation, toxicity was also assessed using the acute bacterial (*Vibrio fischeri*) luminescence bioassay, the chronic microalgal (*Chlorella vulgaris*) growth rate inhibition test and the chronic duckweed (macrophtye, *Lemna minor*) growth inhibition bioassay.

The acid drainage waters from Jervois (Wellington), Jervois (Wood Point), Mobilong and Toora had low pH (5.5, 4.9, 3.6 and 5.4 respectively) and variable conductivity (4.2, 5.1, 27, and 14 mS/cm respectively). Concentrations of dissolved Al, Co, Ni, Zn and Mn exceeded WQG TVs (for hardness correction to 60 mg $CaCO_3$/L) at each of the four sites while concentrations of As and Cd only exceeded TVs at Mobilong and Toora.

Acid drainage water was toxic to at least one freshwater species for each of the four sites. The Jervois (Woods Point) drainage water was the least toxic, with toxicity only observed to cladoceran reproduction (chronic toxicity). Despite similar concentrations of dissolved metals, ammonia, pH and conductivity, the drainage water from the down-river Jervois site at Wellington was more toxic than the Woods Point drainage water. Hence, contaminants other than those measured are causing toxicity to aquatic biota at Wellington or, water quality characteristics at Woods Point are ameliorating toxicity to aquatic biota. All of the toxicity tests (except fish growth) showed toxicity to Mobilong and Toora drainage water. Mobilong drainage water was more toxic to each of the test species (and endpoints measured) than drainage water from Toora. However, the fish survival test showed that Toora drainage water was more toxic than drainage water from Mobilong.

The toxicity of metal-rich particulates from Jervois Wellington at the T-Junction drain (pH 6) and Wellington-1 drain (pH 6.8) were evaluated using the midge *Chironomus tepperi*. The pH of these sediments alone was not expected to adversely affect this organism. Acute toxicity was assessed after 5 days by measuring survival and growth of *C. tepperi*. Chronic toxicity was measured as the number of midge (and their sex) emerging from sediment after 14 days. Metal-rich sediments/precipitates from the Jervois (Wellington) site (pH 6.8) showed a low level of acute toxicity to the midge, *C. tepperi*. However, a high level of chronic toxicity was observed with emergence of midge significantly reduced in T-Junction drain precipitate (pH 6). Wellington-1 drain particulates (6.8) were even more toxic and the ratio of male to female organisms was significantly altered with only male midge observed. Concentrations of acid-extractable metals showed that only nickel concentrations exceeded sediment quality guideline concentrations in the T-Junction precipitate.

The results of this study are based on the one sampling of drainage water from four irrigation sites and the hence the variability of toxicity measured in this study is unknown. This study also utilised laboratory-based toxicity tests and the extrapolation of laboratory-based data to the field sites can be complex as different species and contaminant exposure conditions may be present in the field compared to that utilised in the laboratory. Based on the ecotoxicological assessment of acid-drainage water from Jervois (Wellington and Woods Point), Mobilong and Toora, and, metal-rich particulates from Jervois (Wellington) in this study, a number of recommendations are suggested to further understand the potential impact of acid drainage water entering the Lower Murray. These recommendations include (but not limited to):

(i) - investigating the cause of toxicity to freshwater biota using toxicity identification and evaluation (TIE) techniques, especially at the most toxic site, Jervois (Wellington),
(ii) - the effect of metal-rich particulates on aquatic species such (as fish),
(iii) - assess the ecological impact of acid-drainage water entering the River Murray using field-based assessments (species diversity studies and in situ toxicity testing), and
(iv) - assess the influence of liming of the drainage channels on the toxicity of the discharged waters and precipitates.

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1 Background

There is approximately 5,200 hectares of flood irrigated agriculture protected by a levee bank system on the former floodplain of the River Murray in South Australia, between the townships of Mannum and Wellington. This area comprises 24 individual irrigation areas and is known collectively as the Lower Murray Reclaimed Irrigation Area (LMRIA). Historically, dairy farming is the predominant land use with a smaller area for beef cattle, fodder production and lifestyle farming. The irrigation bays are typically 1.0–1.5 m below the normal river pool level (+0.75 m Australian Height Datum (AHD)), enabling gravity fed flood irrigation. The drainage from irrigation and regional groundwater inputs to the LMRIA is returned to the river using large pumps.

Drought conditions and long-term low inflows from 2006-2010 in the Murray-Darling system led to unprecedented low water levels below Lock 1. During April 2009 the water level in the Lower Murray fell to below -1 m AHD. It is considered that this situation would not have occurred, at least, since the last Ice Age. The low water levels and restricted water allocations during the drought meant that most of the LMRIA was not able to be watered for substantial periods of time and the groundwater table dropped substantially (1-3 m) from pre-drought levels (Leyden et al. 2012). The heavy clay soils subsequently salinised, dried and cracked, causing major damage to the irrigation bays and associated infrastructure, and major socio-economic impacts.

Since 2010, water levels have recovered in the Lower Murray and irrigation has recommenced in a limited manner in the LMRIA. In some cases water has flooded back onto pasture through cracks and fissures in the soil profile. Water quality monitoring by the South Australian Environment Protection Authority (EPA) in late February 2011 found acid drainage water being returned to the river from thirteen irrigation areas (comprising 3,500ha) in the LMRIA (Figure 1). Subsequent investigations have identified the widespread distribution of severe soil acidity in the LMRIA region (Fitzpatrick et al. 2012). This acidity has been generated as a result of oxidation of acid sulfate soils due to the unprecedented low water table levels under the LMRIA during the drought.

The acid drainage water being returned to the River Murray is in the range of pH 2-5 (Leyden et al. 2012). The acid drainage water also contains high levels of soluble and toxic metals and metalloids with large-scale and multiple exceedances of drinking water supply (for aluminium, arsenic, cadmium, iron, manganese, nickel) and water quality guidelines (WQGs) for aquatic ecosystem (for aluminium, arsenic, boron, cadmium, chromium, cobalt, copper, iron, manganese, nickel, zinc; ANZECC/ARMCANZ, 2000). Previous research has observed that these LMRIA drainage discharges can change the river water quality for nutrients (Mosley and Fleming 2010). If large-scale exceedances of WQGs occur in the river, it is possible that the same will occur at the Lower Lakes.

Sampling of the acid drainage plume shows that after dilution and neutralisation in the zone of initial mixing (<25 m from discharge point), the plume sinks to the bottom of the river due to the higher density (salinity), and travels downstream over 1.5 km. The plume appears to widen and mix upwards with river water with distance downstream. Current pH values in the bottom water are within water quality guideline limits (pH 6.5 - 9.0) due to neutralisation in the initial discharge zone. However it is possible that the dissolved and colloidal metals and precipitates present in this neutralised bottom water are at concentrations that may be toxic and further testing is required. When lower flow conditions return in the summer months, or the next drought, there will be much less dilution so there is a greater risk that acidic water could be found in the main channel. The result would be that a spatially more extensive risk zone and potentially increased impacts on drinking water supply offtakes and ecosystems.

CSIRO has recently undertaken an investigation of the behaviour and potential impacts of acid drainage plumes being discharged to the Lower Murray, from a chemistry and water quality guidelines perspective (Simpson et al., 2013). The study focused on drainage waters from five sites within the

LMRIA: Toora, Mobilong, Long Flat, and Jervois (Woods Point and Wellington ends). The research investigated the concentrations and forms of metals following dilution and neutralisation of the drainage water with river water. It confirmed the high concentrations of many potentially toxic metals in the drainage waters, but indicated that the dissolved concentrations of these metals would decrease rapidly upon mixing with River Murray water as a result of precipitation and adsorption to particulate phases. However, the dissolved concentrations of Co, Cu, Ni and Zn exceeded WQGs in many waters even following dilutions of 1:100. These metals represent a potential risk to the aquatic environment. Furthermore, the precipitates that formed were rich in metals and represented a risk to benthic organisms when deposited on the surface of sediments. The precipitates were also an ongoing source of metals being released back into the dissolved phase.

1.1 Research needs

The request for research into the potential ecotoxicological risk posed by the discharge of the drainage waters to the River Murray has arisen due to the presence of dissolved metals at concentrations that exceed WQGs and metal-rich precipitates that occur in the mixed waters.

Broad management objectives for the project were to;

- (a) - assess the ecotoxicological risk of drainage waters following dilution and natural neutralisation with river water and,
- (b) - assess the risk posed to benthic organisms by the metal-rich precipitates that form as a result of these inputs that may deposit on sediments within the river system.

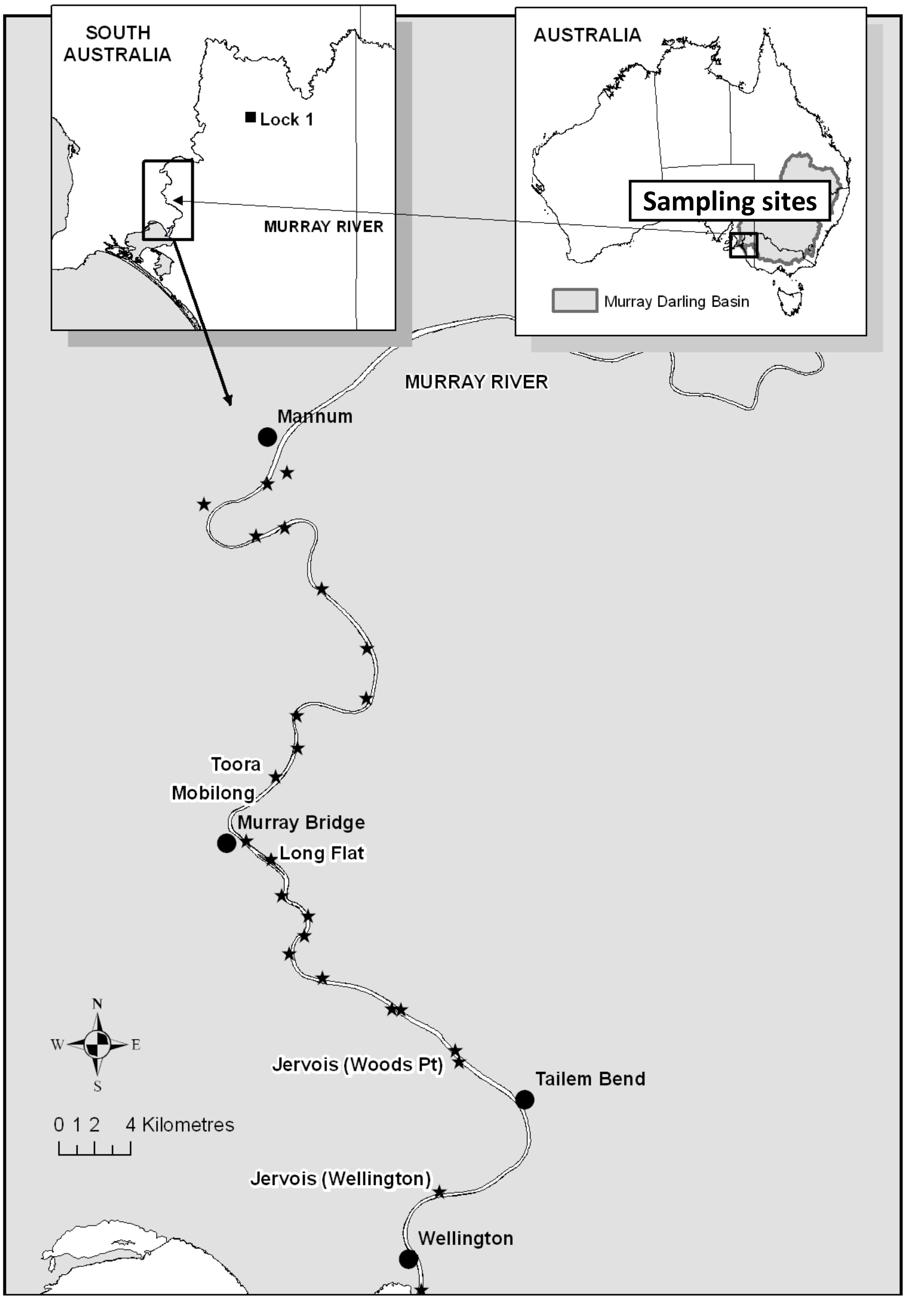


Figure 1. Map of LMRIA acidic and neutral drain discharges including the five LMRIA locations where drainage water was sampled. Major drinking water off-takes are located at Mannum, Murray Bridge and Tailem Bend

2 Research Approach and Methods

2.1 Approach to research

The aim of this research was to provide information on the toxicity and bioavailability of acid drainage water from the LMRIA following dilution and neutralisation of pH with River Murray water. Recent studies have shown that when diluted and mixed with River Murray water, the pH of the drainage waters increases while concentrations of dissolved metals decrease (Simpson et al. 2013, Leyden et al. 2012), however, the bioavailability and toxicity of the metals to aquatic biota was unknown. In addition, the precipitates formed after neutralisation settle on bottom sediments within the river, potentially exposing benthic biota to contaminants.

The following management questions and considerations undertaken in this study were developed through consultation between Dr Luke Mosley and Peter Goonan at the SA EPA and Dr Stuart Simpson at CSIRO in August 2012.

a. Ecotoxicological risk posed by dissolved metals to aquatic organisms

A range of species and endpoints (e.g. acute lethality; chronic growth, reproduction) were considered for use in the aquatic ecotoxicology assessment, including algae (*Chlorella vulgaris*) duckweed (*Lemna minor*), a cladoceran (*Ceriodaphnia*); a shrimp (*Paratya australiensis*); tadpoles of a native frog (*Lymnodynastes* or *Littoria* species); and early life stages of native fish (*Melanotaenia fluviatilis* (Murray Rainbow) or *Maccullochella peelii* (Murray cod)). From these, the most appropriate species were considered to be the cladoceran, shrimp and native fish.

Based on the study of metal chemistry and potential impacts of acid-drainage plumes (Simpson et al., 2013), it was suggested that three to five different drainage waters (including Mobilong and Woods Point) should be tested at different dilutions. The dilutions chosen were expected to represent a worse-case scenario for dilution of the drainage waters with River Murray water. The diluted drainage waters would generally be within the pH range measured in the bottom plumes (pH 6.5-9). Dilutions of the acid drainage water tested included a range of biological response effects (no effect to complete effect). This range was expected to capture both waters where toxic effects occur and those with no effects occurring due to the drainage water inputs being sufficiently diluted.

For each toxicity test, the dissolved metal concentrations were analysed in dilutions of acid drainage water at the start and at the end of the test (or before renewal of test solutions) to monitor the changes in dissolved metal concentrations. Water quality measurements were also made and included pH, temperature, conductivity, and dissolved oxygen (DO) concentrations.

As part of a CSIRO-based investigation, toxicity tests with bacteria, microalgae and duckweed were also undertaken and the results have been included in this report. This testing approach will allow ‘safe dilutions’ to be determined for acid drainage water.

b. Ecotoxicological risk posed to benthic organisms by the metal-rich precipitates

With respect to the effects of the metal-rich precipitates on benthic organisms, the most appropriate test species was considered to be the midge (*Chironomus teperri*) in which the test endpoints are sub-chronic (growth, emergence, survival and sex-ratios). An alternative would be to use the snail (*Potamopyrgus antipodarum*), but this was not considered to be as robust or sensitive a measure of potential risks arising from acid drainage.

It was proposed that precipitates generated from 2-3 sites (with one site replicated) be investigated initially. The sites and preparation of the precipitates used in the toxicity tests were based on results and methods described in the recent water chemistry study (Simpson et al., 2013), and additional in-river monitoring of precipitates undertaken by the SA EPA.

The precipitates that form during the oxidation and neutralisation of the drainage waters may settle at the bottom of the river in dense flocs, or be transported downstream where they are likely to mix with other suspended solids and deposit in low energy environments near the river banks and deeper holes in the river. It was considered unlikely that benthic organisms will colonise the zone of highest deposition of these precipitates. The exposure of benthic organisms to the precipitates is more likely to occur at locations where these have been diluted to some degree with natural sediments. This study focused on the maximum hazard posed by the deposited precipitates by exposing the organisms to undiluted precipitates.

The intended output from this component of this study was to provide information on the level of deposition of precipitates that would be considered unhealthy for the benthic ecosystem. Chemical analyses (dilute acid-extractable metals) were used to help establish cause-effect relationships.

2.2 Sampling

2.2.1 ACID DRAINAGE WATERS

The sampling program was executed by Merrin Adams and Hai Doan (CSIRO), and David Palmer (SA EPA). The initial sampling campaign was undertaken on October 22, 2012 at the drainage pump discharge point to the River Murray from the Toora, Mobilong, Woods Point, and Wellington irrigation areas shown in Figure 1.

Sample bottles were rinsed with 10% nitric acid followed by a thorough rinse with Milli-Q water.

At each site, three acid-washed 20-L carboys were rinsed with drainage water before being filled with drainage water. Twenty nine 20-L containers of River Murray water were also collected from the Thiele Reserve boat ramp immediately for use as dilution and control water in toxicity tests. While Murray River water is usually collected from the river banks at the Mobilong site, easy access to river water was required to collect the large volumes required in this study. Therefore, river water was collected from the Thiele Reserve boat ramp, a site also upstream from the township of Murray Bridge. Due to the large volume of river water that needed to be collected, a 2" centrifugal pump (petrol powered) with 8 m suction hose was used to fill the carboys. The pump was flushed with river water for approximately 5 min and each carboy was rinsed with river water prior to filling. One carboy was filled with river water by hand (no pump) to determine if any potential contamination had been introduced to the river water by using the pump.

The water pH, temperature, electrical conductivity (EC), SPC (specific conductance), redox potential (ORP), DO concentration (mg/L, % saturation) and total dissolved solids (TDS) were measured at the time of sampling using a calibrated water quality probe (YSI 556). Alkalinity and acidity (mg/L $CaCO_3$) were measured in the field using a test kit (HACH Model AL/AC-DT).

Samples were transported to CSIRO laboratory at Urrbrae, Adelaide where the samples were stored at 4˚C prior to undertaking toxicity testing. Subsamples were transported to CSIRO, Lucas Heights, Sydney, for metal analysis and ALS Laboratories, Sydney, for physico-chemical and nutrient analysis.

2.2.2 METAL-RICH PARTICULATES

Drainage channel sediment was collected by Luke Mosley (SA EPA) and David Palmer (SA EPA) on April 4, 2013. Material deposited in the sediment traps (placed on the Jervois Wellington Drain Buoy line where the acid drainage enters the main river channel) was originally selected as a good representation of sediment entering the River Murray and hence for use in the toxicity assessment however, the quantity of material required to carry out the testing could not be collected from traps. Therefore, sediment from the Jervois (Wellington) drainage channel was collected. Attempts to collect sediment close to the sediment traps were not successful due to the presence of vegetation in the drain. After a number of sites along the drainage channel were trialled, 10-15 kg of Jervois (Wellington) T-Junction drain precipitates and drain particulates (Wellington-1), were selected for use in the midge toxicity test.

Prior to initiating the toxicity assessment, the sediment was analysed for dilute acid-extractable metals (AEM) to ensure that the metal concentrations in the sediment represented that which have been measured previously.

For the Chironomid toxicity tests, metal-rich particulates were sieved (425 µm) to remove large matter and the pH of the interstitial water was measured (pH of 6). This was above the desired pH limit of >5.5 (ideally pH 6.5) and therefore, no pH adjustments were made.

River Murray sediment (8-10 kg) was also collected by Hai Doan (CSIRO) at a location upstream from the LMRIA sites on 6 February 2013 for use in dilution of the contaminated sediments.

2.3 Analytical Protocols

2.3.1 WATER FILTRATION

Water samples for dissolved metal analysis were filtered through acid-washed 0.45 µm Millipore membrane filters using polycarbonate filtration apparatus (Sartorius). All filtration assemblies were rigorously cleaned before processing each sample by first filtering 100 mL volumes of 10% v/v nitric acid solution followed by ca. 150 mL of MQ water, and finally, a 50 mL volume of sample. The 50 mL volume of sample was swirled in the top and bottom compartments of the filtration rig to pre-treat the filtration rig, before being poured into the filtrate receiving bottle, shaken to pre-treat the bottle, and discarded to waste. The volume of sample filtered through each filter depended on the turbidity and was generally between 50-250 mL.

2.3.2 NUTRIENT AND PHYSICO-CHEMICAL ANALYSIS

River Murray water and the acidic drainage waters were characterised for a range of physical and chemical parameters by ALS Environmental Division, Sydney. Analyses included alkalinity, acidity, sulfate (as SO_4), chloride, major ions (dissolved), ammonia, nitrite, nitrite + nitrate, total Kjeldahl nitrogen, total phosphorus, reactive phosphorus and dissolved organic carbon. River Murray water samples were preserved collected in appropriate bottles and pre-treated as required depending on the analysis required. A brief description of the methods used to measure each parameter is presented in the test reports (Appendix A).

2.3.3 METAL ANALYSIS

Dissolved metals analysis

Filtered (0.45 µm) water samples were acidified to 0.2% with concentrated nitric acid (TracePur) and metal concentrations determined by inductively coupled plasma-mass spectrometry (ICPMS) (Agilent, 7500CE) or inductively coupled plasma-atomic emission spectrometry (ICP-AES) (CIROS, Spectro) using the operating conditions recommended by the manufacturer and described in CSIRO Method C-209. Metals analysed included Al, Ag, As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Zn and major ions Na, K, Ca and Mg.

Dilute acid-extractable metals (AEM) analyses of sediments and precipitates

To ascertain the fraction of total metals present in dilute acid-extractable metal forms (AEM, targeting the less-crystalline, or recently precipitated, metals associated with (hydr)oxides and carbonates) a cold 1 M HCl digest was employed (Linge, 2008). The AEM fraction was determined by digesting the wet solid in 1 M HCl (~1 g/100 ml) for 60 min, followed by filtration (<0.45 µm). The concentrations of dissolved metals in the acid extract were determined by ICP-MS and ICP-AES as described above.

Quality control for metal analyses

Precision of analytical procedures was assessed by measuring at least 10% of the water and sediment samples in duplicate. Matrix interferences in the water sample and sediment samples were checked by measuring spike recoveries in at least 10% of the each sample type.

Reference materials from the National Research Council Canada (NRC) were also analysed with each batch of samples whenever a suitable reference material was available. The following reference materials were used: NWTM-24.3 (LGC Standards) for metals in fresh waters, and PACS-2 for total recoverable metals in sediments.

2.4 Toxicity tests

All water toxicity tests were carried out on unfiltered acidic drainage water from Toora, Mobilong and Jervois (Woods Point and Wellington) and serially diluted with unfiltered River Murray water. The samples were not adjusted or manipulated in any way before testing so that serial dilutions mimicked the natural neutralisation of acidic drainage water in the River Murray. With previous studies showing that dissolved metal concentration decrease after mixing with river water, test solutions were renewed where possible throughout the duration of the test (generally every 48-72 h) to maintain or re-establish concentrations of dissolved metals achieved upon initial mixing.

2.4.1 CLADOCERAN IMMOBILISATION AND REPRODUCTION TESTS

Toxicity tests with the waterflea, *Ceriodaphnia dubia*, measured both acute (immobilisation) and chronic (reproduction) toxicity of the acidic drainage waters. Cultures of *C. dubia* were cultured at CSIRO, Adelaide in demineralised water (DMW).

The acute bioassay measuring immobilisation of *C. dubia* over 48 h follows the OECD guideline 202 (OECD 2004) with minor modifications (Table 1). Acid drainage water sample was diluted with River Murray water to achieve concentrations of 0.15 to 100% (where 100% is undiluted acid drainage water). Each concentration was dispensed, in triplicate, into 50-mL glass beakers (containing 25 mL test solution). River Murray water and DMW were also prepared in triplicate for use as control treatments. Five *C. dubia* neonates (<24 h old) were added to each vial and incubated at 20 ± 1˚C (16:8 h light:dark) using cool white fluorescent lamps. After 48 h, the number of alive and immobilised (dead) neonates was counted. Test solutions were not renewed (i.e. a static test) during the 48 h exposure. Four acute cladoceran toxicity tests were carried out on 24 Oct (Toora), 30 Oct (Wellington), 6 Nov (Woods Point) and 13 Nov (Mobilong) 2012.

Reproduction of *C. dubia* was assessed over 9 days and is summarised in Table 2 and based on the OECD Test Guideline 211 (1984a and 2012) for *Daphnia magna*. Acid drainage water was diluted with River Murray water to achieve drainage water concentrations of 0.1 to 100% (undiluted). Tests were carried out in 200-mL beakers containing 100 mL of test solution with each treatment prepared in triplicate. Two control treatments were also prepared with River Murray water and DMW, each prepared in triplicate. Ten neonates (< 24 h old) were added to each beaker and incubated at 25 ± 1˚C with a photoperiod of 16:8 light:dark cycle. Daphnids were fed the microalgal *Pseudokirchneriella subcapitata* on days 2, 4 and 6 and 8. During the 8-d test duration, test solutions were renewed on two occasions (days 2 and 4). After 9 days, the number of surviving daphnids and the number of young generated were counted. Initial chronic toxicity tests were carried out on January and February 2013 did not meet quality assurance criteria and chronic tests were repeated in March 2013.

The pH, DO, electrical conductivity and temperature were measured at the beginning and end of the bioassay, and when test solutions were renewed.

A control consisting of DMW and the reference toxicant, copper, were also tested for quality assurance purposes.

The 48-h EC10 and EC50 estimates were determined with the non-linear interpolation method using ToxCalc v5.0 (Tidepool Scientific Software). The concentration of sample tested that caused no significant toxicity (NOEC) and the lowest concentration of test material causing significant toxicity (LOEC) were determined by performing the Steels Many-One Rank test.

Table 1. Summary of the test condition for the acute *Ceriodaphnia dubia* immobilisation bioassay

Test Parameter	Test Condition
Test type	Static, non-renewal
Test duration	48 h
Temperature	25 ± 1°C
Light quality	cool-white fluorescent tube lighting
Light intensity	800 ± 160 Lux
Photoperiod	16 h light : 8 h dark
Test chamber size	50 mL beacker
Test solution volume	25 mL
Age of test organisms	Less than 24 h old
No. of organisms per replicate	5
No. of replicates per treatment	3
No. of organisms per treatment	15
Feeding regime	None
Dilution water	QA: Demineralised water (DMW) prepared by mixing 20% Perrier mineral water with deionised water) Samples: River Murray water
Test concentrations	7
Control treatments	DMW and River Murray water
Endpoint	Immobilisation
Test acceptability criterion	≥ 90% survival in controls. Reference toxicant EC50 within Cusum chart control limits

Table 2. Summary of the test condition for the chronic *Ceriodaphnia dubia* reproduction bioassay

Test Parameter	Test Condition
Test type	Semi-static (renewal every 48 h)
Test duration	9 d
Temperature	25 ± 1°C
Light quality	cool-white fluorescent tube lighting
Light intensity	800 ± 160 Lux
Photoperiod	16 h light : 8 h dark
Test chamber size	200 mL beaker
Test solution volume	100 mL
Renewal of test solutions	Four times, every alternate day(days 2, 4, 6and 8)
Age of test organisms	Less than 24-h old
No. of organisms per replicate	1
No. of replicates per treatment	10
No. of organisms per treatment	10
Feeding regime	Fed *Pseudokirchneriella subcapitata* on days 2, 4, 6 and 8
Dilution water	QA: Deminalised water (DMW) prepared by mixing 20% Perrier mineral water with deionised water Samples: River Murray water
Test concentrations	7
Control treatments	MHW and River Murray water
Endpoint	Number of young generated
Test acceptability criterion	≥ 80% survival of original daphnids in the control treatment. Reference toxicant EC50 within Cusum chart control limits. Control treatments with ≥15 neonates in three broods

2.4.2 SHRIMP SURVIVAL AND OXIDATIVE STRESS RESPONSE

This acute test measures the survival of *Paratya australiensis* shrimp over a 96-h exposure to drainage waters. The test is described in Kumar et al (2010) (Table 3). After 96 h, oxidative stress in *P. australiensis* was also assessed.

The freshwater shrimp *Paratya australiensis* were obtained from Aquablue Seafood, NSW, and acclimated in 60-L aquariums and feed twice daily (fish wafers and Hikari Tropical® sinking wafers) for at least two weeks prior to use in toxicity tests.

The test was undertaken in 1-L borosilicate glass beakers containing 800 mL (per replicate) of test solution with each concentration prepared in duplicate. Two controls (MHW and River Murray water) were also prepared in duplicate. The shrimps were isolated at random and transferred to the test solutions using a fish net. Ten shrimps were added to each test vessel and incubated at a temperature of 21 ± 1°C on a 16 h light and 8 h dark cycle for 96 h. Each test vessel was examined at 48 h and 96 h for shrimp mortality. In addition, test solutions were renewed at 48 h. Death was assumed when animals lost orientation and there was no movement of the legs or scaphognathite. The pH, conductivity and dissolved oxygen were measured in each treatment at the beginning and end of the test and when test solutions were renewed. Toora and Wellington samples were tested on October 29 and samples Woods Point and Mobilong were tested on November 5.

Greater than 90% survival in the control is required to achieve minimum acceptability. Due to limited number of test animals available, a reference toxicant test was not able to be carried out with the drainage water tests and duplicate replicates were utilised in place of the standard triplicate replicates for each treatment tested.

The 96-h EC10 and EC50 estimates were determined with the non-linear interpolation method or with the maximum Likelihood-Probit method using ToxCalc v5.0 (Tidepool Scientific Software). The highest concentration of sample tested that caused no significant toxicity (NOEC) and the lowest concentration of test material causing significant toxicity (LOEC) were determined with Dunnett's test, Steels Many-One Rank test or Bonferroni test.

Shrimp that were alive after 96 hours exposures to the drainage water were processed for oxidative stress enzyme analyses. Shrimp exposed to river water for 96 h were used as controls. All data were presented as mean±standard deviation. Briefly, frozen shrimp were defrosted on ice, homogenised in buffer (0.1M KH_2PO_4, pH 7.4) and centrifuged at 10,000 g for 20 min at 4^oC. These homogenates were then used to measure GST and GR, activities in assays adapted for use with a microplate reader (*Thermo Multiskan Ascent" UV plate reader*). Protein content was determined according to Bradford (1976) using bovine serum albumin as standard. Absorbance was recorded at 595 nm.

Glutathione reductase (GR): Principle of the assay is based on the reduction of GSSG (oxidised Glutathione form) by β-Nicotinamide adenine dinucleotide phosphate reduced form (NADPH) in the presence of glutathione reductase. The activity is measured by the increase in absorbance caused by the reduction of 5'-dithiobis(2-nitrobenzoic acid) [DTNB] at 405 nm. GR activity was expressed in mU mg^{-1} protein (Smith, I.K., *et al.*, Analyt. Biochem., 175, 408-413 (1988)). GST activity was assayed at 340 nm by measuring the increase in absorbance using 1-chloro-2,4-dinitrobenzene (CDNB) as the substrate according to Habig et al. (1974). One unit of GST activity was defined as the formation of 1 µmol of conjugated product per minute. The extinction coefficient 9.6 $mM\text{-}^1cm^{-1}$ of CDNB was used for the calculation.GST activity was expressed in mU mg^{-1} protein.

Table 3. Summary of test conditions for the shrimp *Paratya australiensis* survival bioassay

Test Parameter	Test Condition
Test type	Semi-static
Test duration	96 h
Temperature	21 ± 1^oC
Light quality	cool-white fluorescent tube lighting
Light intensity	800 ± 160 Lux
Photoperiod	16 h light : 8 h dark
Test chamber size	1000 mL
Test solution volume	800 mL
Renewal of test solutions	Once (48 h)
Age/size of test organisms	1-4 cm
No. of organisms per replicate	10
No. of replicates per treatment	2
No. of organisms per treatment	20
Feeding regime	Shrimp not fed during exposure period
Test chamber cleaning	Not required
Test chamber aeration	Aeration provided
Dilution water	QA: Moderately hard water (MHW, 230 mg $CaCO_3$/L) Samples: River Murray water
Test concentrations	7
Control treatments	MHW and River Murray water
Endpoint	Survival – movement observed
Test acceptability criterion	$\leq$10% mortality in controls; Dissolved oxygen >60%

2.4.3 FISH SURVIVAL, GROWTH AND MALFORMATIONS TESTS

This sub-chronic toxicity test measures the number of imbalanced (loss of ability to balance) aquacultured Murray Cod fish *Maccullochella peelii*, after exposure to drainage water for 7 d. Growth and observations of malformations were also measured to identify the effect of drainage water on fish early life development. The toxicity test was based on the methods of OECD Guideline 204 (1984b) and summarised in Table 4.

Larval fish of *M. peelii* were obtained and toxicity tests undertaken in 300-mL beakers containing 100 mL test solution. Eight to ten concentrations of each drainage water sample was prepared by dilution with River Murray water (0.25-100%). Controls consisting of River Murray water from two separate carboys (A and B) and 50% River Murray water diluted with MHW were also prepared. Each treatment was prepared in triplicate and ten fish fry were randomly added to each test vessel. Test vessels were incubated at 21 ± 1˚C on a 16 h light:8h dark light cycle. Test solutions were renewed at 48, 96 and 144 h by replacing the test solution with freshly diluted drainage water and the number of surviving (imbalanced) fish counted. After 2, 4 and 7 days, dead fish were removed from test vessels and preserved for growth and malformations measurements. Water quality parameters (pH, conductivity and DO) were also measured. The test was terminated after 7 days. Fish were euthanased by the addition of 1MS222 (ethyl 3-aminobenzoate methanesulfonate (Sigma) and immediately fixed in 10% buffered formalin. Toxicity tests with fish were carried out on 27 October (all samples). The test was acceptable if there was ≥90% balanced fish fry in the controls.

The 7-d LC10 and LC50 estimates were determined with the non-linear interpolation method using ToxCalc v5.0 (Tidepool Scientific Software). The highest concentration of sample tested causing no significant toxicity (NOEC) and the lowest concentration of test material causing significant toxicity (LOEC) were determined by the Steels Many-One Rank test.

Table 4. Summary of test conditions for the fish *Maccullochella peelii* (aquacultured) survival test

Test Parameter	Test Condition
Test type	Static non-renewal
Test duration	7 d
Temperature	21 ± 1°C
Light quality	cool-white fluorescent tube lighting
Light intensity	800 ± 160 Lux
Photoperiod	16 h light : 8 h dark
Test chamber size	300 mL
Test solution volume	100 mL
Renewal of test solutions	Days 2 4 and 6
Age of test organisms	Larval stage with yolk sac
No. of organisms per replicate	10
No. of replicates per treatment	3
No. of organisms per treatment	30
Feeding regime	Fish larvae not fed during exposure period as yolk sac provides nutrition to the growing fish larvae
Test chamber cleaning	Not required
Test chamber aeration	Not required
Dilution water	River Murray water
Test concentrations	8-10
Control treatments	MHW and River Murray water (undiluted and 50% dilution with MHW)
Endpoint	Survival (Imbalance – loss of swimming ability) and growth as length measurements
Test acceptability criterion	$\leq$10% imbalance in controls

2.4.4 MIDGE SURVIVAL AND LARVAL DEVELOPMENT TEST – SEDIMENT TOXICITY TEST

The acute and chronic toxicity of drainage channel sediment to the midge, *Chironomus tepperi* were assessed. Survival and growth of midge larvae after 5 days and and adult emergence and sex-ratios (chronic) was measured over 10 days and test methods are summarised in Table 5.

Larvae from aquaria-raised midges were used for the toxicity tests. Five days prior to testing, egg masses were collected from cultures maintained at CSIRO, Adelaide, and placed in 1 L beakers (2 egg masses/beaker) with 800 mL of moderately hard water (MHW: 220 - 300 µS/cm, pH 6.9 to 7.9, DO >60%) containing 7.5 g of artificial substrate (shredded tissue). Over the next 5 days, egg masses in these beakers were aerated continuously, fed twice with ground fish flakes (4 g/100 mL), and incubated under constant temperature conditions (21 ± 1°C) with a 16:8 h light:dark photoperiod using cool-white fluorescent lamps (10-20 µmol photons/s/m^2). Five-day-old larvae at second instar larval stage were used for testing. The cultures were considered suitable for use in toxicity tests if they provided a constant supply of larvae, if the larvae were healthy and behaved normally, and if mortality was ≤ 10%. A copper reference toxicity test (water only exposures) was run at the start of whole sediment bioassays using the same batch of larvae for 48 h to ensure their good health.

For the growth and survival bioassay, ten 5-d old midge larvae were added to each replicate beaker containing ca. 140 g (wet weight) of 425 µm sieved sediment and 200 mL MHW (or River Murray water), with 4 replicates per treatment. Each beaker was maintained under the conditions described above. After 5 d, and prior to pupation, midge larvae from each replicate were removed by sieving the sediments and collecting live midge larvae. These larvae were fixed in 10% buffered formalin and processed for their length measurements using the image analyses system. Larval development (that is, emergence from sediment) was determined after ten 5-d old midge larvae were added to beakers containing ca. 140 g (wet weight) of 425 µm sieved sediment and 200 mL MHW (4 replicates per treatment). Beakers were incubated for 10 d at 21˚C (16:8 h light:dark) and the number of emerging adult *C. tepperi,* and their sex, was measured daily.

The pH and electrical conductivity were measured at the beginning and end of the bioassay, while DO and temperature in the test solutions were measured daily.

The 5-d growth and survival and, 10-d larval development test endpoints were determined with the non-linear interpolation method using ToxCalc v5.0 (Tidepool Scientific Software). The highest concentration of sample tested causing no significant toxicity (NOEC) and the lowest concentration of test material causing significant toxicity (LOEC) were determined by the Steels Many-One Rank test.

A reference toxicant test, copper, was also carried out using *C. tepperi* larvae from the same batch of cultures used in the sediment bioassay.

Table 5. Summary of test conditions for the midge *Chironomus tepperi* bioassays

Test Parameter	Test Condition
Test type	Semi-static- renewal of overlying water
Test duration	Survival and growth: 5 d Larval development: 10 d
Temperature	21 ± 1°C
Light quality	cool-white fluorescent tube lighting
Light intensity	800 ± 160 Lux
Photoperiod	16 h light : 8 h dark
Test chamber size	400 mL
Test solution volume	140 g sediment plus 200 mL MHW
Age of test organisms	Second instar larvae
No. of organisms per replicate	10
No. of replicates per treatment	8 (4 for growth and survival and 4 for emergence and sex-ratios)
No. of organisms per treatment	80 (40 for growth and survival and 40 for emergence and sex-ratios)
Feeding regime	Midges not fed during exposure period
Test chamber aeration	Aeration provided
Dilution water/overlying water	Moderately hard water (MHW 140-150 mg $CaCO_3$/L)
Control sediment	River Murray sediment
Test concentrations	none
Endpoint	Acute: survival Chronic: 5-day larval growth and development as emergence over 10 days
Test acceptability criterion	≥80% survival in controls; Reference toxicant LC50 within cusum limits

3 Results and Discussion

3.1 Characterisation of River Murray water and acid drainage water

The drainage waters had a pH of 3.6 to 5.5 and acidity of 19 to 361 mg $CaCO_3$/L. Specific electrical conductivity was 14 and 27 mS/cm in the Toora and Mobilong drainage water respectively and 4 to 5 mS/cm at the two Jervois sites (Woods Point and Wellington) (Table 6). Acidity ranged from 19 to 361 mg $CaCO_3$/L with Mobilong having the highest acidity value (Table 7).

Total ammonia concentrations ranged from 1.9 to 5.1 mg N/L, exceeding the water quality guideline (WQG) trigger value (TV) of 0.9 mg N/L. DOC ranged from 6 to 17 mg/L (Table 8).

In general, an increase in water hardness results in a decrease in bioavailability of some metals (including Cd, Cr(III), Cu, Pb, Ni and Zn). Hence, WQG TVs were also calculated for a water hardness equivalent to the water hardness of the River Murray water (~50 mg CaCO3/L). Concentrations of dissolved Al, Co, Ni, Zn and Mn exceeded WQG TVs (for hardness correction to 60 mg $CaCO_3$/L) at each of the four sites, while concentrations of As and Cd only exceeded TVs at Mobilong and Toora (Table 9). Acidic drainage water from Mobilong had the highest concentrations of metals and metalloids, except for copper. Jervois (Woods Point) had the highest copper concentration (8.1 µg/L) followed by Mobilong (4.6 µg/L). Water from Toora contained the second highest concentration of metals and metalloids. The Jervois sites (Woods Point and Wellington), had relatively similar metal and metalloid concentrations with aluminium showing the greatest difference between the two sites (Jervois-Wellington has an aluminium concentration 2.3 times higher than that for Jervois-Woods Point).

Concentrations of dissolved metals and metalloids (Ag, As, Cd, Co, Cr, Cu, Ni, Pb, Zn and Mn) in the River Murray water were below (or for copper, equal to) WQG TV (for waters of 30 mg $CaCO_3$/L) and guideline concentrations corrected for a water hardness of 60 mg CaCO3/L (Table 9). The exception to this was aluminium.

Concentrations of metals and metalloids in River Murray water were measured in water collected via the pump and by hand (without a pump) at the Thiele Reserve boat ramp. Concentrations of Co, Cr, Zn and Fe were also slightly elevated in River Murray water collected using the pump however the concentrations were low and below WQG TVs. Surprisingly, aluminium concentrations in river water collected via the pump (630 µg/L) was 25 times higher than that measured in river water collected by hand (25 µg/L). In light of this finding, all of the remaining river water sub-samples collected in 20-L carboys via the pump was measured for metals to determine if aluminium concentrations were in fact elevated due to the use of the pump to collect the river water. Analysis of River Murray water from the remaining 22 carboys collected for use in the toxicity tests showed that aluminium concentrations were in a suitable range (58 ± 21 µg/L). The aluminium concentrations in the control waters (river water) in each toxicity test ranged from 48 to 800 µg/L (results of the metal analysis are presented in Appendix B). Despite the high concentration of aluminium in the control waters in some of the toxicity tests, the response of cladocerans, shrimp and fish in control water still met test acceptability criteria (see section 3.2.2). In summary, the collection of river water via the centrifugal pump is not expected to confound the ecotoxicological results obtained in this study however an alternative method to collect large volumes of river water should be considered in future studies.

Table 6. Locations and physico-chemical characteristics of the LMRIA drainage waters (at collection)

Site	Location	Location		Date	Time	pH	Temp	EC	SPC	ORP	DO	DO	TDS
		Easting	Northing				°C	µS/cm	µS/cm	mV	mg/L	%	ppk
RIVER MURRAY	Thiele Reserve (boat ramp)	343142	6113958	22/10/12	14:30	–	–	–	–	–	–	–	–
TOORA	Toora	345457	6116019	22/10/12	13:10	5.41	22.2	13400	14200	80	4.2	50	9.2
MOBILONG	Mobilong	344229	6129757	22/10/12	13:35	3.55	23.1	25700	26700	430	4.4	57	17
JERVOIS (WOODS POINT)	Jervois	355752	6099978	22/10/12	12:00	4.89	21.6	4910	5124	140	3.4	41	3.3
JERVOIS (WELLINGTON)	Jervois	354678	6092698	22/10/12	11:10	5.46	21.5	3940	4240	23	2.5	29	2.7

GIS Locations: (GDA84, Zone 54). NR = not reported. NA = not applicable.

Table 7. Concentrations of alkalinity, acidity, major anions and cations in acid drainage water

Site	Alkalinity mg $CaCO_3$/L	Acidity mg $CaCO_3$/L	Chloride mg/L	Sulfate mg/L	Sodium mg/L	Potassium mg/L	Calcium mg/L	Magnesium mg/L
RIVER MURRAY	54	2	20	7	18	4	10	6
TOORA	<1	99	3730	3420	2230	85	464	552
MOBILONG	<1	361	8040	3920	4600	88	700	845
JERVOIS (WOODS POINT)	27	19	804	1490	640	26	206	204
JERVOIS (WELLINGTON)	<1	42	818	967	612	20	140	137

Filtered (<0.45 µm). Alkalinity (total) = bicarbonate alkalinity (hydroxide and carbonate alkalinity <1 mg/L).

Table 8. Concentrations of nitrogen, phosphate and total organic carbon in acid drainage water

Site	Total Nitrogen mg N/L	Total Kjeldahl-N mg N/L	Nitrate+Nitrite mg N/L	Nitrate mg N/L	Total Ammonia mg N/L	Unionised Ammonia µg N/L (@22˚C)	Unionised Ammonia µg N/L (@22˚C)	Reactive Phosphorus mg P/L	DOC mg/L
RIVER MURRAY	0.4	0.4	<0.01	<0.01	0.02	–	$9.1x10^{-4}$ @ pH 8.0	0.02	5
TOORA	5.3	5.0	0.05	0.05	3.56	0.43 @ pH 5.41	0.16 @ pH 8.0	<0.01	13
MOBILONG	3.6	3.6	0.28	0.28	5.10	0.008 @ pH 3.55	0.23 @ pH 8.0	<0.01	17
JERVOIS (WOODS POINT)	3.3	3.1	0.17	0.17	2.12	0.075 @ pH 4.89	0.096 @ pH 8.0	<0.01	6
JERVOIS (WELLINGTON)	3.1	3.0	0.11	0.11	1.93	0.25 @ pH 5.46	0.088 @ pH 8.0	<0.01	14

Filtered (<0.45 µm). Phosphate = reactive phosphorus.

Table 9. Concentrations of dissolved metals in acid drainage water

Site	Al	Ag	As	Cd	Co	Cr	Cu	Ni	Pb	Zn	Fe	Mn
	µg/L										mg/L	
RIVER MURRAY 1 (collected by pump)	**630**	<0.1	1.2	<0.1	0.11	0.5	1.4	1.4	0.3	2.1	0.57	0.002
RIVER MURRAY 2 (collected by hand)	25	<0.1	1.2	<0.1	0.03	<0.1	1.0	1.2	0.2	0.5	0.17	0.001
TOORA	**555**	<0.1	**390**	**0.6**	**285**	1.4	0.3	**280**	0.1	**160**	30	**4.1**
MOBILONG	**15600**	<0.1	**650**	**2.4**	**610**	3.6	**4.6**	**620**	2.7	**378**	59	**10.0**
JERVOIS (WOODS POINT)	**167**	<0.1	1.7	0.3	**94**	0.5	**8.1**	**123**	0.2	**84**	0.9	**2.4**
JERVOIS (WELLINGTON)	**390**	<0.1	1.4	0.2	**107**	0.8	0.7	**97**	0.2	**61**	1.81	**2.8**
WQG (95%PC; TV ~30 g $CaCO_3$/L) [a]	55	0.05	13	0.2	1.4	3.3	1.4	11	3.4	8	NV	1.9
WQG (hardness=60) [b]	**55**	**0.05**	**13**	**0.36**	**1.4**	**5.9**	**2.5**	**20**	**8.2**	**14**	**NV**	**1.9**

River Murray 1 = river water collected via pump

River Murray 2 = river water collected by hand

[a] WQG (95%PC) = ANZECC/ARMCANZ (2000) WQG trigger value (TV) for 95% species protection applicable to freshwaters of hardness 30 mg $CaCO_3$/L. Values provided are without hardness correction. As(V) = 13 µg/L / As(III) = 24 µg/L, Cr(VI) = 1 µg/L / Cr(III) = 3.3 µg/L. NV = no guideline value. **Blue** when >WQG trigger value.

[b] Hardness-adjusted WQGs for Cd, Cr, Cu, Ni, Pb and Zn applicable to fresh waters (Appendix C). TV = no hardness adjustment applicable and trigger value applies.

3.2 Toxicity of acid drainage water to aquatic biota

3.2.1 METAL CONCENTRATIONS IN TOXICITY TESTS

The River Murray control water, the undiluted drainage water from each site and a series of dilutions of the drainage water were analysed for dissolved metals. Dilutions selected included those that caused no-effect and complete effect on cladocerans (acute toxicity tests), shrimp and fish (Appendix A).

Undiluted drainage water from each site was analysed in the shrimp toxicity tests. These test solutions were prepared within 14 days after the samples were collected and concentrations of dissolved metals were compared to that originally measured in the acid drainage waters. For each of the four drainage water samples, concentrations of Cr, Mn, Co, Ni and Zn remained similar (within 20%) to that originally measured. However, concentrations of dissolved iron had decreased substantially in all four drainage waters, particularly in drainage water from Jervois Wellington and Woods Point (Toora, decreased by 30%; Mobilong, decreased by 37%; Woods Point, decreased by 97%, Wellington decreased by 89%). The concentrations of dissolved copper increased in all four drainage water by a factor 2 (Mobilong and Woods Point), 10 (Wellington) and 33 (Toora) resulting in copper concentrations of 7 to 15 µg Cu/L. Concentrations of arsenic decreased substantially in drainage waters that originally showed high arsenic concentrations (Mobilong and Toora) with concentrations decreasing to below the WQG value of 13 µg As/L. Aluminium concentrations in Jervois (Woods Point)water decreased by about a third from 170 to 63 µg Al/L (Al remained stable in the other three drainage waters). Cadmium increased in Woods Point water to 4 µg Cd/L. The metal decreases could be due to formation and aggregation of iron and aluminium colloids and precipitates, and scavenging of other metals on these surfaces (Simpson et al. 2013).

Where dissolved metal concentrations were higher than detection limits, the concentration of dissolved metals in diluted drainage water in each toxicity test were plotted (Toora shown in Figure 2 and Figure 3; Mobilong, Jervois (Woods Point and Wellington end) are shown in Appendix A). The metals Co, Ni and Mn showed a linear relationship, with concentrations decreasing linearly with increasing dilution of drainage water, which was consistent with the findings of Simpson et al. (2013). A relationship between copper concentrations and diluted drainage water was not observed for any of the four drainage waters, but the concentrations were generally quite low. Zinc concentrations decreased linearly with increasing drainage water in shrimp and cladoceran tests. The exception was for the fish test.

Despite undiluted drainage water having a pH of <5.5, concentrations of some dissolved metals were likely to vary over time as particulates formed. Where possible, drainage water were renewed with freshly prepared diluted drainage water after 48 or 96 h during the toxicity tests. While flow-through test designs would ensure a continuous exposure to the initial high concentrations of dissolved metals, this is difficult to achieve within the scope of traditionally designed toxicity tests. In addition, the rapid decrease in dissolved metal concentrations after mixing with River Murray water (particularly aluminium and iron) poses a challenge in providing a continuous exposure to dissolved metal contaminants. Hence renewal of test solutions was undertaken where possible. In addition, toxicity tests were carried out with unfiltered drainage water and river water to ensure metal-rich particulates were present in the toxicity test solutions. This approach mimics somewhat the situation in the LMRIA discharge zones, where periodic pumping (and exposure of aquatic organisms to contaminants) of acid drainage occurs into the River Murray where the drainage pumps are typically run at night and then switched off during the day when electricity costs are higher.

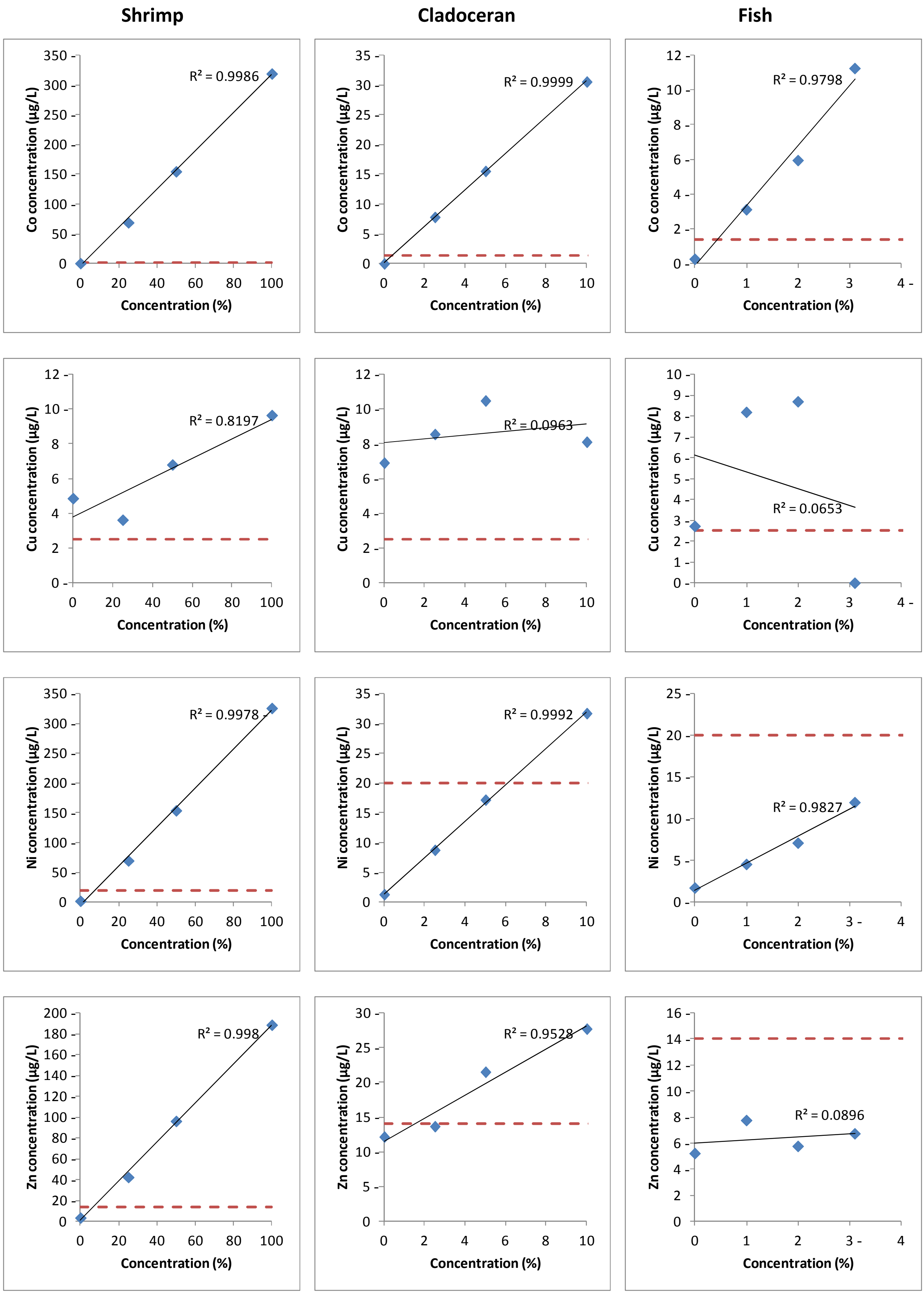


Figure 2. Relationship between concentration of Toora acid drainage water and concentration of dissolved metals (Co, Cu, Ni and Zn) in control (0%) and three concentrations of acid drainage water in toxicity tests with shrimp, cladocerans and fish. Red lines show the water quality trigger value.

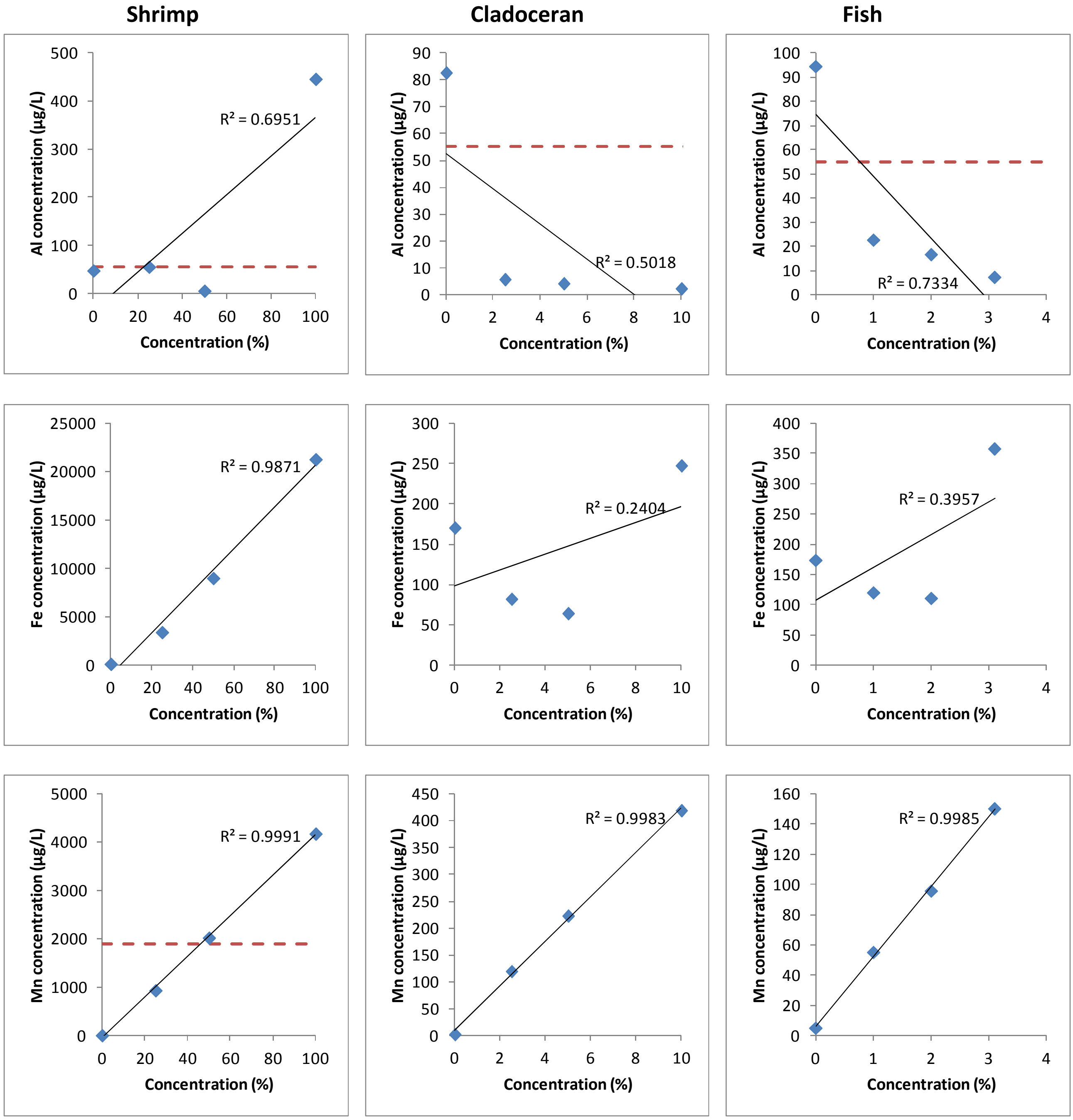


Figure 3. Relationship between concentration of Toora acid drainage water and concentration of dissolved metals (Al, Fe and Mn) in control (0%) and three concentrations of acid drainage water in toxicity test with shrimp, cladocerans and fish. Red lines show the water quality trigger value.

3.2.2 ACUTE AND CHRONIC TOXICITY TO CLADOCERAN, SHRIMP AND FISH

Quality assurance

Acute toxicity tests with the cladoceran *C. dubia* were carried out on four occasions. Mobilisation of organisms in the control (DMW) treatment ranged from 87% to 100% and, 87% to 93% in the River Murray water. The reference toxicant, copper, was toxic to *C. dubia* in each of the four toxicity tests with EC50 values ranging from 8.7 to 12 µg Cu/L.

Chronic toxicity tests with the *C. dubia* showed good reproduction with three broods (groups of offspring) produced by each adult female over 9 days in River Murray water. The average total number of young produced by each individual female was 18.2 in undiluted river water and 19 in 50% river water (diluted with DMW). An acute toxicity test with copper was run on three occasions throughout the chronic toxicity test for quality assurance purposes. The survival of *C. dubia* in control (DMW) water was ≥90% and the EC50 value for copper ranged from 7.0 to 8.7 µg Cu/L indicating that the cladocerans were healthy and responding reproducibly to a known contaminant.

Toxicity tests with the shrimp *P. australiensis* were carried out on two occasions. The survival of shrimp in the standard control (DMW) treatment was 75% and 95% while survival in River Murray water was slightly better with 95% and 100% survival respectively. While survival of shrimp in the first test did not meet the acceptable criteria of ≥90%, this was not significantly less than the survival obtained in the River Murray water (95%) indicating that the organisms used in the test were healthy. Due to the low numbers of shrimp available for use in the toxicity, only duplicate treatments were tested and a reference toxicant was not included. DO concentrations remained above 5.2 mg/L (>60% saturation).

The aquacultured fish larvae, *M. peelii*, showed excellent survival in control (DMW) and River Murray water with 93% and 93-100% survival respectively, exceeding the test criteria of ≥90%. A reference toxicant test with fish was not carried out in this study.

In light of the fact that measured dissolved Al concentrations were variable and in some cases exceeded the WQG trigger value, the response of cladocerans, shrimp and fish in River Murray control treatments were the same or exceeded (better then) the response obtained in the standard QA control treatment. This indicates that the River Murray water was not toxic to any of the organisms used in this study and hence any toxicity observed could be attributed to the acid drainage water and/or its dilution with River Murray water.

Acid drainage water

Toxicity values from toxicity test with cladocerans, shrimp and fish are shown in Table 10 with concentration-response curves shown in Figure 4. Oxidative stress measurements of glutathione reductase and glutathione-S-transferase in shrimp are also presented (Figure 5 and Figure 6) along with growth measurements on surviving fish from the fish survival test (Figure 7).

As part of a separate CSIRO investigation, additional toxicity tests were carried out on the four acid drainage waters. Toxicity tests included measuring inhibition in bacteria luminescence (Microtox, *Vibrio fischeri*), growth inhibition in the microalga *Chlorella vulgaris* and, growth inhibition in the macrophyte *Lemna minor* (duckweed). These results are shown in Table 11 and Appendix B.

Jervois - Woods Point

Acid drainage water from Jervois Woods Point was not toxic (no significant inhibition in biological endpoint measured) to shrimp survival, fish survival and growth, and, cladoceran survival. However, chronic toxicity to the cladoceran, *C. dubia*, was observed at concentrations of drainage water as low as 1.6% (1 in 63 dilutions). The pH of undiluted drainage water at the start of each toxicity test (6.5-6.8) was slightly higher than that measured at collection (4.9) however the conductivity of the drainage water remained relatively unchanged (4.6-4.8 mS/cm). The pH and conductivity of the 1.6% concentration in the cladoceran toxicity test was 7.0 and 320 µS/cm respectively and well within the species tolerances and water quality guidelines. In addition, dissolved metal concentrations at 1.6% drainage water are well below WQG trigger values for dissolved metals. Hence the 30% reduction in cladoceran reproduction observed could be attributed to other contaminants (organics) and/or metal-rich particulates that do not pass through the 0.45 µm filter and hence not measured in the dissolved metal fraction. At high concentrations of Woods Point drainage water (lower dilutions) conductivity may start to contribute to toxicity however the extent is unknown.

Of the additional toxicity tests carried out with microalgae, macrophyte and bacteria, toxicity was only observed in the microalgal test and only in undiluted drainage water. Microalgal toxicity tests are carried out on filtered (0.45 µm) water samples to ensure naturally present phytoplankton were excluded from the sample and to aid in the determination of algal growth by eliminating interfering particulate material. In undiluted drainage water, algal growth was inhibited by 30% and it is unlikely that this inhibition is due to pH (7.2) or conductivity (4.7 µS/cm) as these two parameters were within the tolerance range for *Chlorella vulgaris* (M. Adams unpublished). Hence, this small but significant inhibition in algal growth may be due to other contaminants such as metals.

Oxidative stress measurements, glutathione reductase and glutathione-S-transferase, in shrimp that survived 96 h exposures showed that neither of these stress response biomarkers were altered after exposure to drainage water at these dilutions when compared to that in the river water control treatments.

Jervois - Wellington

At the start of each toxicity test, undiluted drainage water had a pH of 4.3 to 6.4 and conductivity of 4 to 4.3 mS/cm indicating that the pH varied by about 1 pH unit higher and lower than the pH originally measured (5.5) while conductivity remained stable.

Drainage water from the Wellington site was not acutely toxic to shrimp, but toxicity was observed to cladocerans (acute and chronic toxicity) and fish survival. There was no decrease in the growth of surviving fish at drainage water concentrations of ≤2%. Acute toxicity to cladocerans (immobilisation) and chronic toxicity to fish (survival) was observed with 50% inhibition (EC50 value) at 10% and 2% drainage water respectively. Dilutions to as low as 1 to 4% drainage water (1 in 100 to 1 in 25 dilutions) showed a small but significant toxicity to cladocerans and fish which corresponds to a pH of approximately 7.5 and conductivity of around 360 µS/cm. At these dilutions, water quality parameters where within tolerance limits for cladocerans and fish and dissolved metal concentrations were below WQG TV. The exception was cobalt which at 4% would be 4 µg/L (TV = 1.4 µg Co/L). Chronic toxicity to cladocerans was observed at even greater dilutions of drainage water (0.1% to 0.78%) and this toxicity is unlikely to be due to pH, conductivity or dissolved metals as these measurements were within (or expected to be within) tolerance limits for chronic toxicity to *C. dubia*.

Toxicity test with microalgae showed that only undiluted drainage water was toxic and caused complete inhibition in algal growth. The low pH is likely to be causing toxicity to the algae as the pH measured in undiluted drainage water was 4.3 and a pH of 4.1 is known to cause complete inhibition in algal growth. The conductivity of Wellington drainage water is within acceptable growth limits and hence unlikely to be contributing to toxicity. The toxicity observed to bacteria is also likely to be due to the low pH. Wellington drainage water was not toxic to the macrophyte (which had a pH of 6.4 at the time of testing).

Oxidative stress in shrimp exposed to Wellington drainage water was not different to that in the control treatments.

Mobilong

Undiluted drainage water from Mobilong had the lowest pH, highest conductivity and highest concentrations of dissolved metals and was toxic to cladocerans (survival and reproduction), shrimp (survival), fish (survival), microalgae (growth), macrophyte (growth) and bacteria at a range of dilutions. Mobilong drainage water was not observed to be toxic to growth of fish larvae. Toxicity is likely to be dominated by the low pH and high conductivity however some dissolved metal concentrations remained above WQG TVs when pH and conductivity alone were expected to have little influence on toxicity. This was particularly evident in the acute toxicity test with cladocerans where a 5% drainage water concentration caused approximately 50% inhibition in cladoceran survival despite the pH being within tolerance levels (7.0), conductivity was likely to be near the limit of tolerance (1.8 mS/cm) and the metals Co (~30 µg/L), Ni (~30 µg/L) and Zn (~20 µg/L) remained above WQG TVs in diluted drainage water of 1.4, 20 and 14 µg/L respectively. Chronic toxicity to cladocerans was also observed at dilutions of 0.1% to 0.8% drainage water. The pH (7.9) and conductivity (410-620 µS/cm) of diluted drainage water is likely to be within tolerance limits for the cladoceran and metal concentrations at these dilutions were well below their respective TVs (suggesting that other contaminants and/or metal-rich particulates may be contributing to the observed toxicity.

The oxidative stress measurements of glutathione reductase and glutathione-S-transferase were not affected in shrimp after exposure to Mobilong drainage water.

Toora

Undiluted drainage water from Toora used in toxicity tests had pH values of 3.5 to 4.9, lower than that originally measured at collection (5.4). The conductivity remained stable at 14 mS/cm.

For a range of dilutions, toxicity was observed to all test species and all test endpoints measured, except growth of surviving fish in the fish toxicity test. The most sensitive test was chronic toxicity to cladocerans which showed that at dilutions of 0.3% to 1.3% drainage water, contaminants are causing a reduction in cladoceran reproduction as the pH and conductivity is likely to be within tolerance limits. At these concentrations, dissolved metal concentrations are below WQG TVs, except Co (~3 µg/L; TV = 1.4 µg/L). However, at higher concentrations of drainage water (lower dilutions) high conductivity may be playing a role in toxicity to cladocerans. Hence, metal-rich particulates and/or other contaminants are likely to be causing toxicity. Toora drainage water was also quite toxic to fish with death of all fish observed after exposure to dilutions ≥3% drainage water after only 48 h. The pH and conductivity of drainage water was within the tolerance limits for fish and hence toxicity could be attributed to other factors. Acute toxicity to cladocerans may also be due to low pH and metals, but not conductivity. The toxicity observed to shrimp survival, microalgal growth and macrophyte growth is likely to be caused by the low pH and high conductivity of the sample.

Measurements of oxidative stress in shrimp showed that neither glutathione reductase nor glutathione-S-transferase were affected after exposure to Toora drainage water.

Summary

In light of the toxicity data generated for acid drainage water from Jervois sites (Wellington and Woods Point), Mobilong and Toora, a few main observations were identified.

Acid drainage water was toxic to at least one freshwater species for each of the four sites sampled. Woods Point was the least toxic site with toxicity only observed to cladoceran reproduction (chronic toxicity).

Acid drainage water from Jervois (Wellington) was more toxic than drainage water from Jervois (Woods Point) with toxicity observed to cladocerans (immobilisation and reproduction), fish (survival) and microalgae (growth). This was surprising considering that the pH, conductivity and concentrations of dissolved metals and ammonia were similar in both drainage water samples. This suggests that other contaminants and/or metal-rich particulates may be causing toxicity to freshwater biota in the Jervois (Wellington) drainage water. Other water quality parameters in Jervois (Woods Point) drainage water that may have protective effects (ameliorating toxicity) could also be considered.

All of the toxicity tests (except fish growth) showed toxicity to Mobilong and Toora drainage water. Mobilong drainage water was more toxic to each of the test species (and endpoints measured) than drainage water from Toora. This was expected because Mobilong had the lowest pH, highest conductivity and highest concentrations of dissolved metals. However, there was one exception; toxicity of drainage water to fish survival. The fish survival test showed that Toora drainage water was four times more toxic (EC50 = dilution to 2.2%) than drainage water from Mobilong (EC50 = 8.8%). This suggests that the fish were responding to something other than the water quality parameters mentioned above. While it is likely that metal-rich particulates were present in both drainage waters, the composition of the metal-rich particulates may be different at the different sites and this may also be the cause of the higher toxicity observed at the Toora site.

Dissolved metals, low pH and high conductivity may not be the major cause of toxicity to aquatic biota. While water quality guidelines exist for most of these individual parameters, comparison of individual dissolved metal concentrations, pH and conductivity does not take into account potential interactive effects of multiple metals and other factors that can affect the bioavailability and toxicity of contaminants to aquatic biota. In addition, the toxicity of precipitates in neutralised (diluted) drainage waters may be a significant cause of toxicity to some aquatic biota. This is especially the case for fish where studies have shown that aluminium particulates are toxic by interfering with fish gills (Gensemer and Playle 1999). While sediment quality guidelines are available for some individual metals, these cannot be applied to particulates in the water column because there are different species and routes-of-uptake in the water column compared to that in the sediments.

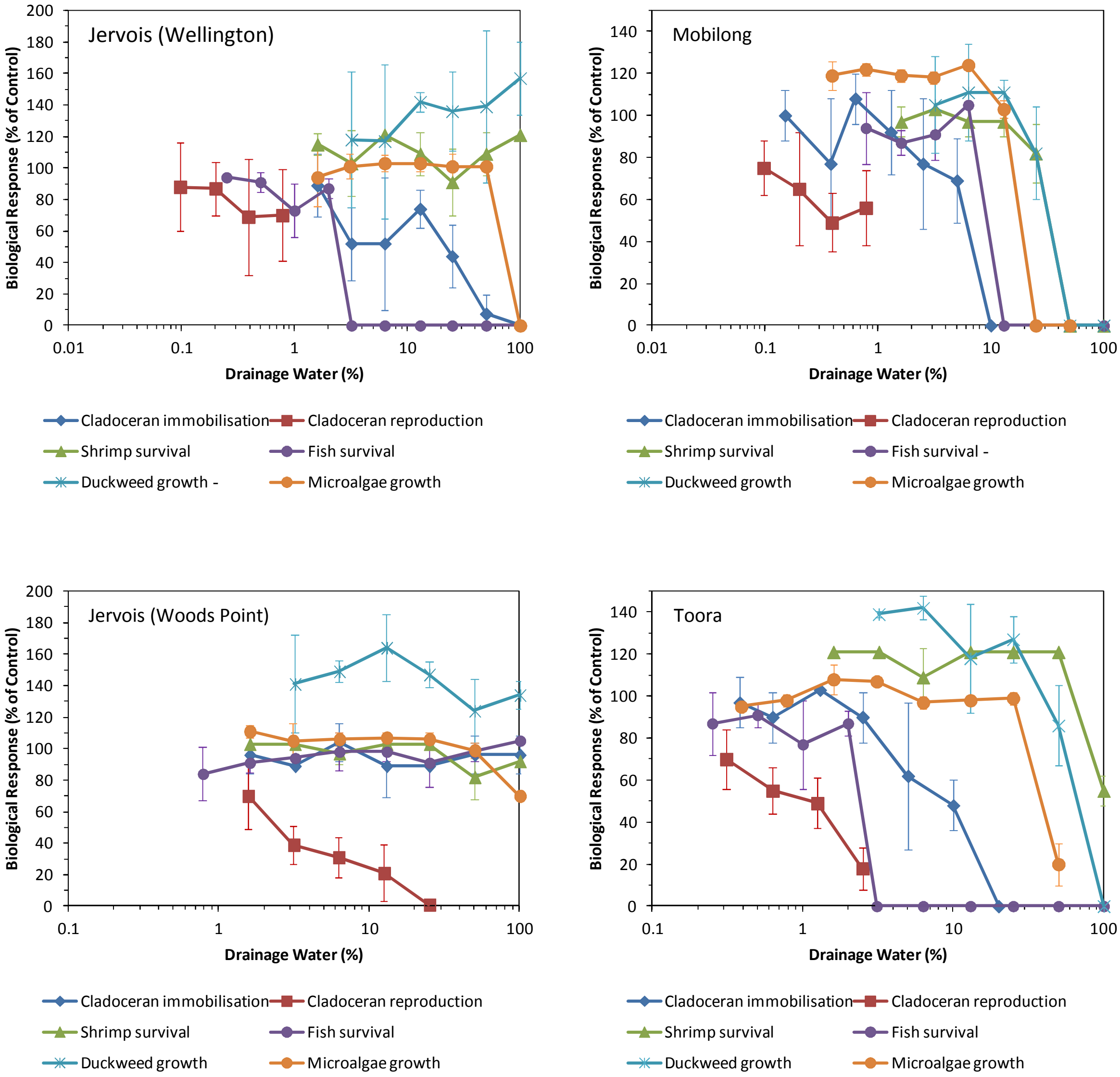


Figure 4. Concentration-response curves showing the toxicity of acid drainage water to cladocerans (immobilisation and reproduction), shrimp and fish (survival). For all curves, the control response (0% drainage water) is equal to a biological response of 100%

Table 10. Toxicity of unfiltered acidic drainage water to cladocerans, shrimp and fish

Sample/Toxicity test	Test Type	EC50[a] (%)	EC10[b] (%)	LOEC[c] (%)	NOEC[d] (%)
Jervois (Wellington)					
Cladoceran immobilisation (48 h)	Acute toxicity	10 (0.02-43)	3.7	3.1	1.6
Cladoceran reproduction (9 d)	Chronic toxicity	NC	0.083 (0.04-0.33)	0.78	0.39
Shrimp survival (5 d)	Acute toxicity	>100	>100	>100	100
Shrimp oxidative stress (5 d)	Biomarker	>100			
Fish survival (7 d)	Chronic toxicity	2.0 (1.6-2.3)	CND	1	0.5
Fish growth (7 d)	Chronic toxicity	>100			
Fish malformations (7 d)	Biomarker	TBD	TBD	TBD	TBD
Jervois (Woods Point)					
Cladoceran immobilisation (48 h)	Acute toxicity	>100	>100	>100	100
Cladoceran reproduction (9 d)	Chronic toxicity	2.6 (2.1-3.1)	0.52 (0.35-0.95)	1.6	<1.6
Shrimp survival (5 d)	Acute toxicity	>100	67[#]	>100	100
Shrimp oxidative stress (5 d)	Biomarker	>100			
Fish survival (7 d)	Chronic toxicity	>100	>100	>100	100
Fish growth (7 d)	Chronic toxicity	>100			
Fish malformations (7 d)	Biomarker	TBD	TBD	TBD	TBD
Mobilong					
Cladoceran immobilisation (48 h)	Acute toxicity	4.2 (3.2-5.5)	CND	10	5
Cladoceran reproduction (9 d)	Chronic toxicity	NC	0.038 (0.028-0.083)	0.097	<0.097
Shrimp survival (5 d)	Acute toxicity	30 (26-35)	25[#]	25	13
Shrimp oxidative stress (5 d)	Biomarker	>100			
Fish survival (7 d)	Chronic toxicity	8.8 (8.5-9.2)	CND	13	6.3
Fish growth (7 d)	Chronic toxicity	>100			
Fish malformations (7 d)	Biomarker	TBD	TBD	TBD	TBD
Toora					
Cladoceran immobilisation (48 h)	Acute toxicity	7.4 (5.0-10)	3.7 (1.1-4.7)	10	5
Cladoceran reproduction (9 d)	Chronic toxicity	1.1 (0.6-1.5)	0.10 (0.08-0.19)	0.31	<0.31
Shrimp survival (5 d)	Acute toxicity	96 (73->100)	CND	100	50
Shrimp oxidative stress (5 d)	Biomarker	>100			
Fish survival (7 d)	Chronic toxicity	2.2 (1.8-2.6)	CND	3.1	2
Fish growth (7 d)	Chronic toxicity	>100			
Fish malformations (7 d)	Biomarker	TBD	TBD	TBD	TBD

[a] Concentration of effluent to cause 50% effect/inhibition
[b] Concentration of effluent to cause 10% effect/inhibition
[c]. lowest concentration tested that caused a significant (p≤0.05) effect
[d] highest concentration tested that caused no significant (p≤0.05) effect
[e] dilution required to achieve 'no effect' concentration (using NOEC values)
[#] Poor reliability (due to lack of concentration dependant response or an all-or-nothing response curve)
TBD = To be determined; CND = Could not be determined, or reliable value could not be extrapolated; NC = Not calculated (death of cladocerans observed at concentrations greater than 0.78%)

Table 11. Toxicity of acid drainage water to bacteria, microalgae and a macrophyte

Sample/Toxicity test	Test Type	EC50[a] (%)	EC10[b] (%)	LOEC[c] (%)	NOEC[d] (%)
Jervois (Wellington)					
Microtox (bacterial luminescence)	Acute toxicity	5-min >83	22 (14-33)	21	10
		15-min >83	46 (41-51)	42	21
Microalgal growth inhibition[a]	Chronic toxicity	75	55	100	50
Macrophyte growth inhibition	Chronic toxicity	>100	>100	>100	100
Jervois (Woods Point)					
Microtox (bacterial luminescence)	Acute toxicity	5-min >83	45	83	42
		15-min >83	>83	>83	83
Microalgal growth inhibition	Chronic toxicity	>100	57 (35-66)	100	50
Macrophyte growth inhibition	Chronic toxicity	>100	>100	>100	100
Mobilong					
Microtox (bacterial luminescence)	Acute toxicity	5-min 14 (13-14)	3.4 (1.0-5.0)	2.8	1.4
		15-min 15 (14-16)	5.7 (0.1-8.5)	1.4	0.69
Microalgal growth inhibition	Chronic toxicity	18 (18-19)	12 (9.7-14)	25	13
Macrophyte growth inhibition	Chronic toxicity	34 (27-38)	18 (11-29)	50	25
Toora					
Microtox (bacterial luminescence)	Acute toxicity	5-min 48 (44-51)	20 (15-26)	22	11
		15-min 54 (53-56)	26 (21-29)	43	22
Microalgal growth inhibition	Chronic toxicity	40 (38-44)	27 (26-28)	50	25
Macrophyte growth inhibition	Chronic toxicity	63 (55-71)	31 (0-36)	100	50

[a] Microalgal toxicity tests were carried out on filtered (0.45 µm) water samples diluted with filtered (0.45 µm) River Murray water.

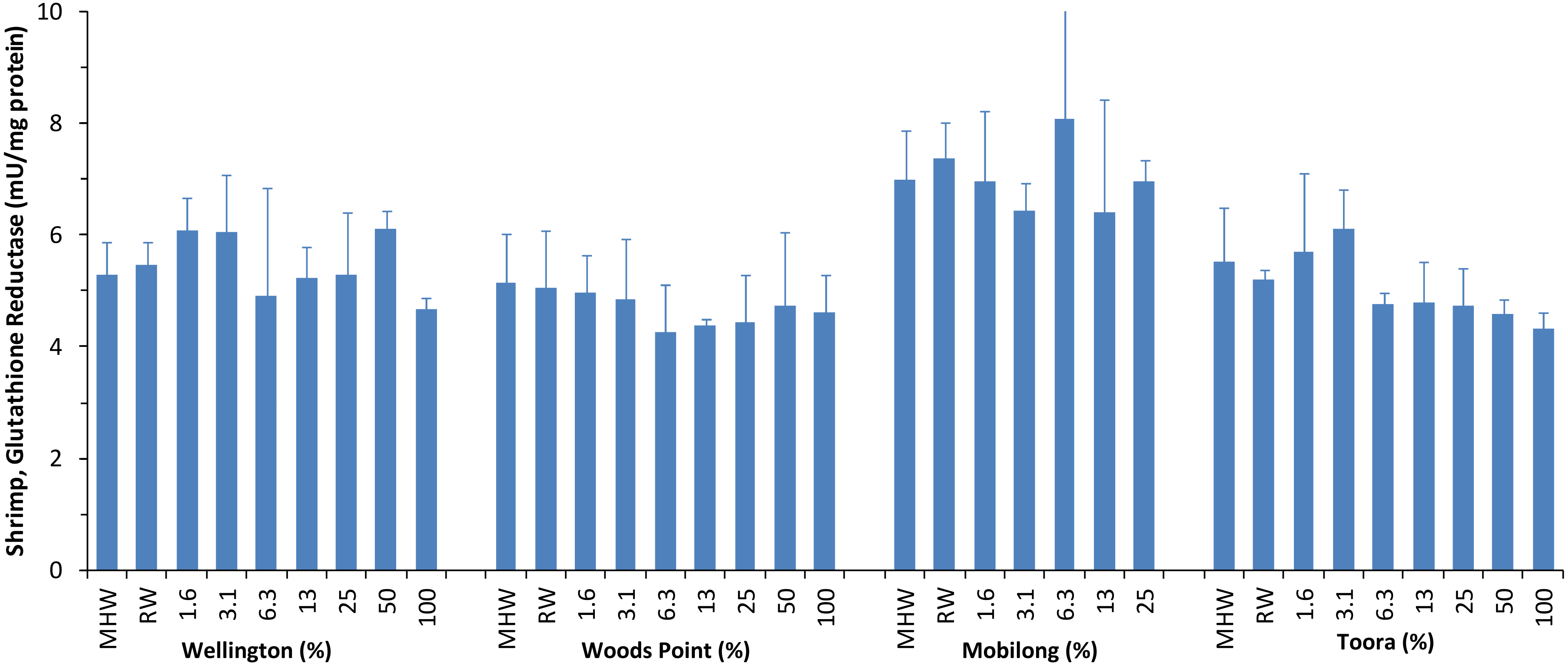


Figure 5. Oxidative stress (glutathione reductase) in the shrimp *Paratya australiensis* after a 96-h exposure to acid drainage water. MHW = moderately hard water; RW = River Murray water; Error bars represent 1 standard deviation

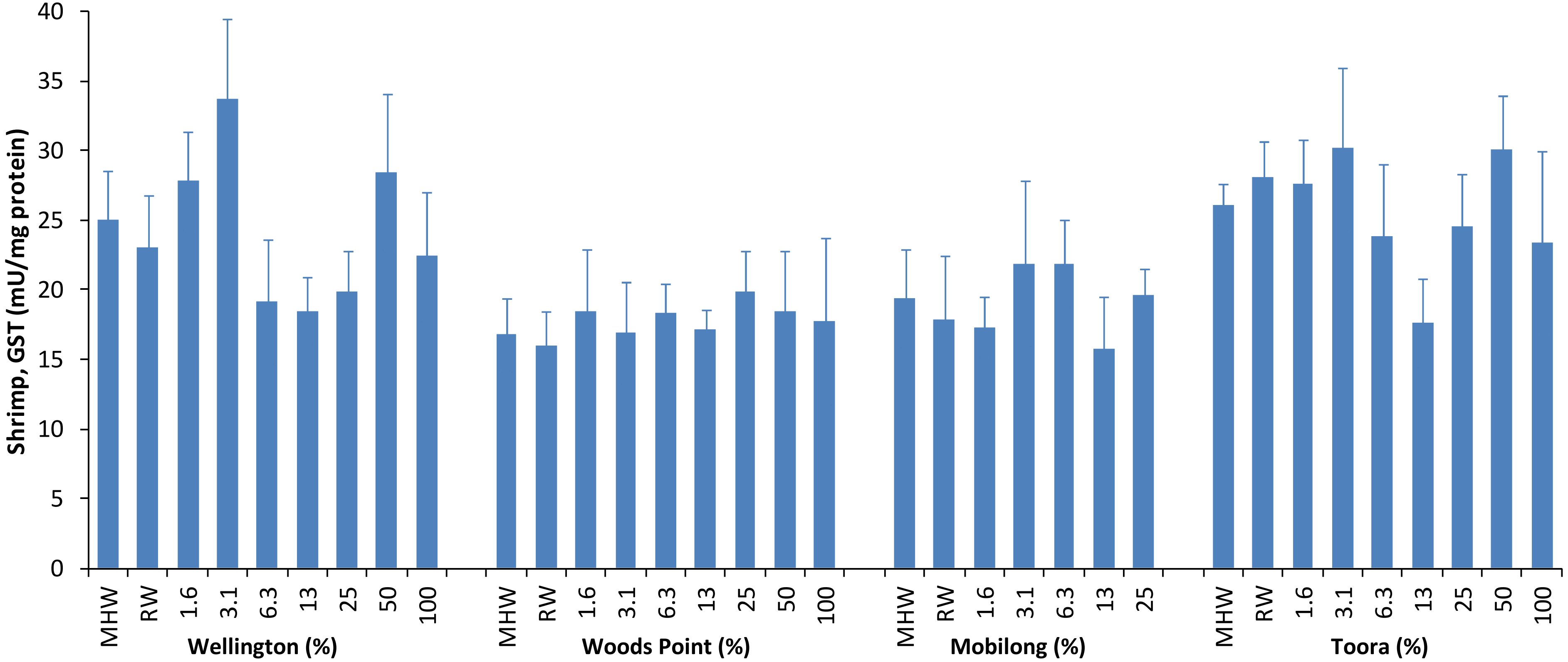


Figure 6. Oxidative stress (glutathione-S-transferase) in the shrimp *Paratya australiensis* after a 96-h exposure to acid drainage water. MHW = moderately hard water; RW = River Murray water; Error bars represent 1 standard deviation

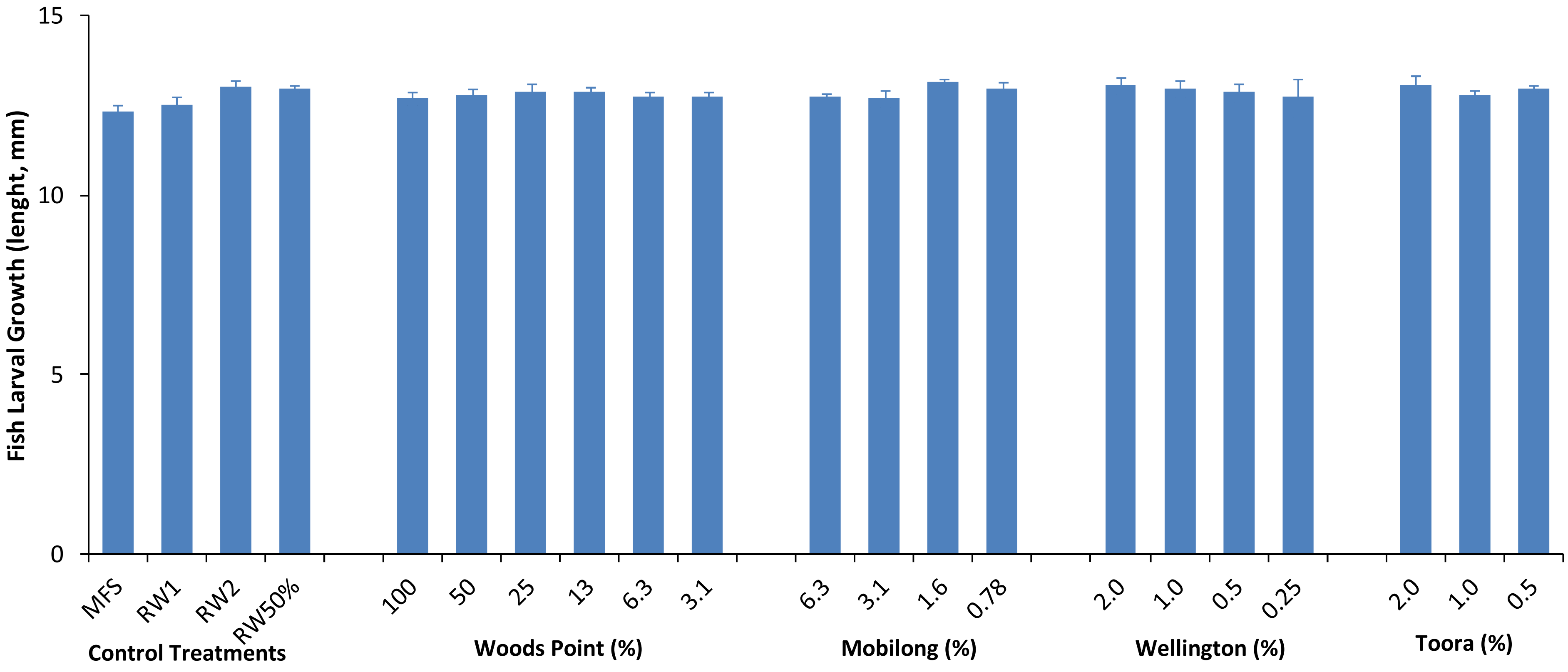


Figure 7. Toxicity of acid drainage water to the growth of surviving Murray Cod after 7 days. Error bars represent 1 standard deviation.

3.3 - Toxicity of metal-rich precipitates from the Jervois Wellington site

3.3.1 METAL CONCENTRATIONS IN JERVOIS SEDIMENT AND PRECIPITATES

Sediments/precipitates from near the Jervois-Wellington drain at the T-Junction (pH 6) and at Wellington 1 (pH 6.8) were selected for use in the midge survival and larval development test. Reference sediment was collected from upstream in the River Murray. Concentrations of metals in sediment/precipitate are shown in (Table 12). The concentration of nickel in the T-Junction (63 mg/kg) was the only metal concentration exceeding the sediment quality trigger value and ISQG-High value (52 mg/kg).

Table 12. Dilute acid-extractable metal concentrations and pH of the Jervois-Wellington drain particulates and precipitates (collected 4 April, 2013)

Sample	Al	As	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Sn	V	Zn	pH
							mg/kg							
Jervois - Drain: T-Junction Precipitate (A) Dig. (Avg) -	5220	4.9	1.5	27	2.3	12	40000	96	**63**	9.7	0.0	32	104	6.0 -
Jervois - Wellington 1 Drain - 16/4/13 Dig. (Avg) -	1850	13	<1	21	1.2	16	12000	1090	13	13	1.7	44	39	6.8 -
Trigger value (TV) [a]	NV	20	1.5	NV	80	65	NV	NV	21	50	5	NV	200	NA -
ISQG-High [b]	NV	70	10	NV	370	270	NV	NV	52	220	70	NV	410	NA -

[a] Trigger value (TV) = ANZECC/ARMCANZ (2000) SQG-low trigger value (TV) for 95% species protection. Blue when >SQG trigger value.

[b] ISQG-High = ANZECC/ARMCANZ (2000) SQG-high trigger value (TV) for 95% species protection. Red when > SQG-high value. NV = no guideline value.

3.3.2 TOXICITY OF PRECIPITATES/SEDIMENTS TO THE MIDGE *CHIRONOMUS TEPPERI*

The T-Junction and the Wellington-1 drain sediment/precipitates were acutely toxic to midge with a small but significant ($p \leq 0.05$) decrease in growth (89% of control) and survival (75% of control) respectively (Table 13). A high level of chronic toxicity (Figure 8) was observed with emergence of midge significantly reduced in the T-Junction drain sediment/precipitates. The Wellington-1 drain particulates were even more toxic and the ratio of male to female organisms was significantly altered.

For metals were TVs are available, only concentrations of acid –extractable Ni exceeded the TV and ISQG-High value. The cause of the reduced midge growth in T-Junction precipitates and decrease in survival of midge in the Wellington-1 drain sediment is uncertain. The precipitates were orange-brown and x-ray diffraction results indicated they were predominantly comprised of the Fe oxyhydroxysulfate mineral, schwertmannite. However, iron, aluminium and manganese are generally of low toxicity in sediments to benthic biota; hence the lack of a sediment trigger value for these metals. Given that a high proportion of total metals (in particular Al, Fe and Mn) in acid drainage sediments and precipitates (Simpson et al., 2013) is present in a form that is easily extracted with acid (acid extractable metal), they cannot be ruled out as a possible contributor to the observed toxicity to midge.

Table 13. Toxicity of Jervois Wellington T-Junction Precipitate and Drain sediment to the midge *Chironomus tepperi*

Sample	Acute Toxicity	
	5-d Survival (%)	5-d Growth (length, µm)
Control – Murray River (Upstream)	100 ± 0	15.0 ± 1.5
Jervois T-Junction Drain Precipitate	95 ± 6	13.3 ± 2.3[a]
Jervois Wellington Drain	75 ± 10[a]	15.3 ± 1.8

[a] significantly less than the control (p≤0.05)

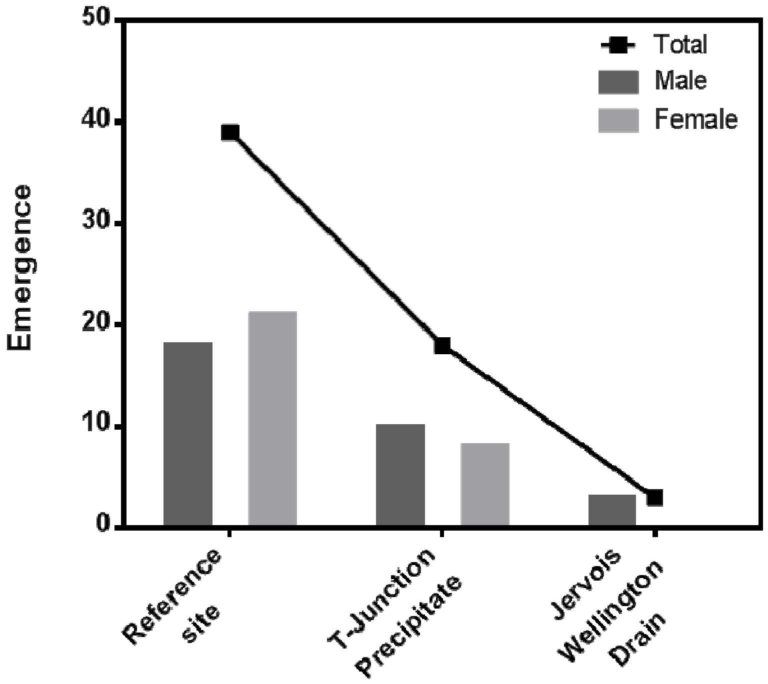


Figure 8. Chronic toxicity of River Murray sediment, Jervois Wellington T-Junction precipitates and drain particulates to the midge *Chironomus tepperi* after 10 days: reproduction (emergence) and sex

Quality assurance

Midge survival in the control after 5 days was 100% indicating that the midge used in the experiments were healthy.

4 Summary and Conclusions

- Concentrations of dissolved Al, Co, Ni, Zn and Mn exceeded WQG TVs (for hardness correction to 60 mg $CaCO_3$/L) at each of the four sites while concentrations of As and Cd only exceeded TVs at Mobilong and Toora.

- The drainage waters from all four sites were toxic to at least one freshwater species. The drainage water from Woods Point was the least toxic, with toxicity only observed to cladoceran reproduction (chronic toxicity).

- The drainage water from Jervois at Wellington was more toxic than Jervois at Woods Point, despite similar dissolved metal concentrations, pH and conductivity. Hence, contaminants other than these are causing toxicity to aquatic biota or, water quality characteristics in Jervois (Woods Point) is ameliorating toxicity to aquatic biota.

- All of the toxicity tests (except fish growth) showed toxicity to Mobilong and Toora drainage water. Mobilong drainage water was more toxic to each of the test species (and endpoints measured) than drainage water from Toora. However, the fish survival test showed that Toora drainage water was more toxic than drainage water from Mobilong.

- The presence and effect of other contaminants (e.g. endocrine disrupting chemicals) and metal-rich particulates in acid drainage water (and diluted acid drainage water after mixing with River Murray water) cannot be excluded as contributors to toxicity at specific sites (e.g. Wellington) and to individual species (e.g. fish, *M. peelii,* cladoceran *C. dubia*) and should be investigated further.

- Metal-rich sediments/precipitates from a drain at Jervois (Wellington end) exhibited a low level of acute toxicity to the midge, *C. tepperi*. A high level of chronic toxicity was observed with emergence of midge significantly reduced in T-Junction drain precipitate. Wellington-1 drain particulates were even more toxic and the ratio of male to female organisms was significantly altered.

5 Recommendations

- The cause of toxicity for the drainage water at Jervois (Wellington end) is unknown and further investigations should include identifying other contaminants (e.g. endocrine disrupting chemicals) and the effect of metal-rich precipitates (e.g. measure toxicity of filtered and unfiltered water) on sensitive freshwater biota (e.g. chronic toxicity to cladocerans). This could use toxicity identification and evaluation (TIE) techniques.

- The higher than expected toxicity to fish survival from Toora drainage water was also unexpected and further investigations may include the effect of metal-rich precipitates on *M. peelii* and other species. The histopathology of fish gills exposed to aluminium precipitates is a particular important area to investigate.

- The relevance of the species used for assessing the potential risk posed by the drainage water entering the River Murray should be further assessed to ensure that test results can be extrapolated reliably to the receiving environment. This study used sensitive species and robust test methods to assess the bioavailability of contaminants. In this study, the cladoceran *Ceriodaphnia dubia* represented zooplankton and a possible interaction between algae-zooplanton-fish and was the most sensitive species to acid drainage water however the extrapolation of toxicity test data to the field should be validated with field-based studies.

- Results from the toxicity tests, and chemical analysis, should be validated using field-based assessments. For example, *in situ* toxicity tests measuring endpoints such as survival can be undertaken along with measuring the bioaccumulation of contaminants in organisms (e.g midge, yabbies). Further biodiversity assessments in the receiving environment should also be undertaken.

- The use of high conductivity and low pH water as controls that are diluted to the same concentration as the test species dilutions is recommended to better ascertain the impact of physic-chemical stressors on the organisms tested.

- A high chronic toxicity to midge was observed for the metal-rich precipitates collected from Jervois Wellington. This test should be undertaken on precipitates collected from sediment traps located immediately before release into the River Murray. Testing these precipitates would enable a more relevant assessment of precipitates ultimately released in the river.

- The ANZECC/ARMCANZ water quality guidelines require review for aluminium, particularly in relation to deriving guideline value(s) for aluminium toxicity in lower pH water and where aluminium particulates are present (e.g. acid extractable particulate aluminium).

- Liming of the drainage channels has been investigated as a means of lowering the dissolved metal concentrations prior to discharge of the drainage waters to the River Murray, and an assessment of how liming alters the toxicity of the discharges waters and precipitates should be undertaken.

References

ANZECC/ARMCANZ (2000). Australia and New Zealand Guidelines for Fresh and Marine Water Quality. Australian and New Zealand Environment and Conservation Council, Agriculture and Resource Management Council of Australia and New Zealand, Canberra, ACT, Australia.

Fitzpatrick RW, Shand P, Simpson S, Grocke S, Raven M, Baker AKM, Mosley LM and Self PG (2012). *Assessment of re-flooded Acid Sulfate Soil environments at Long Flat, Jervois, Toora and Pompoota in the Lower Murray Reclaimed Irrigation Area (LMRIA).* CSIRO Land and Water Science Report No 03/2012, November 2012. 220 pp.

Gensemer, R.W. and Playle, R.C. (1999) The bioavailability and toxicity of aluminium in aquatic environments. Critical Reviews in Environmental Science and Technology, 29: 315-450.

Kumar, A., Correll, R., Grocke, S. and Bajet, C. (2010). Toxicity of selected pesticides to freshwater shrimp, *Paratya australiensis* (Decopda: Atyidae): use of time series acute toxicity data to predict chronic lethality. Ecotoxicology and Environmental Safety, 73, 360-369.

Leyden E, Palmer D, Scott P, Zammit B and Mosley L (2012). Lower Murray River Irrigated Area Acidification Risk Project: Preliminary monitoring report 2011–12. Environment Protection Authority, South Australia. Available at: http://www.epa.sa.gov.au/xstd_files/Water/Report/Preliminary%20Report_LMRIA_Final.pdf

Linge KL (2008). Trace element determination by ICP-AES and ICP-MS: developments and applications reported during 2006 and 2007. *Geostandards and Geoanalytical Research* 32, 435-468.

Mosley L.M and Fleming N 2010, Pollutant Loads returned to the Lower Murray River from flood-irrigated agriculture, Water Air and Soil Pollution, 211, 475–487.

OECD (1984a). OECD Guideline 211 for testing of chemicals, *Daphnia* magna reproduction test, adopted 1984.

OECD (1984b). OECD Guideline 204 for testing of chemicals, Fish, prolonged toxicity test: 14-day study, adopted 1984.

OECD (2004). OECD Guideline 202 for testing of chemicals, *Daphnia* sp., Acute immobilisation test, adopted 2004.

OECD (2012). OECD Guideline 211 for testing of chemicals, *Daphnia* magna reproduction test, adopted 2012.

Simpson SL, Vardanega C, Jarolimek C, Angel BA. (2013). Behaviour and potential impacts of acid drainage plumes being discharged to the Lower Murray. Water for a Healthy Country Flagship Report. 69 pages.

Glossary and Acronyms

µg/g: microgram per gram

µg/L: microgram per litre

Acute toxicity: Effects resulting from exposure (usually short-term) over a small part of the organism's life span e.g. mortality, enzyme inhibition.

AEM: Dilute acid-extractable metal (usually using 1 M HCl, 60 min)

ANZECC: Australian and New Zealand Environment and Conservation Council.

ARD: Acid rock drainage

ARMCANZ: Agriculture and Resource Management Council of Australia and New Zealand.

Bioassay: a test used to evaluate the relative potency of a chemical by measuring its effect on a living organism relative to a control.

Bioavailable: Able to be taken up by organisms.

Chelex: A metal-binding agent used for speciation analyses

Chronic toxicity: Effects over a significant portion of the organism's life span e.g. effects on growth and reproduction.

Control: Part of an experimental procedure that is ideally exactly like the treated part except that it is not subject to the test conditions. It is used as a standard of comparison, to check that the outcome of the experiment is a reflection of the test conditions and not of some unknown general factor.

dM: Dissolved metal (<0.45 µm filterable)

DO: Dissolved oxygen.

DOC: Dissolved organic carbon (analysed using a TOC analyser).

Ecotoxicology: The science dealing with the adverse effects of chemicals, physical agents and natural products on populations and communities of living organisms

EC50 (or EC10): The toxicant concentration that is expected to cause one or more specified effects in 50% (or 10%) of a group of organisms under specified conditions.

Guideline: Numerical concentration limit or narrative statement to support and maintain a designated water use.

ICP-AES: Inductively coupled plasma atomic emission spectroscopy

ICP-MS: Inductively coupled plasma mass spectroscopy

ISQG-High: Interim sediment quality guideline high value (from ANZECC/ARMCANZ, 2000)

Level of protection: The acceptable level of change from a defined reference condition.

LOEC: the lowest concentration tested to have a significant effect on an organism(s).

LOR: Limit of reporting.

mg/kg: milligram per kilogram

mg/L: milligram per litre

NATA: National Association of Testing Authorities, Australia

NOEC: the highest concentration tested to have no significant effect on an organism(s).

Overlying water: The water above the sediment at a collection site or in a test chamber.

pH: The intensity of the acidic or basic character of a solution, defined as the negative logarithm of the hydrogen ion concentration of a solution.

POC: Particulate organic carbon

QA/QC: Quality assurance/quality control.

Quality assurance (QA): The implementation of checks on the success of quality control (e.g. replicate samples, analysis of samples of known concentration).

Quality control (QC): The implementation of procedures to maximise the integrity of monitoring data (e.g. cleaning procedures, contamination avoidance, sample preservation methods).

Sediment: Unconsolidated mineral and organic particulate material that has settled to the bottom of aquatic environments.

sM: suspended metal concentration (particulate metals in TSS)

SA EPA: South Australia Environment al Protection Agency

Speciation: Measurement of different chemical forms or species of an element in a solution or solid.

SQG: Sediment quality guideline.

TDS: Total dissolved solids

TRM: Total recoverable metals in solid sample

TOC: Total organic carbon.

Toxicity: The inherent potential or capacity of a material to cause adverse effects in a living organism.

Toxicity test: The means by which the toxicity of a chemical or other test material is determined. A toxicity test is used to measure the degree of response produced by exposure to a specific level of stimulus (or concentration of chemical).

TSS: Total suspended solids

TV: Trigger value (from ANZECC/ARMCANZ, 2000).

WQG: Water quality guideline.

Appendix A Chemical Analysis

Dissolved metals, physico-chemistry and nutrient analysis in River Murray water and acid drainage water

To: Merrin Adams, Project Leader Ecotoxicology, CSIRO Land and Water
Address: CSIRO Land and Water, Locked Bag 2007, Kirrawee, NSW, 2232
Email: merrin.adams@csrio.au

Sample Labels	**Sample I.D.:**	**Date sampled**	**Ag (µg/L)**	**Al (µg/L)**	**As (µg/L)**	**Ca (mg/L)**	**Cd (µg/L)**	**Co (µg/L)**	**Cr (µg/L)**	**Cu (µg/L)**	**Fe (mg/L)**	**K (mg/L)**	**Mg (mg/L)**	**Mn (mg/L)**	**Na (mg/L)**	**Ni (µg/L)**	**Pb (µg/L)**	**Zn (µg/L)**
CE245-1	Murray River 1	22/10/2012	<0.1	626	1.2	8.3	<0.1	0.11	0.5	1.4	0.57	2.9	4.8	0.002	15.5	1.4	0.3	2.1
CE245-2	Murray River 2	22/10/2012	<0.1	25	1.2	8.4	<0.1	0.03	<0.1	1.0	0.17	2.7	4.5	0.001	15.7	1.2	0.2	0.5
CE245-3	Wellington	22/10/2012	<0.1	386	1.4	129	0.2	107	0.8	0.7	1.81	16	117	2.80	441	97	0.2	61
CE245-4	Woods Pt	22/10/2012	<0.1	167	1.7	192	0.3	94	0.5	8.1	0.9	21	168	2.41	467	123	0.2	84
CE245-5	Toora	22/10/2012	<0.1	555	2.2	391	0.6	285	1.4	0.3	30.3	64	434	4.09	1500	280	0.1	160
CE245-6	Mobilong	22/10/2012	<0.1	16600	10	---	2.3	605	3.7	4.6	58.5	74	786	9.95	Over - range	613	2.8	375
CE245-6 DUP	Mobilong	22/10/2012	<0.1	14600	11	---	2.4	615	3.5	4.6	---	---	---	---		618	2.6	381
CE245-6 Average	Mobilong	22/10/2012	<0.1	15600	11	648	2.4	610	3.6	4.6	---	---	---	---		616	2.7	378
LOD (3σ)			*0.10*	*0.1*	*0.2*	*0.3*	*0.1*	*0.3*	*0.1*	*0.1*	*0.002*	*0.2*	*0.1*	*0.0*	*0.4*	*0.2*	*0.1*	*0.2*

			% Spike recovery															
			Ag	**Al**	**As**	**Ca**	**Cd**	**Co**	**Cr**	**Cu**	**Fe**	**K**	**Mg**	**Mn**	**Na**	**Ni**	**Pb**	**Zn**
CE245-6	% Spike Recovery Mobilong	22/10/2012	71	71	101		91	102	104	94	---	---	---	---	---	88	81	80
			C-209	C-209	C-209	C-229	C-209	C-209	C-209	C-209	C-229	C-229	C-229	C-229	C-229	C-209	C-209	C-209

Environmental Division

CERTIFICATE OF ANALYSIS

Work Order	: **ES1226640**	Page	: 1 of 4
Client	: **CSIRO ENERGY TECHNOLOGY**	Laboratory	: Environmental Division Sydney
Contact	: MERRIN ADAMS	Contact	: Client Services
Address	: NEW ILLAWARRA ROAD, LUCAS HEIGHTS LOCKED BAG 2007 KIRRAWEE NSW 2232	Address	: 277-289 Woodpark Road Smithfield NSW Australia 2164
E-mail	: merrin.adam@csiro.au	E-mail	: sydney@alsglobal.com
Telephone	: +61 02 9710 6800	Telephone	: +61-2-8784 8555
Facsimile	: +61 02 9710 6831	Facsimile	: +61-2-8784 8500
Project	: ----	QC Level	: NEPM 1999 Schedule B(3) and ALS QCS3 requirement
Order number	: ----		
C-O-C number	: ----	Date Samples Received	: 09-NOV-2012
Sampler	: ----	Issue Date	: 15-NOV-2012
Site	: ----		
		No. of samples received	: 5
Quote number	: ----	No. of samples analysed	: 5

This report supersedes any previous report(s) with this reference. Results apply to the sample(s) as submitted. All pages of this report have been checked and approved for release.

This Certificate of Analysis contains the following information:

- General Comments
- Analytical Results

NATA Accredited Laboratory 825

Accredited for compliance with ISO/IEC 17025.

WORLD RECOGNISED **ACCREDITATION**

Signatories

This document has been electronically signed by the authorized signatories indicated below. Electronic signing has been carried out in compliance with procedures specified in 21 CFR Part 11.

Signatories	*Position*	*Accreditation Category*
Ankit Joshi	Inorganic Chemist	Sydney Inorganics
Celine Conceicao	Senior Spectroscopist	Sydney Inorganics
Sarah Millington	Senior Inorganic Chemist	Sydney Inorganics

General Comments

The analytical procedures used by the Environmental Division have been developed from established internationally recognized procedures such as those published by the USEPA, APHA, AS and NEPM. In house developed procedures are employed in the absence of documented standards or by client request.

Where moisture determination has been performed, results are reported on a dry weight basis.

Where a reported less than (<) result is higher than the LOR, this may be due to primary sample extract/digestate dilution and/or insufficient sample for analysis.

Where the LOR of a reported result differs from standard LOR, this may be due to high moisture content, insufficient sample (reduced weight employed) or matrix interference.

When sampling time information is not provided by the client, sampling dates are shown without a time component. In these instances, the time component has been assumed by the laboratory for processing purposes.

Key : CAS Number = CAS registry number from database maintained by Chemical Abstracts Services. The Chemical Abstracts Service is a division of the American Chemical Society.
LOR = Limit of reporting
^ = This result is computed from individual analyte detections at or above the level of reporting

- **It has been noted that Ammonia is greater than TKN for sample ID MOBILING, however this difference is within the limits of experimental variation.**

Analytical Results

Sub-Matrix: **WATER**			*Client sample ID*	**RIVER MURRAY**	**WELLINGTON**	**WOODS PT**	**TOORA**	**MOBILING**
			Client sampling date / time	23-OCT-2012 15:00	23-OCT-2012 15:00	23-OCT-2012 15:00	23-OCT-2012 15:00	23-OCT-2012 15:00
Compound	*CAS Number*	*LOR*	*Unit*	**ES1226640-001**	**ES1226640-002**	**ES1226640-003**	**ES1226640-004**	**ES1226640-005**
ED037P: Alkalinity by PC Titrator								
Hydroxide Alkalinity as CaCO3	DMO-210-001	1	mg/L	<1	<1	<1	<1	<1
Carbonate Alkalinity as CaCO3	3812-32-6	1	mg/L	<1	<1	<1	<1	<1
Bicarbonate Alkalinity as CaCO3	71-52-3	1	mg/L	**54**	<1	**27**	<1	<1
Total Alkalinity as CaCO3	----	1	mg/L	**54**	<1	**27**	<1	<1
ED038A: Acidity								
Acidity as CaCO3	----	1	mg/L	**2**	**42**	**19**	**99**	**361**
ED041G: Sulfate (Turbidimetric) as SO4 2- by DA								
Sulfate as SO4 - Turbidimetric	14808-79-8	1	mg/L	**7**	**967**	**1490**	**3420**	**3920**
ED045G: Chloride Discrete analyser								
Chloride	16887-00-6	1	mg/L	**20**	**818**	**804**	**3730**	**8040**
ED093F: Dissolved Major Cations								
Calcium	7440-70-2	1	mg/L	**10**	**140**	**206**	**464**	**700**
Magnesium	7439-95-4	1	mg/L	**6**	**137**	**204**	**552**	**845**
Sodium	7440-23-5	1	mg/L	**18**	**612**	**640**	**2230**	**4600**
Potassium	7440-09-7	1	mg/L	**4**	**20**	**26**	**85**	**88**
EK055G: Ammonia as N by Discrete Analyser								
Ammonia as N	7664-41-7	0.01	mg/L	**0.02**	**1.93**	**2.12**	**3.56**	**5.10**
EK057G: Nitrite as N by Discrete Analyser								
Nitrite as N	----	0.01	mg/L	<0.01	<0.01	<0.01	<0.01	<0.01
EK058G: Nitrate as N by Discrete Analyser								
Nitrate as N	14797-55-8	0.01	mg/L	<0.01	**0.11**	**0.17**	**0.05**	**0.28**
EK059G: Nitrite plus Nitrate as N (NOx) by Discrete Analyser								
Nitrite + Nitrate as N	----	0.01	mg/L	<0.01	**0.11**	**0.17**	**0.05**	**0.28**
EK061G: Total Kjeldahl Nitrogen By Discrete Analyser								
Total Kjeldahl Nitrogen as N	----	0.1	mg/L	**0.4**	**3.0**	**3.1**	**3.6**	**5.0**
EK062G: Total Nitrogen as N (TKN + NOx) by Discrete Analyser								
^ **Total Nitrogen as N**	----	0.1	mg/L	**0.4**	**3.1**	**3.3**	**3.6**	**5.3**
EK067G: Total Phosphorus as P by Discrete Analyser								
Total Phosphorus as P	----	0.01	mg/L	**0.07**	**0.06**	**0.02**	**0.04**	**0.03**
EK071G: Reactive Phosphorus as P by discrete analyser								
Reactive Phosphorus as P	----	0.01	mg/L	**0.02**	<0.01	<0.01	<0.01	<0.01
EN055: Ionic Balance								
Total Anions	----	0.01	meq/L	**1.79**	**43.2**	**54.2**	**176**	**308**
Total Cations	----	0.01	meq/L	**1.88**	**45.4**	**55.6**	**168**	**307**
Ionic Balance	----	0.01	%	----	**2.46**	**1.20**	**2.53**	**0.27**

Analytical Results

Sub-Matrix: **WATER**

			Client sample ID	RIVER MURRAY	WELLINGTON	WOODS PT	TOORA	MOBILING
			Client sampling date / time	23-OCT-2012 15:00	23-OCT-2012 15:00	23-OCT-2012 15:00	23-OCT-2012 15:00	23-OCT-2012 15:00
Compound	CAS Number	LOR	Unit	ES1226640-001	ES1226640-002	ES1226640-003	ES1226640-004	ES1226640-005
EP002: Dissolved Organic Carbon (DOC)								
Dissolved Organic Carbon	----	1	mg/L	**5**	**14**	**6**	**17**	**13**

QUALITY CONTROL REPORT

Work Order	: **ES1226640**	Page	: 1 of 7
Client	: **CSIRO ENERGY TECHNOLOGY**	Laboratory	: Environmental Division Sydney
Contact	: MERRIN ADAMS	Contact	: Client Services
Address	: NEW ILLAWARRA ROAD, LUCAS HEIGHTS LOCKED BAG 2007 KIRRAWEE NSW 2232	Address	: 277-289 Woodpark Road Smithfield NSW Australia 2164
E-mail	: merrin.adam@csiro.au	E-mail	: sydney@alsglobal.com
Telephone	: +61 02 9710 6800	Telephone	: +61-2-8784 8555
Facsimile	: +61 02 9710 6831	Facsimile	: +61-2-8784 8500
Project	: ----	QC Level	: NEPM 1999 Schedule B(3) and ALS QCS3 requirement
Site	: ----		
C-O-C number	: ----	Date Samples Received	: 09-NOV-2012
Sampler	: ----	Issue Date	: 15-NOV-2012
Order number	: ----		
		No. of samples received	: 5
Quote number	: ----	No. of samples analysed	: 5

This report supersedes any previous report(s) with this reference. Results apply to the sample(s) as submitted. All pages of this report have been checked and approved for release.

This Quality Control Report contains the following information:

- Laboratory Duplicate (DUP) Report; Relative Percentage Difference (RPD) and Acceptance Limits
- Method Blank (MB) and Laboratory Control Spike (LCS) Report; Recovery and Acceptance Limits
- Matrix Spike (MS) Report; Recovery and Acceptance Limits

NATA Accredited Laboratory 825

Accredited for compliance with ISO/IEC 17025.

WORLD RECOGNISED ACCREDITATION

Signatories

This document has been electronically signed by the authorized signatories indicated below. Electronic signing has been carried out in compliance with procedures specified in 21 CFR Part 11.

Signatories	*Position*	*Accreditation Category*
Ankit Joshi	Inorganic Chemist	Sydney Inorganics
Celine Conceicao	Senior Spectroscopist	Sydney Inorganics
Sarah Millington	Senior Inorganic Chemist	Sydney Inorganics

General Comments

The analytical procedures used by the Environmental Division have been developed from established internationally recognized procedures such as those published by the USEPA, APHA, AS and NEPM. In house developed procedures are employed in the absence of documented standards or by client request.

Where moisture determination has been performed, results are reported on a dry weight basis.

Where a reported less than (<) result is higher than the LOR, this may be due to primary sample extract/digestate dilution and/or insufficient sample for analysis.

Where the LOR of a reported result differs from standard LOR, this may be due to high moisture content, insufficient sample (reduced weight employed) or matrix interference.

Key : Anonymous = Refers to samples which are not specifically part of this work order but formed part of the QC process lot
CAS Number = CAS registry number from database maintained by Chemical Abstracts Services. The Chemical Abstracts Service is a division of the American Chemical Society.
LOR = Limit of reporting
RPD = Relative Percentage Difference
# = Indicates failed QC

Laboratory Duplicate (DUP) Report

The quality control term Laboratory Duplicate refers to a randomly selected intralaboratory split. Laboratory duplicates provide information regarding method precision and sample heterogeneity. The permitted ranges for the Relative Percent Deviation (RPD) of Laboratory Duplicates are specified in ALS Method QWI-EN/38 and are dependent on the magnitude of results in comparison to the level of reporting: Result < 10 times LOR:- No Limit; Result between 10 and 20 times LOR:- 0% - 50%; Result > 20 times LOR:- 0% - 20%.

Sub-Matrix: **WATER**

				Laboratory Duplicate (DUP) Report					
Laboratory sample ID	*Client sample ID*	*Method: Compound*	*CAS Number*	*LOR*	*Unit*	*Original Result*	*Duplicate Result*	*RPD (%)*	*Recovery Limits (%)*
ED037P: Alkalinity by PC Titrator (QC Lot: 2590268)									
EN1204285-001	Anonymous	ED037-P: Hydroxide Alkalinity as CaCO3	DMO-210-001	1	mg/L	<1	<1	0.0	No Limit
		ED037-P: Carbonate Alkalinity as CaCO3	3812-32-6	1	mg/L	<1	<1	0.0	No Limit
		ED037-P: Bicarbonate Alkalinity as CaCO3	71-52-3	1	mg/L	159	159	0.0	0% - 20%
		ED037-P: Total Alkalinity as CaCO3	----	1	mg/L	159	159	0.0	0% - 20%
ES1226448-001	Anonymous	ED037-P: Hydroxide Alkalinity as CaCO3	DMO-210-001	1	mg/L	<1	<1	0.0	No Limit
		ED037-P: Carbonate Alkalinity as CaCO3	3812-32-6	1	mg/L	<1	1	0.0	No Limit
		ED037-P: Bicarbonate Alkalinity as CaCO3	71-52-3	1	mg/L	110	109	0.0	0% - 20%
		ED037-P: Total Alkalinity as CaCO3	----	1	mg/L	110	111	1.1	0% - 20%
ED037P: Alkalinity by PC Titrator (QC Lot: 2590271)									
ES1226640-002	WELLINGTON	ED037-P: Hydroxide Alkalinity as CaCO3	DMO-210-001	1	mg/L	<1	<1	0.0	No Limit
		ED037-P: Carbonate Alkalinity as CaCO3	3812-32-6	1	mg/L	<1	<1	0.0	No Limit
		ED037-P: Bicarbonate Alkalinity as CaCO3	71-52-3	1	mg/L	<1	<1	0.0	No Limit
		ED037-P: Total Alkalinity as CaCO3	----	1	mg/L	<1	<1	0.0	No Limit
ED038A: Acidity (QC Lot: 2595882)									
ES1226468-001	Anonymous	ED038: Acidity as CaCO3	----	1	mg/L	15	14	6.9	0% - 50%
ED041G: Sulfate (Turbidimetric) as SO4 2- by DA (QC Lot: 2589934)									
ES1226467-001	Anonymous	ED041G: Sulfate as SO4 - Turbidimetric	14808-79-8	1	mg/L	64	61	5.7	0% - 20%
ES1226640-001	RIVER MURRAY	ED041G: Sulfate as SO4 - Turbidimetric	14808-79-8	1	mg/L	7	7	0.0	No Limit
ED045G: Chloride Discrete analyser (QC Lot: 2589932)									
ES1226425-007	Anonymous	ED045G: Chloride	16887-00-6	1	mg/L	26	26	0.0	0% - 20%
ES1226640-001	RIVER MURRAY	ED045G: Chloride	16887-00-6	1	mg/L	20	20	0.0	0% - 20%
ED093F: Dissolved Major Cations (QC Lot: 2589931)									
ES1226425-007	Anonymous	ED093F: Calcium	7440-70-2	1	mg/L	<1	<1	0.0	No Limit
		ED093F: Magnesium	7439-95-4	1	mg/L	2	3	0.0	No Limit
		ED093F: Sodium	7440-23-5	1	mg/L	11	12	0.0	0% - 50%
		ED093F: Potassium	7440-09-7	1	mg/L	<1	<1	0.0	No Limit
ES1226640-002	WELLINGTON	ED093F: Calcium	7440-70-2	1	mg/L	140	150	7.2	0% - 20%
		ED093F: Magnesium	7439-95-4	1	mg/L	137	148	7.5	0% - 20%
		ED093F: Sodium	7440-23-5	1	mg/L	612	618	1.0	0% - 20%
		ED093F: Potassium	7440-09-7	1	mg/L	20	20	0.0	0% - 20%
EK055G: Ammonia as N by Discrete Analyser (QC Lot: 2594027)									
ES1226519-001	Anonymous	EK055G: Ammonia as N	7664-41-7	0.01	mg/L	0.03	0.03	0.0	No Limit
ES1226640-003	WOODS PT	EK055G: Ammonia as N	7664-41-7	0.01	mg/L	2.12	2.06	3.1	0% - 20%
EK057G: Nitrite as N by Discrete Analyser (QC Lot: 2589933)									
ES1226467-001	Anonymous	EK057G: Nitrite as N	----	0.01	mg/L	0.01	0.01	0.0	No Limit

Sub-Matrix: **WATER**

				Laboratory Duplicate (DUP) Report					
Laboratory sample ID	*Client sample ID*	*Method: Compound*	*CAS Number*	*LOR*	*Unit*	*Original Result*	*Duplicate Result*	*RPD (%)*	*Recovery Limits (%)*
EK057G: Nitrite as N by Discrete Analyser (QC Lot: 2589933) - continued									
ES1226640-001	RIVER MURRAY	EK057G: Nitrite as N	----	0.01	mg/L	<0.01	<0.01	0.0	No Limit
EK059G: Nitrite plus Nitrate as N (NOx) by Discrete Analyser (QC Lot: 2594025)									
ES1226519-001	Anonymous	EK059G: Nitrite + Nitrate as N	----	0.01	mg/L	0.35	0.33	5.9	0% - 20%
ES1226640-003	WOODS PT	EK059G: Nitrite + Nitrate as N	----	0.01	mg/L	0.17	0.17	0.0	0% - 50%
EK061G: Total Kjeldahl Nitrogen By Discrete Analyser (QC Lot: 2593716)									
ES1226635-002	Anonymous	EK061G: Total Kjeldahl Nitrogen as N	----	0.1	mg/L	3.0	3.0	0.0	0% - 20%
ME1201821-001	Anonymous	EK061G: Total Kjeldahl Nitrogen as N	----	0.1	mg/L	17.2	16.2	6.1	0% - 20%
EK067G: Total Phosphorus as P by Discrete Analyser (QC Lot: 2593717)									
ES1226635-002	Anonymous	EK067G: Total Phosphorus as P	----	0.01	mg/L	3.37	3.32	1.5	0% - 20%
ME1201821-001	Anonymous	EK067G: Total Phosphorus as P	----	0.01	mg/L	8.94	9.48	5.9	0% - 20%
EK071G: Reactive Phosphorus as P by discrete analyser (QC Lot: 2589935)									
ES1226541-001	Anonymous	EK071G: Reactive Phosphorus as P	----	0.01	mg/L	0.02	0.02	0.0	No Limit
ES1226640-005	MOBILING	EK071G: Reactive Phosphorus as P	----	0.01	mg/L	<0.01	<0.01	0.0	No Limit
EP002: Dissolved Organic Carbon (DOC) (QC Lot: 2593139)									
ES1226611-005	Anonymous	EP002: Dissolved Organic Carbon	----	1	mg/L	2	2	0.0	No Limit
ES1226612-001	Anonymous	EP002: Dissolved Organic Carbon	----	1	mg/L	81	80	1.2	0% - 20%

Method Blank (MB) and Laboratory Control Spike (LCS) Report

The quality control term Method / Laboratory Blank refers to an analyte free matrix to which all reagents are added in the same volumes or proportions as used in standard sample preparation. The purpose of this QC parameter is to monitor potential laboratory contamination. The quality control term Laboratory Control Sample (LCS) refers to a certified reference material, or a known interference free matrix spiked with target analytes. The purpose of this QC parameter is to monitor method precision and accuracy independent of sample matrix. Dynamic Recovery Limits are based on statistical evaluation of processed LCS.

Sub-Matrix: **WATER**

				Method Blank (MB) Report	*Laboratory Control Spike (LCS) Report*			
					Spike	*Spike Recovery (%)*	*Recovery Limits (%)*	
Method: Compound	*CAS Number*	*LOR*	*Unit*	*Result*	*Concentration*	*LCS*	*Low*	*High*
ED037P: Alkalinity by PC Titrator (QCLot: 2590268)								
ED037-P: Total Alkalinity as CaCO3	----	1	mg/L	----	200 mg/L	92.6	74	110
ED037P: Alkalinity by PC Titrator (QCLot: 2590271)								
ED037-P: Total Alkalinity as CaCO3	----	1	mg/L	----	200 mg/L	91.0	74	110
ED038A: Acidity (QCLot: 2595882)								
ED038: Acidity as CaCO3	----	1	mg/L	<1	20 mg/L	100	92	110
ED041G: Sulfate (Turbidimetric) as SO4 2- by DA (QCLot: 2589934)								
ED041G: Sulfate as SO4 - Turbidimetric	14808-79-8	1	mg/L	<1	25 mg/L	110	84	124
ED045G: Chloride Discrete analyser (QCLot: 2589932)								
ED045G: Chloride	16887-00-6	1	mg/L	<1	1000 mg/L	95.6	84	122
ED093F: Dissolved Major Cations (QCLot: 2589931)								
ED093F: Calcium	7440-70-2	1	mg/L	<1	50 mg/L	93.2	85	111
ED093F: Magnesium	7439-95-4	1	mg/L	<1	50 mg/L	102	87	111
ED093F: Sodium	7440-23-5	1	mg/L	<1	50 mg/L	90.9	79	109
ED093F: Potassium	7440-09-7	1	mg/L	<1	50 mg/L	98.8	86	112
EK055G: Ammonia as N by Discrete Analyser (QCLot: 2594027)								
EK055G: Ammonia as N	7664-41-7	0.01	mg/L	<0.01	1.00 mg/L	96.7	89	113
EK057G: Nitrite as N by Discrete Analyser (QCLot: 2589933)								
EK057G: Nitrite as N	----	0.01	mg/L	<0.01	0.5 mg/L	100	87	119
EK059G: Nitrite plus Nitrate as N (NOx) by Discrete Analyser (QCLot: 2594025)								
EK059G: Nitrite + Nitrate as N	----	0.01	mg/L	<0.01	0.5 mg/L	102	86	124
EK061G: Total Kjeldahl Nitrogen By Discrete Analyser (QCLot: 2593716)								
EK061G: Total Kjeldahl Nitrogen as N	----	0.1	mg/L	<0.1	5 mg/L	88.3	70	130
EK067G: Total Phosphorus as P by Discrete Analyser (QCLot: 2593717)								
EK067G: Total Phosphorus as P	----	0.01	mg/L	<0.01	4.42 mg/L	92.5	70	130
EK071G: Reactive Phosphorus as P by discrete analyser (QCLot: 2589935)								
EK071G: Reactive Phosphorus as P	----	0.01	mg/L	<0.01	0.50 mg/L	105	86	124
EP002: Dissolved Organic Carbon (DOC) (QCLot: 2593139)								
EP002: Dissolved Organic Carbon	----	1	mg/L	<1	10 mg/L	101	78	122

Matrix Spike (MS) Report

The quality control term Matrix Spike (MS) refers to an intralaboratory split sample spiked with a representative set of target analytes. The purpose of this QC parameter is to monitor potential matrix effects on analyte recoveries. Static Recovery Limits as per laboratory Data Quality Objectives (DQOs). Ideal recovery ranges stated may be waived in the event of sample matrix interference.

Sub-Matrix: **WATER**

				Matrix Spike (MS) Report			
				Spike	Spike Recovery (%)	Recovery Limits (%)	
Laboratory sample ID	*Client sample ID*	*Method: Compound*	*CAS Number*	*Concentration*	*MS*	*Low*	*High*
ED041G: Sulfate (Turbidimetric) as SO4 2- by DA (QCLot: 2589934)							
ES1226467-001	Anonymous	ED041G: Sulfate as SO4 - Turbidimetric	14808-79-8	10 mg/L	# Not Determined	70	130
ED045G: Chloride Discrete analyser (QCLot: 2589932)							
ES1226425-007	Anonymous	ED045G: Chloride	16887-00-6	250 mg/L	110	70	130
EK055G: Ammonia as N by Discrete Analyser (QCLot: 2594027)							
ES1226519-001	Anonymous	EK055G: Ammonia as N	7664-41-7	1.00 mg/L	92.7	70	130
EK057G: Nitrite as N by Discrete Analyser (QCLot: 2589933)							
ES1226467-001	Anonymous	EK057G: Nitrite as N	----	0.5 mg/L	101	70	130
EK059G: Nitrite plus Nitrate as N (NOx) by Discrete Analyser (QCLot: 2594025)							
ES1226519-001	Anonymous	EK059G: Nitrite + Nitrate as N	----	0.5 mg/L	81.8	70	130
EK061G: Total Kjeldahl Nitrogen By Discrete Analyser (QCLot: 2593716)							
ES1226635-002	Anonymous	EK061G: Total Kjeldahl Nitrogen as N	----	5 mg/L	82.8	70	130
EK067G: Total Phosphorus as P by Discrete Analyser (QCLot: 2593717)							
ES1226635-002	Anonymous	EK067G: Total Phosphorus as P	----	1.00 mg/L	110	70	130
EK071G: Reactive Phosphorus as P by discrete analyser (QCLot: 2589935)							
ES1226541-001	Anonymous	EK071G: Reactive Phosphorus as P	----	0.50 mg/L	99.4	70	130
EP002: Dissolved Organic Carbon (DOC) (QCLot: 2593139)							
ES1226611-006	Anonymous	EP002: Dissolved Organic Carbon	----	100 mg/L	105	70	130

Matrix Spike (MS) and Matrix Spike Duplicate (MSD) Report

The quality control term Matrix Spike (MS) and Matrix Spike Duplicate (MSD) refers to intralaboratory split samples spiked with a representative set of target analytes. The purpose of these QC parameters are to monitor potential matrix effects on analyte recoveries. Static Recovery Limits as per laboratory Data Quality Objectives (DQOs). Ideal recovery ranges stated may be waived in the event of sample matrix interference.

Sub-Matrix: **WATER**

				Matrix Spike (MS) and Matrix Spike Duplicate (MSD) Report						
				Spike	Spike Recovery (%)		Recovery Limits (%)		RPDs (%)	
Laboratory sample ID	*Client sample ID*	*Method: Compound*	*CAS Number*	*Concentration*	*MS*	*MSD*	*Low*	*High*	*Value*	*Control Limit*
ED045G: Chloride Discrete analyser (QCLot: 2589932)										
ES1226425-007	Anonymous	ED045G: Chloride	16887-00-6	250 mg/L	110	----	70	130	----	----
EK057G: Nitrite as N by Discrete Analyser (QCLot: 2589933)										
ES1226467-001	Anonymous	EK057G: Nitrite as N	----	0.5 mg/L	101	----	70	130	----	----
ED041G: Sulfate (Turbidimetric) as SO4 2- by DA (QCLot: 2589934)										
ES1226467-001	Anonymous	ED041G: Sulfate as SO4 - Turbidimetric	14808-79-8	10 mg/L	# Not Determined	----	70	130	----	----
EK071G: Reactive Phosphorus as P by discrete analyser (QCLot: 2589935)										
ES1226541-001	Anonymous	EK071G: Reactive Phosphorus as P	----	0.50 mg/L	99.4	----	70	130	----	----
EP002: Dissolved Organic Carbon (DOC) (QCLot: 2593139)										
ES1226611-006	Anonymous	EP002: Dissolved Organic Carbon	----	100 mg/L	105	----	70	130	----	----

Sub-Matrix: **WATER**

				Matrix Spike (MS) and Matrix Spike Duplicate (MSD) Report						
				Spike	Spike Recovery (%)		Recovery Limits (%)		RPDs (%)	
Laboratory sample ID	Client sample ID	Method: Compound	CAS Number	Concentration	MS	MSD	Low	High	Value	Control Limit
EK061G: Total Kjeldahl Nitrogen By Discrete Analyser (QCLot: 2593716)										
ES1226635-002	Anonymous	EK061G: Total Kjeldahl Nitrogen as N	----	5 mg/L	82.8	----	70	130	----	----
EK067G: Total Phosphorus as P by Discrete Analyser (QCLot: 2593717)										
ES1226635-002	Anonymous	EK067G: Total Phosphorus as P	----	1.00 mg/L	110	----	70	130	----	----
EK059G: Nitrite plus Nitrate as N (NOx) by Discrete Analyser (QCLot: 2594025)										
ES1226519-001	Anonymous	EK059G: Nitrite + Nitrate as N	----	0.5 mg/L	81.8	----	70	130	----	----
EK055G: Ammonia as N by Discrete Analyser (QCLot: 2594027)										
ES1226519-001	Anonymous	EK055G: Ammonia as N	7664-41-7	1.00 mg/L	92.7	----	70	130	----	----

Environmental Division

INTERPRETIVE QUALITY CONTROL REPORT

Work Order : **ES1226640**

Client : CSIRO ENERGY TECHNOLOGY
Contact : MERRIN ADAMS
Address : NEW ILLAWARRA ROAD, LUCAS HEIGHTS
LOCKED BAG 2007
KIRRAWEE NSW 2232

E-mail : merrin.adam@csiro.au
Telephone : +61 02 9710 6800
Facsimile : +61 02 9710 6831

Project : ----
Site : ----
C-O-C number : ----
Sampler : ----
Order number : ----

Quote number : ----

Page : 1 of 9

Laboratory : Environmental Division Sydney
Contact : Client Services
Address : 277-289 Woodpark Road Smithfield NSW Australia 2164

E-mail : sydney@alsglobal.com
Telephone : +61-2-8784 8555
Facsimile : +61-2-8784 8500

QC Level : NEPM 1999 Schedule B(3) and ALS QCS3 requirement

Date Samples Received : 09-NOV-2012
Issue Date : 15-NOV-2012

No. of samples received : 5
No. of samples analysed : 5

This report supersedes any previous report(s) with this reference. Results apply to the sample(s) as submitted. All pages of this report have been checked and approved for release.

This Interpretive Quality Control Report contains the following information:

- Analysis Holding Time Compliance
- Quality Control Parameter Frequency Compliance
- Brief Method Summaries
- Summary of Outliers

Analysis Holding Time Compliance

The following report summarises extraction / preparation and analysis times and compares with recommended holding times. Dates reported represent first date of extraction or analysis and precludes subsequent dilutions and reruns. Information is also provided re the sample container (preservative) from which the analysis aliquot was taken. Elapsed period to analysis represents number of days from sampling where no extraction / digestion is involved or period from extraction / digestion where this is present. For composite samples, sampling date is assumed to be that of the oldest sample contributing to the composite. Sample date for laboratory produced leachates is assumed as the completion date of the leaching process. Outliers for holding time are based on USEPA SW 846, APHA, AS and NEPM (1999). A listing of breaches is provided in the Summary of Outliers.

Holding times for leachate methods (excluding elutriates) vary according to the analytes being determined on the resulting solution. For non-volatile analytes, the holding time compliance assessment compares the leach date with the shortest analyte holding time for the equivalent soil method. These soil holding times are: Organics (14 days); Mercury (28 days) & other metals (180 days). A recorded breach therefore does not guarantee a breach for all non-volatile parameters.

Matrix: **WATER**

Evaluation: ✗ = Holding time breach ; ✓ = Within holding time.

Method *Container / Client Sample ID(s)*	*Sample Date*	*Extraction / Preparation*			*Analysis*		
		Date extracted	*Due for extraction*	*Evaluation*	*Date analysed*	*Due for analysis*	*Evaluation*
ED037P: Alkalinity by PC Titrator							
Clear Plastic Bottle - Natural (ED037-P) RIVER MURRAY, WOODS PT, MOBILING WELLINGTON, TOORA,	**23-OCT-2012**	---	06-NOV-2012	----	**09-NOV-2012**	06-NOV-2012	✗
ED038A: Acidity							
Clear Plastic Bottle - Natural (ED038) RIVER MURRAY, WOODS PT, MOBILING WELLINGTON, TOORA,	**23-OCT-2012**	----	----	----	**14-NOV-2012**	06-NOV-2012	✗
ED041G: Sulfate (Turbidimetric) as SO4 2- by DA							
Clear Plastic Bottle - Natural (ED041G) RIVER MURRAY, WOODS PT, MOBILING WELLINGTON, TOORA,	**23-OCT-2012**	---	20-NOV-2012	----	**09-NOV-2012**	20-NOV-2012	✓
ED045G: Chloride Discrete analyser							
Clear Plastic Bottle - Natural (ED045G) RIVER MURRAY, WOODS PT, MOBILING WELLINGTON, TOORA,	**23-OCT-2012**	---	20-NOV-2012	----	**09-NOV-2012**	20-NOV-2012	✓
ED093F: Dissolved Major Cations							
Clear Plastic Bottle - Natural (ED093F) RIVER MURRAY, WOODS PT, MOBILING WELLINGTON, TOORA,	**23-OCT-2012**	---	30-OCT-2012	----	**09-NOV-2012**	30-OCT-2012	✗
EK055G: Ammonia as N by Discrete Analyser							
Clear Plastic Bottle - Sulfuric Acid (EK055G) RIVER MURRAY, WOODS PT, MOBILING WELLINGTON, TOORA,	**23-OCT-2012**	---	20-NOV-2012	----	**13-NOV-2012**	20-NOV-2012	✓
EK057G: Nitrite as N by Discrete Analyser							
Clear Plastic Bottle - Natural (EK057G) RIVER MURRAY, WOODS PT, MOBILING WELLINGTON, TOORA,	**23-OCT-2012**	---	25-OCT-2012	----	**09-NOV-2012**	25-OCT-2012	✗

Matrix: **WATER**

Evaluation: ✘ = Holding time breach ; ✓ = Within holding time.

Method ***Container** / Client Sample ID(s)*	***Sample Date***	*Extraction / Preparation*			*Analysis*		
		Date extracted	*Due for extraction*	*Evaluation*	***Date analysed***	*Due for analysis*	*Evaluation*
EK059G: Nitrite plus Nitrate as N (NOx) by Discrete Analyser							
Clear Plastic Bottle - Sulfuric Acid (EK059G) RIVER MURRAY, WOODS PT, MOBILING WELLINGTON, TOORA,	**23-OCT-2012**	**---**	20-NOV-2012	----	**13-NOV-2012**	20-NOV-2012	✓
EK061G: Total Kjeldahl Nitrogen By Discrete Analyser							
Clear Plastic Bottle - Sulfuric Acid (EK061G) RIVER MURRAY, WOODS PT, MOBILING WELLINGTON, TOORA,	**23-OCT-2012**	**13-NOV-2012**	20-NOV-2012	✓	**13-NOV-2012**	20-NOV-2012	✓
EK067G: Total Phosphorus as P by Discrete Analyser							
Clear Plastic Bottle - Sulfuric Acid (EK067G) RIVER MURRAY, WOODS PT, MOBILING WELLINGTON, TOORA,	**23-OCT-2012**	**13-NOV-2012**	20-NOV-2012	✓	**13-NOV-2012**	20-NOV-2012	✓
EK071G: Reactive Phosphorus as P by discrete analyser							
Clear Plastic Bottle - Natural (EK071G) RIVER MURRAY, WOODS PT, MOBILING WELLINGTON, TOORA,	**23-OCT-2012**	**---**	25-OCT-2012	----	**09-NOV-2012**	25-OCT-2012	✘
EP002: Dissolved Organic Carbon (DOC)							
Amber DOC Filtered- Sulfuric Preserved (EP002) RIVER MURRAY, WOODS PT, MOBILING WELLINGTON, TOORA,	**23-OCT-2012**	**----**	----	----	**13-NOV-2012**	20-NOV-2012	✓

Quality Control Parameter Frequency Compliance

The following report summarises the frequency of laboratory QC samples analysed within the analytical lot(s) in which the submitted sample(s) was(where) processed. Actual rate should be greater than or equal to the expected rate. A listing of breaches is provided in the Summary of Outliers.

Matrix: **WATER**

Evaluation: × = Quality Control frequency not within specification ; ✓ = Quality Control frequency within specification.

Quality Control Sample Type		*Count*		*Rate (%)*			*Quality Control Specification*
Analytical Methods	*Method*	*QC*	*Regular*	***Actual***	***Expected***	*Evaluation*	
Laboratory Duplicates (DUP)							
Acidity as Calcium Carbonate	ED038	1	7	**14.3**	**10.0**	✓	NEPM 1999 Schedule B(3) and ALS QCS3 requirement
Alkalinity by PC Titrator	ED037-P	3	24	**12.5**	**10.0**	✓	NEPM 1999 Schedule B(3) and ALS QCS3 requirement
Ammonia as N by Discrete analyser	EK055G	2	20	**10.0**	**10.0**	✓	NEPM 1999 Schedule B(3) and ALS QCS3 requirement
Chloride by Discrete Analyser	ED045G	2	20	**10.0**	**10.0**	✓	NEPM 1999 Schedule B(3) and ALS QCS3 requirement
Dissolved Organic Carbon	EP002	2	20	**10.0**	**10.0**	✓	NEPM 1999 Schedule B(3) and ALS QCS3 requirement
Major Cations - Dissolved	ED093F	2	20	**10.0**	**10.0**	✓	NEPM 1999 Schedule B(3) and ALS QCS3 requirement
Nitrite and Nitrate as N (NOx) by Discrete Analyser	EK059G	2	20	**10.0**	**10.0**	✓	NEPM 1999 Schedule B(3) and ALS QCS3 requirement
Nitrite as N by Discrete Analyser	EK057G	2	20	**10.0**	**10.0**	✓	NEPM 1999 Schedule B(3) and ALS QCS3 requirement
Reactive Phosphorus as P-By Discrete Analyser	EK071G	2	11	**18.2**	**10.0**	✓	NEPM 1999 Schedule B(3) and ALS QCS3 requirement
Sulfate (Turbidimetric) as SO4 2- by Discrete Analyser	ED041G	2	20	**10.0**	**10.0**	✓	NEPM 1999 Schedule B(3) and ALS QCS3 requirement
Total Kjeldahl Nitrogen as N By Discrete Analyser	EK061G	2	11	**18.2**	**10.0**	✓	NEPM 1999 Schedule B(3) and ALS QCS3 requirement
Total Phosphorus as P By Discrete Analyser	EK067G	2	11	**18.2**	**10.0**	✓	NEPM 1999 Schedule B(3) and ALS QCS3 requirement
Laboratory Control Samples (LCS)							
Acidity as Calcium Carbonate	ED038	1	7	**14.3**	**5.0**	✓	NEPM 1999 Schedule B(3) and ALS QCS3 requirement
Alkalinity by PC Titrator	ED037-P	2	24	**8.3**	**5.0**	✓	NEPM 1999 Schedule B(3) and ALS QCS3 requirement
Ammonia as N by Discrete analyser	EK055G	1	20	**5.0**	**5.0**	✓	NEPM 1999 Schedule B(3) and ALS QCS3 requirement
Chloride by Discrete Analyser	ED045G	2	20	**10.0**	**10.0**	✓	NEPM 1999 Schedule B(3) and ALS QCS3 requirement
Dissolved Organic Carbon	EP002	1	20	**5.0**	**5.0**	✓	NEPM 1999 Schedule B(3) and ALS QCS3 requirement
Major Cations - Dissolved	ED093F	1	20	**5.0**	**5.0**	✓	NEPM 1999 Schedule B(3) and ALS QCS3 requirement
Nitrite and Nitrate as N (NOx) by Discrete Analyser	EK059G	1	20	**5.0**	**5.0**	✓	NEPM 1999 Schedule B(3) and ALS QCS3 requirement
Nitrite as N by Discrete Analyser	EK057G	1	20	**5.0**	**5.0**	✓	NEPM 1999 Schedule B(3) and ALS QCS3 requirement
Reactive Phosphorus as P-By Discrete Analyser	EK071G	1	11	**9.1**	**5.0**	✓	NEPM 1999 Schedule B(3) and ALS QCS3 requirement
Sulfate (Turbidimetric) as SO4 2- by Discrete Analyser	ED041G	1	20	**5.0**	**5.0**	✓	NEPM 1999 Schedule B(3) and ALS QCS3 requirement
Total Kjeldahl Nitrogen as N By Discrete Analyser	EK061G	3	11	**27.3**	**15.0**	✓	NEPM 1999 Schedule B(3) and ALS QCS3 requirement
Total Phosphorus as P By Discrete Analyser	EK067G	3	11	**27.3**	**15.0**	✓	NEPM 1999 Schedule B(3) and ALS QCS3 requirement
Method Blanks (MB)							
Acidity as Calcium Carbonate	ED038	1	7	**14.3**	**5.0**	✓	NEPM 1999 Schedule B(3) and ALS QCS3 requirement
Ammonia as N by Discrete analyser	EK055G	1	20	**5.0**	**5.0**	✓	NEPM 1999 Schedule B(3) and ALS QCS3 requirement
Chloride by Discrete Analyser	ED045G	1	20	**5.0**	**5.0**	✓	NEPM 1999 Schedule B(3) and ALS QCS3 requirement
Dissolved Organic Carbon	EP002	1	20	**5.0**	**5.0**	✓	NEPM 1999 Schedule B(3) and ALS QCS3 requirement
Major Cations - Dissolved	ED093F	1	20	**5.0**	**5.0**	✓	NEPM 1999 Schedule B(3) and ALS QCS3 requirement
Nitrite and Nitrate as N (NOx) by Discrete Analyser	EK059G	1	20	**5.0**	**5.0**	✓	NEPM 1999 Schedule B(3) and ALS QCS3 requirement
Nitrite as N by Discrete Analyser	EK057G	1	20	**5.0**	**5.0**	✓	NEPM 1999 Schedule B(3) and ALS QCS3 requirement
Reactive Phosphorus as P-By Discrete Analyser	EK071G	1	11	**9.1**	**5.0**	✓	NEPM 1999 Schedule B(3) and ALS QCS3 requirement
Sulfate (Turbidimetric) as SO4 2- by Discrete Analyser	ED041G	1	20	**5.0**	**5.0**	✓	NEPM 1999 Schedule B(3) and ALS QCS3 requirement
Total Kjeldahl Nitrogen as N By Discrete Analyser	EK061G	1	11	**9.1**	**5.0**	✓	NEPM 1999 Schedule B(3) and ALS QCS3 requirement
Total Phosphorus as P By Discrete Analyser	EK067G	1	11	**9.1**	**5.0**	✓	NEPM 1999 Schedule B(3) and ALS QCS3 requirement
Matrix Spikes (MS)							
Ammonia as N by Discrete analyser	EK055G	1	20	**5.0**	**5.0**	✓	ALS QCS3 requirement

Matrix: **WATER**

Evaluation: × = Quality Control frequency not within specification ; ✓ = Quality Control frequency within specification.

Quality Control Sample Type		Count		Rate (%)			Quality Control Specification
Analytical Methods	*Method*	*QC*	*Regular*	***Actual***	***Expected***	*Evaluation*	
Matrix Spikes (MS) - Continued							
Chloride by Discrete Analyser	ED045G	1	20	**5.0**	**5.0**	✓	ALS QCS3 requirement
Dissolved Organic Carbon	EP002	1	20	**5.0**	**5.0**	✓	ALS QCS3 requirement
Nitrite and Nitrate as N (NOx) by Discrete Analyser	EK059G	1	20	**5.0**	**5.0**	✓	ALS QCS3 requirement
Nitrite as N by Discrete Analyser	EK057G	1	20	**5.0**	**5.0**	✓	ALS QCS3 requirement
Reactive Phosphorus as P-By Discrete Analyser	EK071G	1	11	**9.1**	**5.0**	✓	ALS QCS3 requirement
Sulfate (Turbidimetric) as SO4 2- by Discrete Analyser	ED041G	1	20	**5.0**	**5.0**	✓	ALS QCS3 requirement
Total Kjeldahl Nitrogen as N By Discrete Analyser	EK061G	1	11	**9.1**	**5.0**	✓	ALS QCS3 requirement
Total Phosphorus as P By Discrete Analyser	EK067G	1	11	**9.1**	**5.0**	✓	ALS QCS3 requirement

Brief Method Summaries

The analytical procedures used by the Environmental Division have been developed from established internationally recognized procedures such as those published by the US EPA, APHA, AS and NEPM. In house developed procedures are employed in the absence of documented standards or by client request. The following report provides brief descriptions of the analytical procedures employed for results reported in the Certificate of Analysis. Sources from which ALS methods have been developed are provided within the Method Descriptions.

Analytical Methods	*Method*	*Matrix*	*Method Descriptions*
Alkalinity by PC Titrator	ED037-P	WATER	APHA 21st ed., 2320 B This procedure determines alkalinity by automated measurement (e.g. PC Titrate) using pH 4.5 for indicating the total alkalinity end-point. This method is compliant with NEPM (1999) Schedule B(3) (Appdx. 2)
Acidity as Calcium Carbonate	ED038	WATER	APHA 21st ed., 2310 B Acidity is determined by titration with a standardised alkali to an end-point pH of 8.3. This method is compliant with NEPM (1999) Schedule B(3) (Appdx. 2)
Sulfate (Turbidimetric) as SO4 2- by Discrete Analyser	ED041G	WATER	APHA 21st ed., 4500-SO4 Dissolved sulfate is determined in a 0.45um filtered sample. Sulfate ions are converted to a barium sulfate suspension in an acetic acid medium with barium chloride. Light absorbance of the BaSO4 suspension is measured by a photometer and the SO4-2 concentration is determined by comparison of the reading with a standard curve. This method is compliant with NEPM (1999) Schedule B(3) (Appdx. 2)
Chloride by Discrete Analyser	ED045G	WATER	APHA 21st ed., 4500 Cl - G.The thiocyanate ion is liberated from mercuric thiocyanate through sequestration of mercury by the chloride ion to form non-ionised mercuric chloride.in the presence of ferric ions the librated thiocynate forms highly-coloured ferric thiocynate which is measured at 480 nm APHA 21st edition seal method 2 017-1-L april 2003
Major Cations - Dissolved	ED093F	WATER	Major Cations is determined based on APHA 21st ed., 3120; USEPA SW 846 - 6010 The ICPAES technique ionises the 0.45um filtered sample atoms emitting a characteristic spectrum. This spectrum is then compared against matrix matched standards for quantification. This method is compliant with NEPM (1999) Schedule B(3) (Appdx. 2) Sodium Absorption Ratio is calculated from Ca, Mg and Na which determined by ALS in house method QWI-EN/ED093F. This method is compliant with NEPM (1999) Schedule B(3) (Appdx. 2) Hardness parameters are calculated based on APHA 21st ed., 2340 B. This method is compliant with NEPM (1999) Schedule B(3) (Appdx. 2)
Ammonia as N by Discrete analyser	EK055G	WATER	APHA 21st ed., 4500-NH3 G Ammonia is determined by direct colorimetry by Discrete Analyser. This method is compliant with NEPM (1999) Schedule B(3) (Appdx. 2)
Nitrite as N by Discrete Analyser	EK057G	WATER	APHA 21st ed., 4500-NO2- B. Nitrite is determined by direct colourimetry by Discrete Analyser. This method is compliant with NEPM (1999) Schedule B(3) (Appdx. 2)
Nitrate as N by Discrete Analyser	EK058G	WATER	APHA 21st ed., 4500-NO3- F. Nitrate is reduced to nitrite by way of a chemical reduction followed by quantification by Discrete Analyser. Nitrite is determined seperately by direct colourimetry and result for Nitrate calculated as the difference between the two results. This method is compliant with NEPM (1999) Schedule B(3) (Appdx. 2)
Nitrite and Nitrate as N (NOx) by Discrete Analyser	EK059G	WATER	APHA 21st ed., 4500-NO3- F. Combined oxidised Nitrogen (NO2+NO3) is determined by Chemical Reduction and direct colourimetry by Discrete Analyser. This method is compliant with NEPM (1999) Schedule B(3) (Appdx. 2)
Total Kjeldahl Nitrogen as N By Discrete Analyser	EK061G	WATER	APHA 21st ed., 4500-Norg D. 25mL water samples are digested using a traditional Kjeldahl digestion followed by determination by Discrete Analyser. This method is compliant with NEPM (1999) Schedule B(3) (Appdx. 2)
Total Nitrogen as N (TKN + Nox) By Discrete Analyser	EK062G	WATER	APHA 21st ed., 4500-Norg / 4500-NO3-. This method is compliant with NEPM (1999) Schedule B(3) (Appdx. 2)
Total Phosphorus as P By Discrete Analyser	EK067G	WATER	APHA 21st ed., 4500-P B&F This procedure involves sulphuric acid digestion of a 100mL sample to break phosphorus down to orthophosphate. The orthophosphate reacts with ammonium molybdate and antimony potassium tartrate to form a complex which is then reduced and its concentration measured at 880nm using Discrete Analyser. This method is compliant with NEPM (1999) Schedule B(3) (Appdx. 2)

Analytical Methods	*Method*	*Matrix*	*Method Descriptions*
Reactive Phosphorus as P-By Discrete Analyser	EK071G	WATER	APHA 21st ed., 4500-P F Ammonium molybdate and potassium antimonyl tartrate reacts in acid medium with othophosphate to form a heteropoly acid -phosphomolybdic acid - which is reduced to intensely coloured molybdenum blue by ascorbic acid. Quantification is by Discrete Analyser. This method is compliant with NEPM (1999) Schedule B(3) (Appdx. 2)
Ionic Balance by PCT DA and Turbi SO4 DA	EN055 - PG	WATER	APHA 21st Ed. 1030F. The Ionic Balance is calculated based on the major Anions and Cations. The major anions include Alkalinity, Chloride and Sulfate which determined by PCT and DA. The Cations are determined by Turbi SO4 by DA. This method is compliant with NEPM (1999) Schedule B(3) (Appdx. 2)
Dissolved Organic Carbon	EP002	WATER	APHA 21st ed., 5310 B. This method is compliant with NEPM (1999) Schedule B(3) (Appdx. 2)

Summary of Outliers

Outliers : Quality Control Samples

The following report highlights outliers flagged in the Quality Control (QC) Report. Surrogate recovery limits are static and based on USEPA SW846 or ALS-QWI/EN/38 (in the absence of specific USEPA limits). This report displays QC Outliers (breaches) only.

Duplicates, Method Blanks, Laboratory Control Samples and Matrix Spikes

Matrix: **WATER**

Compound Group Name	Laboratory Sample ID	Client Sample ID	Analyte	CAS Number	Data	Limits	Comment
Matrix Spike (MS) Recoveries							
ED041G: Sulfate (Turbidimetric) as SO4 2- by DA	ES1226467-001	Anonymous	**Sulfate as SO4 - Turbidimetric**	14808-79-8	Not Determined	----	**MS recovery not determined, background level greater than or equal to 4x spike level.**

- **For all matrices, no Method Blank value outliers occur.**
- **For all matrices, no Duplicate outliers occur.**
- **For all matrices, no Laboratory Control outliers occur.**

Regular Sample Surrogates

- **For all regular sample matrices, no surrogate recovery outliers occur.**

Outliers : Analysis Holding Time Compliance

This report displays Holding Time breaches only. Only the respective Extraction / Preparation and/or Analysis component is/are displayed.

Matrix: **WATER**

Method	*Extraction / Preparation*			*Analysis*		
Container / Client Sample ID(s)	*Date extracted*	*Due for extraction*	***Days overdue***	*Date analysed*	*Due for analysis*	***Days overdue***
ED037P: Alkalinity by PC Titrator						
Clear Plastic Bottle - Natural RIVER MURRAY, WELLINGTON, WOODS PT, TOORA, MOBILING	----	----	----	09-NOV-2012	06-NOV-2012	**3**
ED038A: Acidity						
Clear Plastic Bottle - Natural RIVER MURRAY, WELLINGTON, WOODS PT, TOORA, MOBILING	----	----	----	14-NOV-2012	06-NOV-2012	**8**
ED093F: Dissolved Major Cations						
Clear Plastic Bottle - Natural RIVER MURRAY, WELLINGTON, WOODS PT, TOORA, MOBILING	----	----	----	09-NOV-2012	30-OCT-2012	**10**
EK057G: Nitrite as N by Discrete Analyser						

Matrix: **WATER**

Method **Container** / Client Sample ID(s)	*Extraction / Preparation* *Date extracted*	 *Due for extraction*	 ***Days overdue***	*Analysis* *Date analysed*	 *Due for analysis*	 ***Days overdue***
EK057G: Nitrite as N by Discrete Analyser - Analysis Holding Time Compliance						
Clear Plastic Bottle - Natural RIVER MURRAY, WELLINGTON, WOODS PT, TOORA, MOBILING	----	----	----	09-NOV-2012	25-OCT-2012	**15**
EK071G: Reactive Phosphorus as P by discrete analyser						
Clear Plastic Bottle - Natural RIVER MURRAY, WELLINGTON, WOODS PT, TOORA, MOBILING	----	----	----	09-NOV-2012	25-OCT-2012	**15**

Outliers : Frequency of Quality Control Samples

The following report highlights breaches in the Frequency of Quality Control Samples.

- **No Quality Control Sample Frequency Outliers exist.**

Dissolved metals in River Murray water

Laboratory I.D.:	Client I.D.	Date sampled:	Al 167.019	As 188.980	B 249.678	Ba 233.527	Cd 228.802	Co 228.615	Cr 205.560	Cu 324.754	Fe 234.350	Mn 257.610	Mo 202.032	Ni 231.604	Pb 220.353	Sr 421.552	Ti 336.122	Ti 337.280	V 292.401	Zn 213.857
LOD (3σ)			*0.1*	*1*	*7*	*0.05*	*0.2*	*0.9*	*0.2*	*1*	*0.4*	*0.1*	*0.1*	*0.5*	*0.2*	*0.1*	*0.03*	*0.03*	*0.2*	*0.4*
TM 24.3	Reference material		34	5	13	13	4	7	5	7	15	8	6	5	6	106	7	7	7	27
TM 24.3 Certified			34.4 ± 5.2	5.21 ± 0.53	15.9 ± 3	13.2 ± 0.8	3.97 ± 0.37	6.29 ± 0.5	5.01 ± 0.49	6.79 ± 0.64	15.4 ± 4.2	8.12 ± 0.72	6.18 ± 0.61	5.12 ± 0.61	5.82 ± 0.45	110 ± 6.2	7.3 ± 0.85	7.3 ± 0.85	7.03 ± 0.51	23.5 ± 3.6
% Recovery			***100***	***95***	***79***	***99***	***102***	***105***	***100***	***97***	***98***	***99***	***98***	***99***	***104***	***97***	***90***	***91***	***101***	***113***
TMDA 52.3	Reference material		305	23	7	144	91	132	168	195	398	193	207	271	358	281	118	119	138	260
TMDA 52.3 Certified			310±25	25.4±3.3	10.7±2.4	148±10.7	90.9±8.1	136±9.1	165±12	197±15	412±38.3	198±14	207±15	274±20	358±29	286±20	120±8	120±8	145±11	263±25
% Recovery			***98***	***92***	***68***	***98***	***100***	***97***	***102***	***99***	***97***	***98***	***100***	***99***	***93***	***98***	***98***	***99***	***95***	***99***
CE250-1	Murray River 1	22/11/2012	47	2	15	27	<0.2	<1	<0.2	2	129	1	0.2	1.5	0.3	82	3.6	3.6	1.5	3
CE250-2	Murray River 2	22/11/2012	81	1	14	26	<0.2	<1	0.3	3	154	2	0.4	1.4	0.5	83	4.8	4.7	1.3	3
CE250-3	Murray River 3	22/11/2012	71	1	13	26	<0.2	<1	0.2	2	152	2	0.3	1.6	1.2	82	4.8	4.7	1.9	3
CE250-4	Murray River 4	22/11/2012	45	2	12	27	<0.2	<1	0.3	2	133	2	0.4	1.6	0.3	83	3.5	3.4	1.7	2
CE250-5	Murray River 5	22/11/2012	77	2	12	27	<0.2	<1	<0.2	2	159	2	0.4	1.5	0.6	82	5.0	4.9	1.7	2
CE250-6	Murray River 6	22/11/2012	84	1	12	27	<0.2	<1	0.2	2	164	2	0.3	1.6	0.3	83	5.0	4.9	1.8	3
CE250-7	Murray River 7	22/11/2012	64	1	10	27	<0.2	<1	0.2	2	151	2	0.3	1.3	0.7	82	4.4	4.3	1.5	2
CE250-8	Murray River 8	22/11/2012	61	1	9	27	<0.2	<1	0.3	2	146	2	0.4	1.5	0.9	83	4.3	4.2	1.5	2
CE250-9	Murray River 9	22/11/2012	63	1	12	27	<0.2	<1	0.2	2	148	2	0.2	1.4	0.8	83	4.2	4.1	1.8	2
CE250-10	Murray River 10	22/11/2012	104	1	10	27	<0.2	<1	<0.2	3	181	2	0.3	1.8	0.8	83	5.7	5.7	1.8	6
CE250-10 Dup	Murray River 10	22/11/2012	105	0	13	27	<0.2	<1	0.2	3	179	2	0.3	1.6	0.4	83	5.6	5.6	1.7	6
CE250-10 Avg	***Murray River 10 Avg***	***22/11/2012***	***104.9***	***1***	***11***	***26.9***	<0.2	<1	***0.2***	***3***	***180.0***	***2***	***0.3***	***1.7***	***0.6***	***83.0***	***5.7***	***5.6***	***1.8***	***6***
CE250-11	Murray River 11	22/11/2012	12	1	11	26	<0.2	<1	<0.2	6	54	1	0.2	1.3	0.5	82	1.2	1.1	0.4	4
CE250-12	Murray River 12	22/11/2012	53	1	8	27	<0.2	<1	0.3	1	138	2	0.3	1.2	0.5	82	3.7	3.7	1.6	2
CE250-13	Murray River 13	22/11/2012	27	0	9	27	<0.2	<1	<0.2	5	94	1	0.3	1.4	0.6	83	2.2	2.3	0.9	3
CE250-14	Murray River 14	22/11/2012	81	1	10	27	<0.2	<1	0.2	2	162	2	0.2	1.6	0.1	82	5.0	4.9	1.7	2
CE250-15	Murray River 15	22/11/2012	56	0	12	26	<0.2	<1	0.2	3	139	2	0.3	1.4	0.7	82	3.9	3.9	1.6	2
CE250-16	Murray River 16	22/11/2012	53	1	11	28	<0.2	<1	0.3	2	144	2	0.3	1.6	-0.2	84	4.1	4.0	1.6	2
CE250-17	Murray River 17	22/11/2012	41	1	11	27	<0.2	<1	0.2	2	132	2	0.1	1.8	-0.2	84	3.6	3.6	1.8	2
CE250-18	Murray River 18	22/11/2012	81	1	10	27	<0.2	<1	0.3	2	163	2	0.4	1.6	0.1	83	4.8	4.7	1.9	2
CE250-19	Murray River 19	22/11/2012	51	1	11	27	<0.2	1	0.2	2	138	2	0.3	1.5	0.5	83	3.8	3.8	1.8	3
CE250-20	Murray River 20	22/11/2012	40	1	13	27	<0.2	<1	0.3	2	128	2	0.3	1.4	-0.2	84	3.4	3.3	1.8	2
CE250-20 Dup	Murray River 20	22/11/2012	39	1	12	27	<0.2	<1	0.3	2	128	2	0.3	1.5	0.1	83	3.4	3.4	1.6	2
CE250-20 Avg	***Murray River 20 Avg***	***22/11/2012***	***39.8***	***1***	***13***	***27.1***	<0.2	<1	***0.3***	***2***	***128.1***	***2***	***0.3***	***1.5***	***0.0***	***83.3***	***3.4***	***3.4***	***1.7***	***2***
CE250-21	Murray River 21	22/11/2012	42	1	9	27	<0.2	<1	0.2	2	128	2	0.4	1.5	0.4	84	3.7	3.6	1.6	3
CE250-22	Murray River 22	22/11/2012	45	1	10	28	<0.2	1	0.2	2	135	1	0.3	1.3	0.5	83	3.7	3.7	1.7	1

Batch: CE250
Analysts: Joshua King and Chad Jarolimek
Date: 18-02-2013

Murray River	**Al**	**As**	**B**	**Ba**	**Cd**	**Co**	**Cr**	**Cu**	**Fe**	**Mn**	**Mo**	**Ni**	**Pb**	**Sr**	**Ti (336)**	**Ti (337)**	**V**	**Zn**
Average	58	1.1	11	27	<0.2	<1	0.2	2.4	140	1.6	0.3	1.5	0.4	83	4.0	4.0	1.6	2.6
1 SD	21	0.5	2	0.5	–	–	0.1	1.1	26	0.3	0.1	0.1	0.3	1	1.0	1.0	0.3	1.0

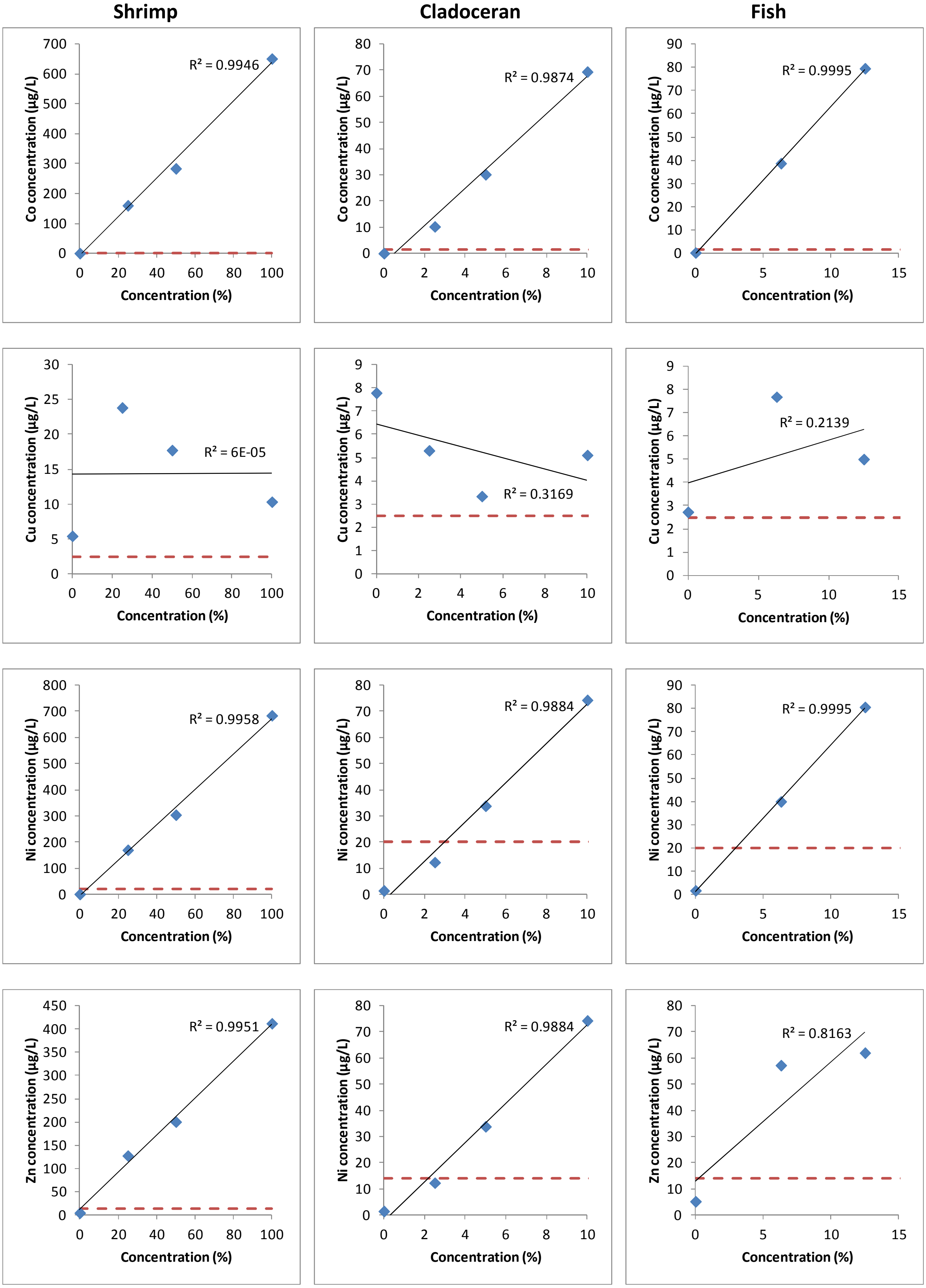
Dissolved metal concentrations in toxicity test solutions

		Al 167.0 Conc. [ug/l] ICP-AES	51 V [2] Conc. [ug/l] ICP-MS	52 Cr [2] Conc. [ug/l] ICP-MS	55 Mn [1] Conc. [ug/l] ICP-MS	Fe 258.6 Conc. [ug/l] ICP-AES	59 Co [2] Conc. [ug/l] ICP-MS	60 Ni [2] Conc. [ug/l] ICP-MS	63 Cu [2] Conc. [ug/l] ICP-MS	66 Zn [2] Conc. [ug/l] ICP-MS	75 As [2] Conc. [ug/l] ICP-MS	78 Se [1] Conc. [ug/l] ICP-MS	95 Mo [2] Conc. [ug/l] ICP-MS	95 Mo [3] Conc. [ug/l] ICP-MS	107 Ag [3] Conc. [ug/l] ICP-MS	111 Cd [3] Conc. [ug/l] ICP-MS	205 Tl [3] Conc. [ug/l] ICP-MS	208 Pb [3] Conc. [ug/l] ICP-MS
	LOD (3σ)	*0.1*	*0.2*	*0.1*	*1*	*0.21*	*0.1*	*0.1*	*3*	*1*	*0.7*	*0.3*	*0.3*	*0.3*	*0.1*	*0.1*	*0.02*	*0.1*
Avg TM24.3		***35.1***	***7.0***	***4.8***	***7.3***	***15.1***	***6.2***	***5.1***	***6.5***	***25.6***	***5.0***	***3.4***	***6.1***	***6.4***	***2.3***	***3.8***	***4.1***	***5.7***
TM 24.3 Certified		34.4 ± 5.2	7.03 ± 0.51	5.01 ± 0.49	8.12 ± 0.72	15.4 ± 4.2	6.29 ± 0.5	5.12 ± 0.61	6.79 ± 0.64		5.21 ± 0.53	3.42 ± 0.55	6.18 ± 0.61	6.18 ± 0.61		3.97 ± 0.37	4.18 ± 0.38	5.82 ± 0.45
% Recovery		***102***	***100***	***96***	***90***	***98***	***99***	***100***	***95***		***96***	***99***	***99***	***103***		***95***	***99***	***98***
Sample Name	Sample ID																	
CE251-1	Shrimp - Murray Rv (0h) 29/10	48	1.6	0.14	10	143	0.61	1.85	5	4	0.5	0.3	0.4	0.4	<0.1	<0.1	<0.02	0.3
CE251-2	Shrimp - Murray Rv (0h) 5/11	800	2.5	0.43	3	668	0.13	1.59	5	5	1.7	<0.3	<0.3	0.3	<0.1	0.1	<0.02	0.9
CE251-3	Shrimp - Toora 25% 0h	56	0.2	0.40	939	3418	69	70	4	42	1.2	<0.3	<0.3	0.2	<0.1	0.1	0.18	0.2
CE251-4	Shrimp - Toora 50% 0h	6	<0.2	0.68	2023	9014	155	154	7	96	0.8	<0.3	<0.3	<0.3	<0.1	0.4	0.20	0.2
CE251-5	Shrimp - Toora 100% 0h	446	0.2	1.21	4175	21278	319	326	10	189	2.8	<0.3	<0.3	<0.3	<0.1	0.6	0.16	1.5
CE251-6	Shrimp - Mobilong 25% 0h	3920	<0.2	0.97	2443	924	160	170	24	128	2.4	<0.3	<0.3	<0.3	<0.1	0.7	0.11	2.8
CE251-7	Shrimp - Mobilong 50% 0h	6963	0.3	1.97	4338	6228	284	304	18	201	4.6	0.6	<0.3	<0.3	<0.1	1.3	0.20	4.1
CE251-8	Shrimp - Mobilong 100% 0h	14303	0.4	4.00	9263	37290	651	684	10	412	11.7	0.8	<0.3	<0.3	<0.1	2.7	0.44	4.2
CE251-9	Shrimp - Woods Point 25% 0h	60	1.3	0.16	607	104	26	36	10	33	0.6	<0.3	<0.3	<0.3	<0.1	0.1	0.02	0.3
CE251-10	Shrimp - Woods Point 50% 0h	24	0.5	0.07	1119	41	48	65	11	50	1.2	<0.3	<0.3	<0.3	<0.1	0.2	0.04	0.2
CE251-10 Dup	Shrimp - Woods Point 50% 0h Dup	24	0.5	0.13	1109	40	49	66	10	52	1.1	<0.3	<0.3	<0.3	<0.1	0.2	0.04	0.2
CE251-11	Shrimp - Woods Point 100% 0h	63	0.2	0.31	2191	33	95	126	15	108	2.0	<0.3	<0.3	<0.3	<0.1	4.0	0.10	1.3
CE251-12	Shrimp - Wellington 25% 0h	11	0.4	0.13	827	44	34	33	4	27	0.3	<0.3	<0.3	<0.3	<0.1	0.1	0.02	0.1
CE251-13	Shrimp - Wellington 50% 0h	26	<0.2	0.27	1371	42	58	54	3	41	0.6	<0.3	<0.3	<0.3	<0.1	0.2	0.03	0.1
CE251-14	Shrimp - Wellington 100% 0h	428	0.3	0.61	2796	215	106	101	7	83	1.8	<0.3	<0.3	<0.3	<0.1	0.3	0.07	0.9
CE251-15	Cerio A - Toora MR 0h	83	1.9	0.01	3	171	0.06	1.32	7	12	1.9	<0.3	<0.3	<0.3	<0.1	<0.1	<0.02	0.7
CE251-16	Cerio A - Toora 2.5% 0h	6	0.8	-0.04	120	82	7.8	8.8	9	14	0.9	<0.3	<0.3	<0.3	<0.1	<0.1	<0.02	0.2
CE251-17	Cerio A - Toora 5% 0h	4	0.4	0.05	223	65	16	17	10	22	0.7	0.4	<0.3	<0.3	<0.1	<0.1	<0.02	0.2
CE251-18	Cerio A - Toora 10% 0h	2	<0.2	0.07	419	248	31	32	8	28	0.4	<0.3	<0.3	<0.3	<0.1	0.1	0.02	<0.1
CE251-19	Cerio A - Mobilong MR 0h	61	1.8	0.04	3	151	0.09	1.49	8	12	1.0	<0.3	0.3	<0.3	<0.1	<0.1	<0.02	0.4
CE251-20	Cerio A - Mobilong 2.5% 0h	29	0.4	0.00	186	40	10	13	5	9	0.4	<0.3	<0.3	<0.3	<0.1	0.1	<0.02	0.1
CE251-20 Dup	Cerio A - Mobilong 2.5% 0h Dup	30	0.4	-0.01	185	39	10	12	5	10	0.6	<0.3	<0.3	<0.3	<0.1	<0.1	0.02	0.1
CE251-21	Cerio A - Mobilong 5% 0h	19	0.2	0.12	497	16	30	34	3	22	0.7	<0.3	<0.3	0.3	<0.1	0.2	0.04	0.1
CE251-22	Cerio A - Mobilong 10% 0h	22	<0.2	0.24	1006	33	69	74	5	56	0.9	0.4	<0.3	<0.3	<0.1	0.2	0.05	0.1
CE251-23	Cerio A - Woods Point MR 0h	535	2.6	0.28	6	494	0.12	1.70	3	3	1.5	<0.3	0.3	<0.3	<0.1	0.1	<0.02	0.7
CE251-24	Cerio A - Woods Point 25% 0h	141	1.3	0.20	561	159	25	34	4	22	1.6	<0.3	<0.3	<0.3	<0.1	<0.1	0.02	0.3
CE251-25	Cerio A - Woods Point 50% 0h	51	0.6	0.18	1095	74	50	67	5	45	1.3	<0.3	<0.3	<0.3	<0.1	0.2	0.04	0.1
CE251-26	Cerio A - Woods Point 100% 0h	71	<0.2	0.23	2332	35	101	135	8	88	1.0	<0.3	<0.3	<0.3	<0.1	0.3	0.09	0.1
CE251-27	Cerio A - Wellington MR 0h	74	2.4	0.05	2	157	0.08	1.3	4	2	0.9	<0.3	0.3	0.3	<0.1	<0.1	<0.02	0.3
CE251-28	Cerio A - Wellington 1.6% 0h	41	1.5	-0.02	44	129	1.77	3.1	3	3	0.9	<0.3	<0.3	0.4	<0.1	0.1	0.02	0.4
CE251-29	Cerio A - Wellington 3.1% 0h	94	1.4	0.07	85	157	3.50	4.6	3	5	1.0	<0.3	0.3	0.1	<0.1	0.0	<0.02	0.2
CE251-30	Cerio A - Wellington 6.3% 0h	17	0.8	0.02	175	90	7.25	7.8	3	6	0.3	<0.3	<0.3	0.5	<0.1	0.3	0.03	0.5
CE251-30 Dup	Cerio A - Wellington 6.3% 0h Dup	16	0.8	-0.05	177	90	7.48	8.0	3	5	0.7	0.4	<0.3	<0.3	<0.1	<0.1	<0.02	0.2
CE251-31	Cerio A - Wellington 12.5% 0h	9	0.7	0.03	347	74	15	14	6	13	1.1	0.3	<0.3	<0.3	<0.1	<0.1	<0.02	0.2
CE251-32	Cerio A - Wellington 25% 0h	10	0.4	-0.03	650	52	29	26	3	15	1.0	<0.3	<0.3	<0.3	<0.1	0.1	0.02	0.1
CE251-33	Cerio A - Wellington 50% 0h	15	0.2	0.21	1336	43	58	51	2	33	0.7	<0.3	<0.3	<0.3	<0.1	0.1	0.03	<0.1
CE251-34	Fish - Murray Rv 29/10	94	1.9	0.07	5	174	0.27	1.7	3	5	1.2	<0.3	<0.3	<0.3	<0.1	<0.1	0.02	0.3
CE251-35	Fish - Toora 1% 0h	23	1.4	-0.05	55	120	3.1	4.6	8	8	0.7	<0.3	<0.3	<0.3	<0.1	0.1	<0.02	0.4
CE251-36	Fish - Toora 2% 0h	17	0.9	0.01	96	111	6.0	7.1	9	6	0.5	<0.3	<0.3	<0.3	<0.1	<0.1	<0.02	0.2
CE251-37	Fish - Toora 3.1% 0h	7	0.8	-0.02	150	358	11	12	2	7	0.5	<0.3	<0.3	<0.3	<0.1	<0.1	<0.02	0.1
CE251-38	Fish - Mobilong 3.1% 0h	7474	<0.2	1.90	4024	13075	302	300	8	192	6.5	<0.3	<0.3	<0.3	<0.1	1.5	0.20	5.5
CE251-39	Fish - Mobilong 6.25% 0h	18	<0.2	0.04	553	528	39	40	8	57	0.8	<0.3	<0.3	<0.3	<0.1	0.2	0.04	0.1
CE251-40	Fish - Mobilong 12.5% 0h	55	<0.2	0.13	1083	1696	79	80	5	61	1.0	<0.3	<0.3	<0.3	<0.1	0.3	0.05	0.1
CE251-40 Dup	Fish - Mobilong 12.5% 0h Dup	56	<0.2	0.15	1091	1716	80	81	5	63	0.7	<0.3	<0.3	<0.3	<0.1	0.3	0.04	0.1
CE251-41	Fish - Woods Point 25% 0h	9	0.7	0.02	610	50	28	37	3	39	1.2	<0.3	<0.3	<0.3	<0.1	0.1	0.01	<0.1
CE251-42	Fish - Woods Point 50% 0h	14	0.6	0.02	1110	86	53	67	10	51	0.6	<0.3	<0.3	<0.3	<0.1	0.1	0.04	<0.1
CE251-43	Fish - Woods Point 100% 0h	48	<0.2	0.13	2146	290	102	127	11	95	1.6	<0.3	<0.3	<0.3	<0.1	0.3	0.08	0.1
CE251-44	Fish - Wellington 1% 0h	243	2.1	0.01	21	278	0.74	2.48	11	6	1.0	<0.3	<0.3	<0.3	<0.1	<0.1	<0.02	1.1
CE251-45	Fish - Wellington 2% 0h	17	1.2	-0.01	252	101	9.9	11	5	12	0.7	<0.3	<0.3	<0.3	<0.1	<0.1	<0.02	0.2
CE251-46	Fish - Wellington 3.1% 0h	31	1.5	0.02	90	120	3.80	5.10	11	15	0.4	<0.3	<0.3	<0.3	<0.1	<0.1	<0.02	0.4
CE251-9 Spk Rec (%)	Shrimp - Woods Point 25% 0h	***98***	***107***	***107***	***81***	***81***	***106***	***106***	***106***	***103***	***105***	***110***	***104***	***105***	***94***	***99***	***100***	***100***
CE251-19 Spk Rec (%)	Cerio A - Mobilong MR 0h	***104***	***100***	***101***	***94***	***94***	***102***	***102***	***102***	***97***	***102***	***113***	***100***	***107***	***103***	***101***	***102***	***102***
CE251-29 Spk Rec (%)	Cerio A - Wellington 3.1% 0h	***110***	***101***	***97***	***94***	***95***	***96***	***98***	***100***	***98***	***97***	***98***	***97***	***99***	***94***	***97***	***97***	***96***
CE251-39 Spk Rec (%)	Fish - Mobilong 6.25% 0h	***92***	***102***	***96***	***87***	***118***	***96***	***98***	***99***	***97***	***96***	***98***	***99***	***99***	***92***	***95***	***94***	***93***

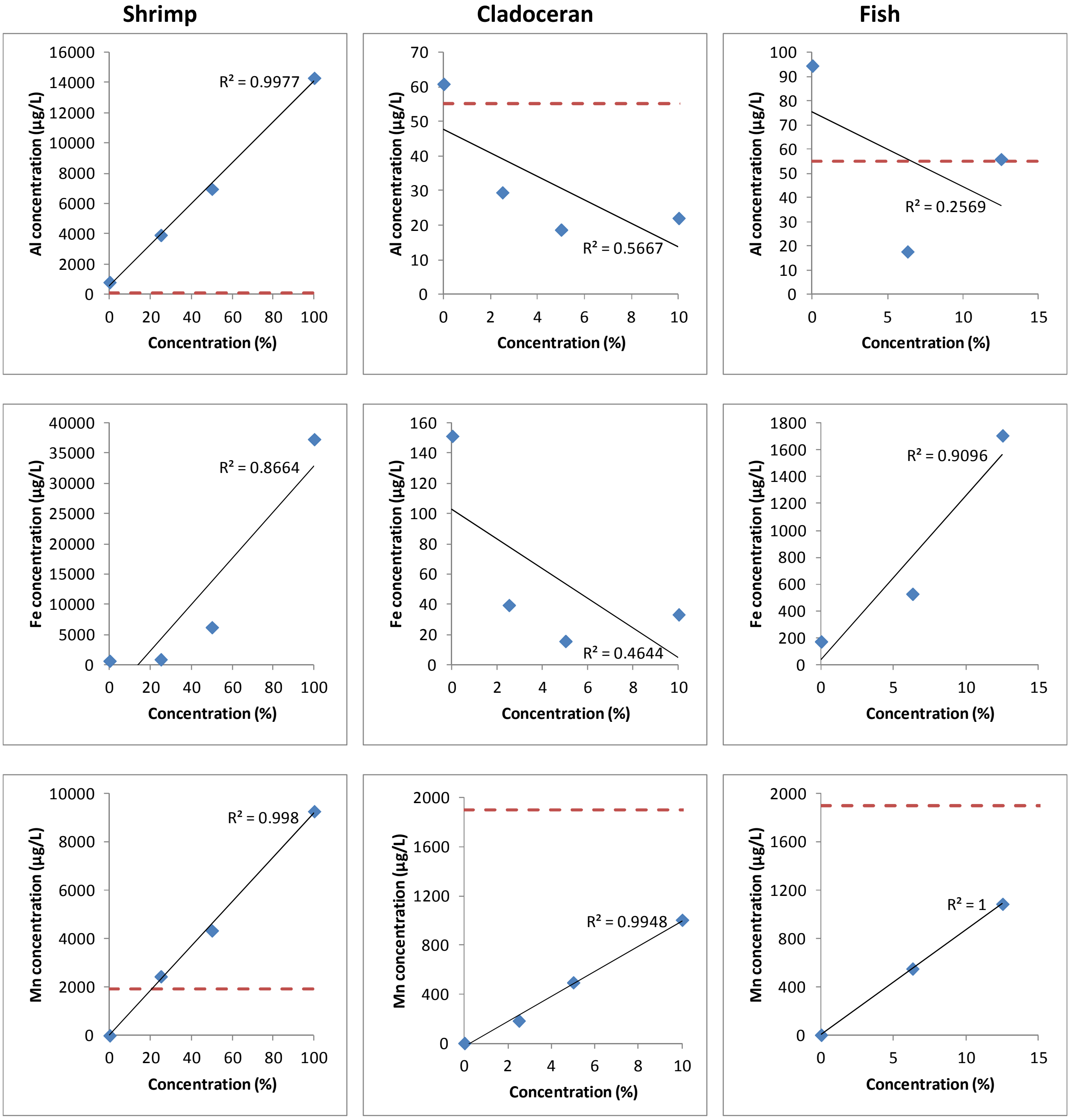
Analysts: Joshua King and Chad Jarolimek
Date: 18-02-13
Batch: CE251

Dissolved metal concentrations in toxicity test solutions

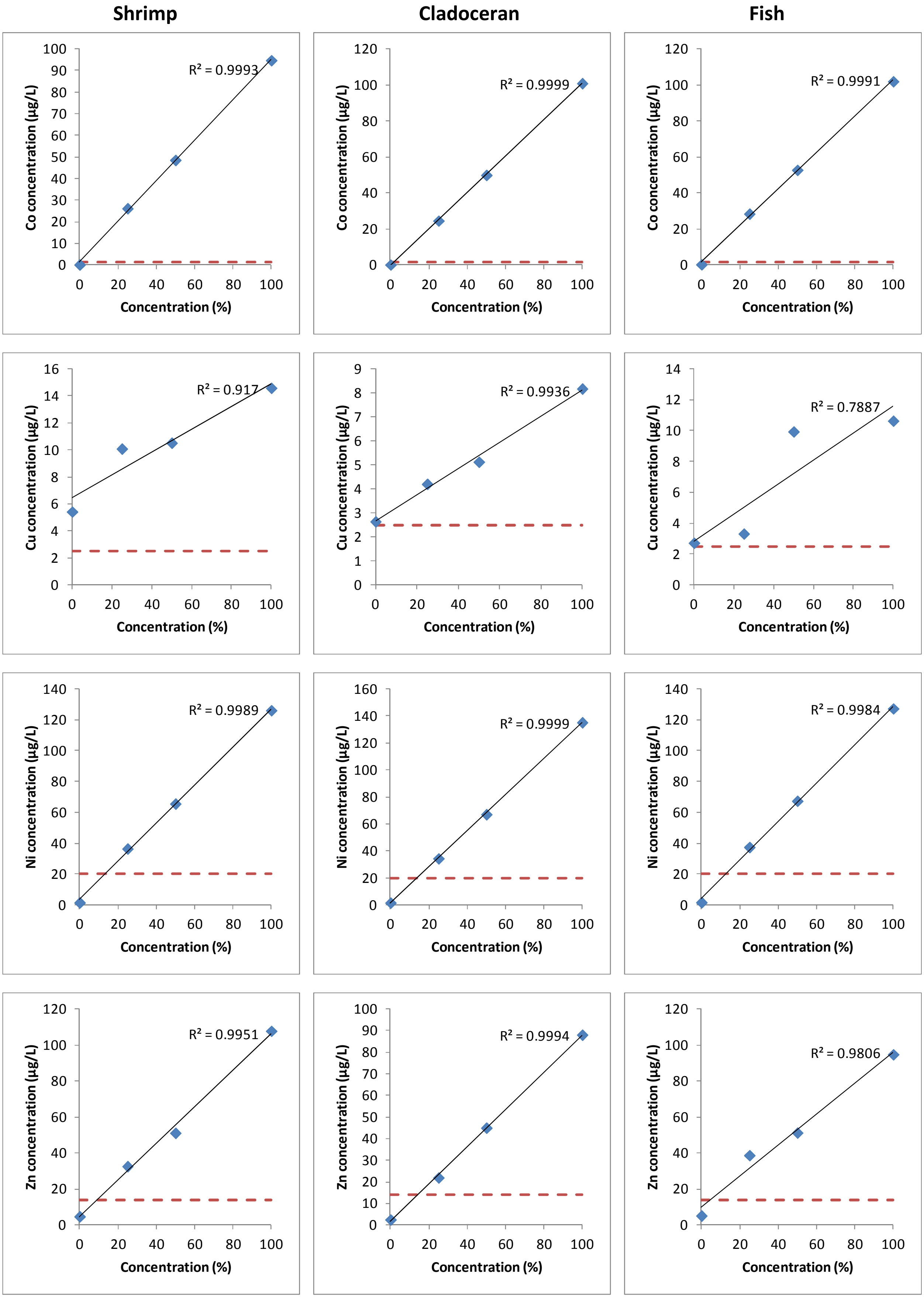
Relationship between concentration of **Mobilong** acid drainage water and concentration of dissolved metals (Co, Cu, Ni, Zn) in control (0%) and three concentrations of acid drainage water in toxicity tests with shrimp, cladocerans and fish. Red lines show the water quality trigger value.



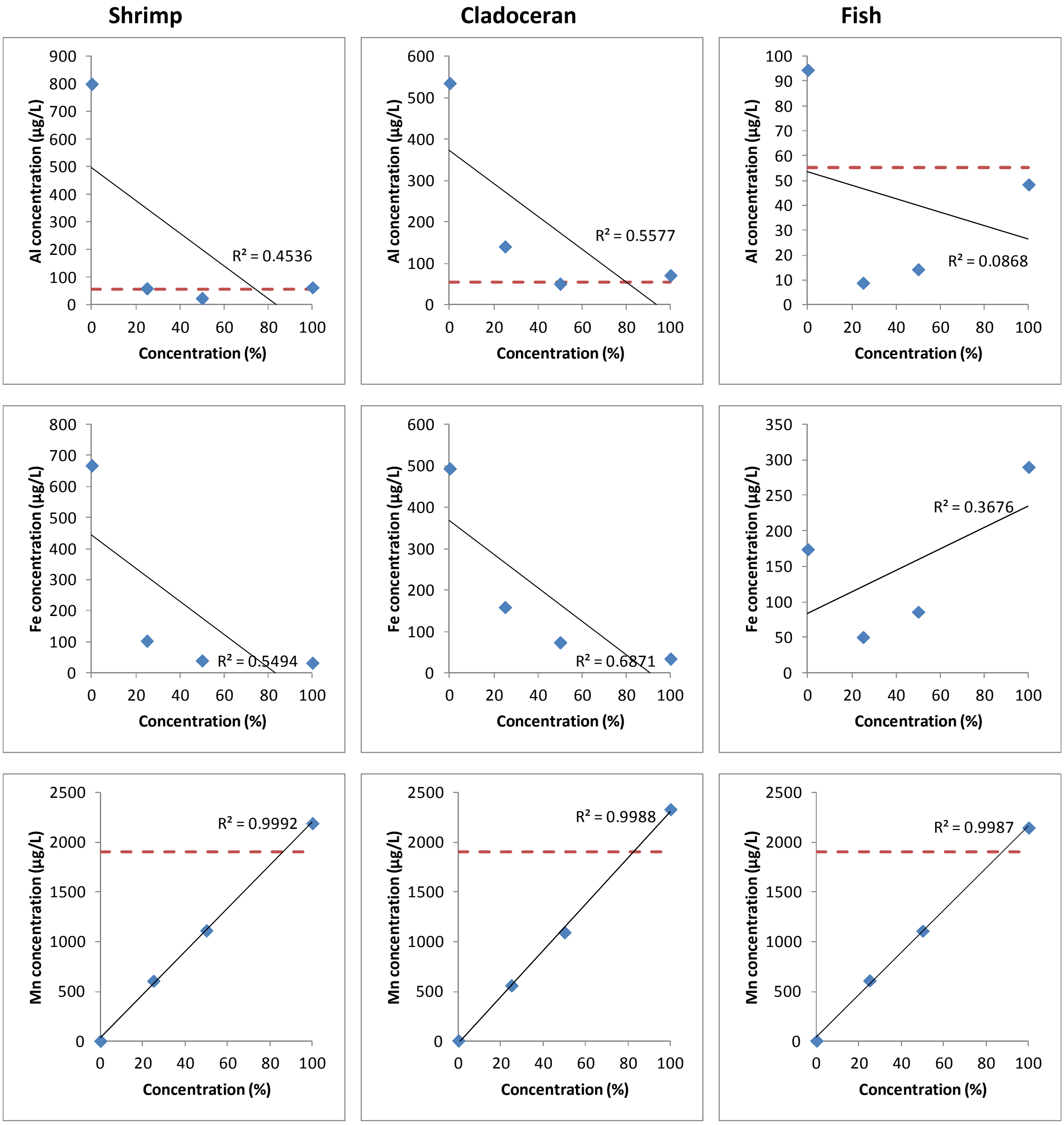
Relationship between concentration of **Mobilong** acid drainage water and concentration of dissolved metals (Al, Fe and Mn) in control (0%) and three concentrations of acid drainage water in toxicity tests with shrimp, cladocerans and fish. Red lines show the water quality trigger value.



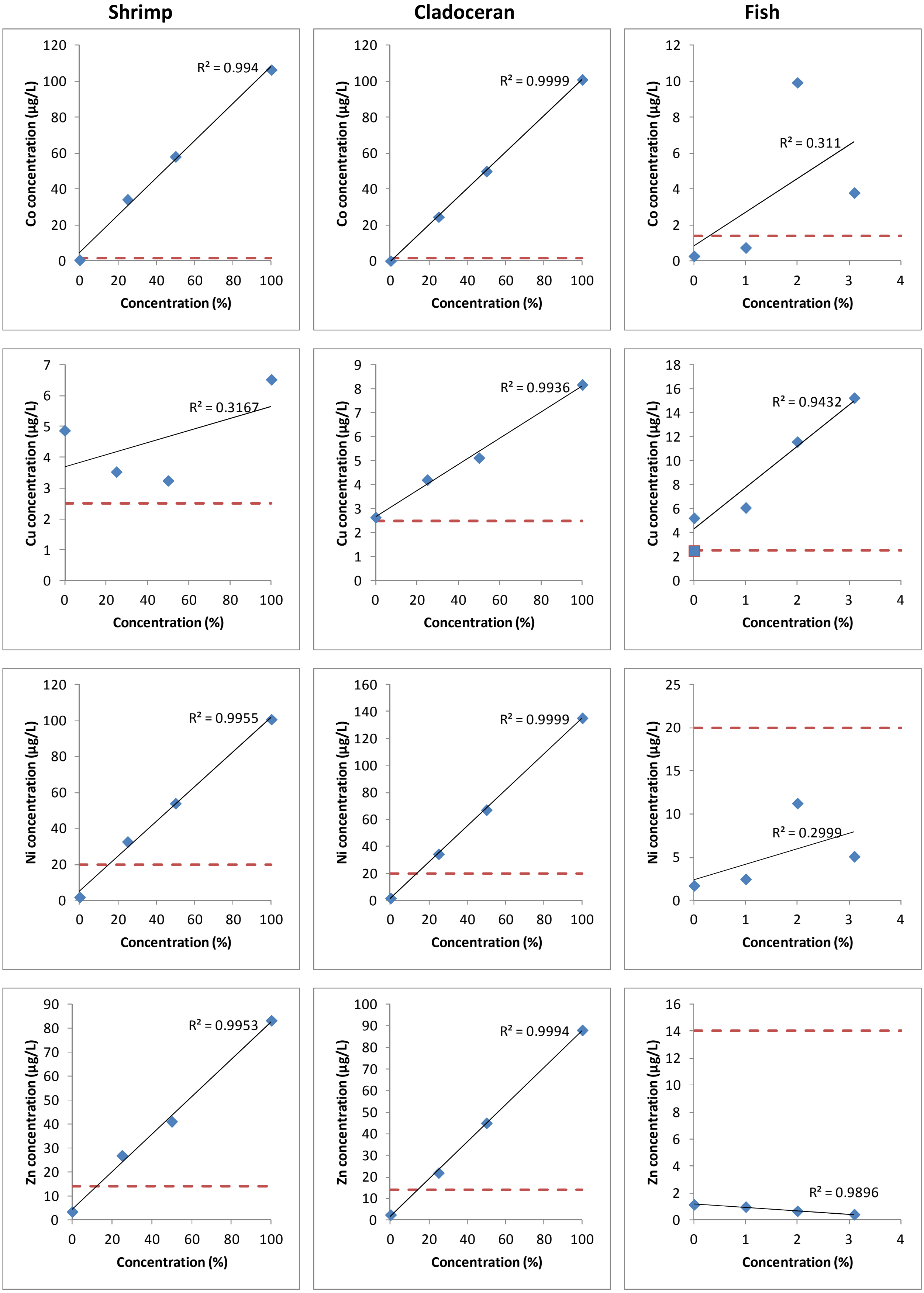
Relationship between concentration of **Jervois (Woods Point)** acid drainage water and concentration of dissolved metals (Co, Cu, Ni, Zn) in control (0%) and three concentrations of acid drainage water in toxicity tests with shrimp, cladocerans and fish. Red lines show the water quality trigger value.



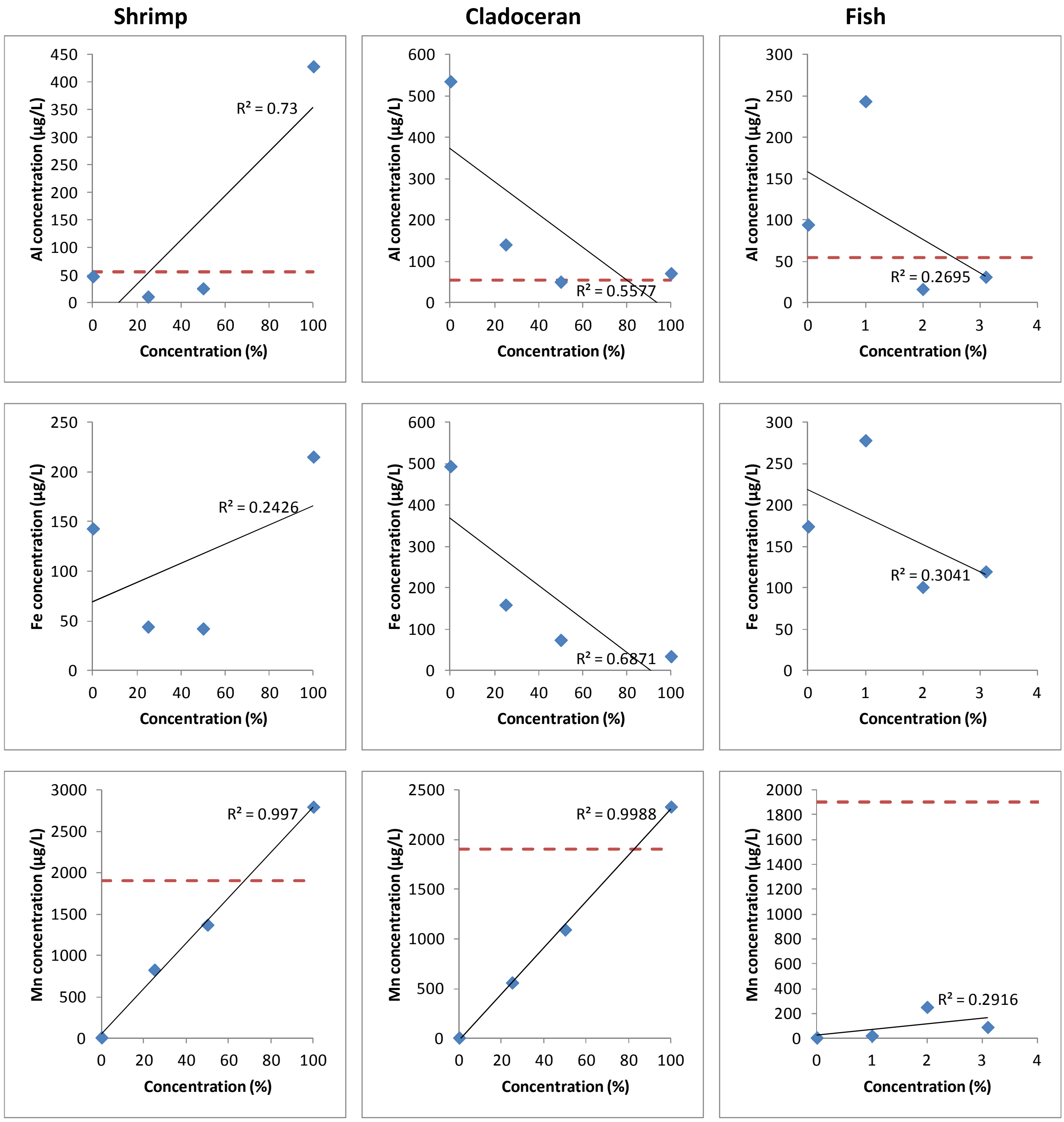
Relationship between concentration of **Jervois (Woods Point)** acid drainage water and concentration of dissolved metals (Al, Fe and Mn) in control (0%) and three concentrations of acid drainage water in toxicity tests with shrimp, cladocerans and fish. Red lines show the water quality trigger value.



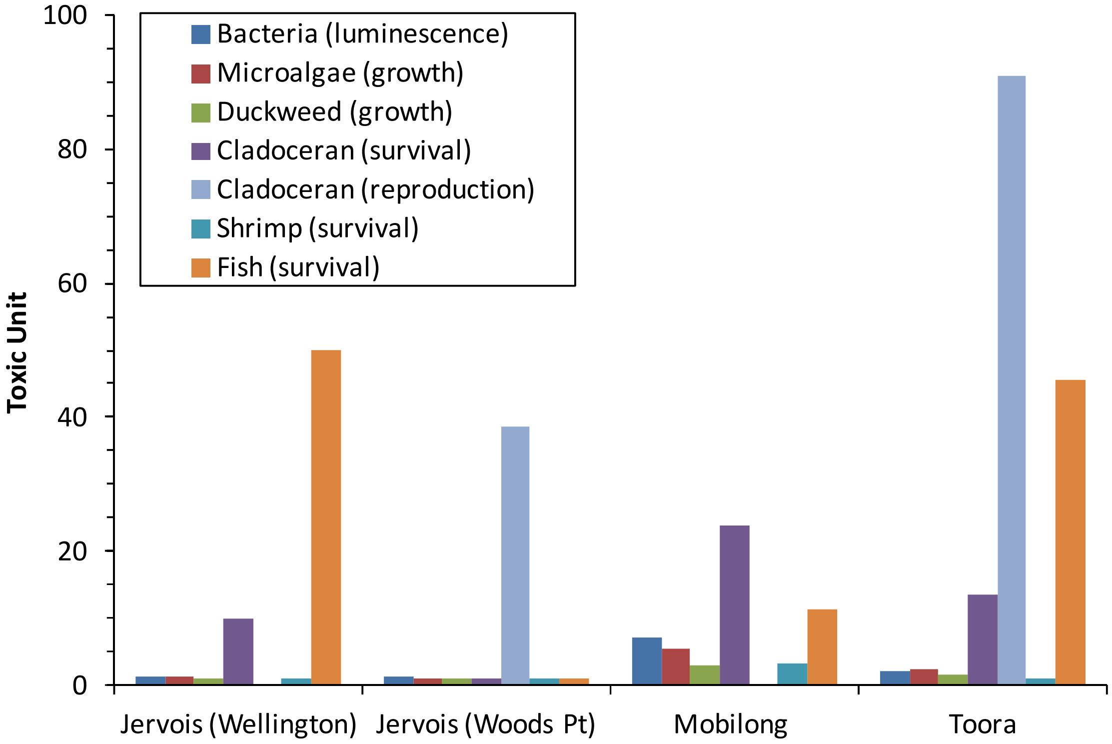
Relationship between concentration of **Jervois (Wellington)** acid drainage water and concentration of dissolved metals (Co, Cu, Ni, Zn) in control (0%) and three concentrations of acid drainage water in toxicity tests with shrimp, cladocerans and fish. Red lines show the water quality trigger value.



Relationship between concentration of **Jervois (Wellington)** acid drainage water and concentration of dissolved metals (Al, Fe and Mn) in control (0%) and three concentrations of acid drainage water in toxicity tests with shrimp, cladocerans and fish. Red lines show the water quality trigger value.



Appendix B Toxicity Test Results



Toxicity of acid-drainage water to freshwater biota. Toxic Unit (TU) is the inverse of the EC50 value (100/EC50), i.e. the higher the TU value the more toxic the sample. Toxic Unit values for cladoceran reproduction tests with Jervois (Wellington) and Mobilong could not be determined due to a poor concentration-response curve around the EC50 value, however, this test was consistently the most sensitive (most toxic) to all four acid drainage water samples. Toxic unit values shown as 1 are actually <1 (i.e. EC50 value >100%).

Raw data from the Cladoceran immobilisation bioassay

Cerio Acute Test

Test material: Toora **Date:** 24/10/2012 - 26/10/2012

Test organism: *Ceriodaphnia dubia* **Time:**

	After 48 hours																			
	# Alive				# Dead							Water Quality at 0hr				Water Quality at 48hr			
Concentration	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**Av # Alive**	**Av # Dead**	**TOTAL #**	**pH**	**DO**	**Cond**	**Temp**	**pH**	**DO**	**Cond**	**Temp**
Control DMW	5	5	5		0	0	0		5.0	0.0					19	7.93	5.11	135.6	19
River Water	5	4	5		0	1	0		4.7	0.3		7.97	5.74	218.3		8.1	5.05	251	
0.38%	5	4	5		0	1	0		4.7	0.3		7.95	5.71	273		8.12	5.07	271	
0.63%	5	4	4		0	1	1		4.3	0.7		7.93	5.64	331		8.14	5.11	328	
1.25%	5	5	5		0	0	0		5.0	0.0		7.88	5.77	453		8.14	5.03	441	
2.50%	5	4	4		0	1	1		4.3	0.7		7.82	6.19	714		8.11	4.94	653	
5%	5	2	2		0	3	3		3.0	2.0		7.67	5.95	1159		8.06	5.25	1070	
10%	2	2	3		3	3	2		2.3	2.7		7.5	6.28	1978		7.98	5.01	1705	
20%	0	0	0		5	5	5		0.0	5.0		7.23	5.63	3.52ms		7.84	4.65	3270	

Test material: Wellington **Date:** 30/10/2012 - 1/11/12

Test organism: *Ceriodaphnia dubia* **Time:**

	After 48 hours																			
	# Alive				# Dead							Water Quality at 0hr				Water Quality at 48hr			
Concentration	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**Av # Alive**	**Av # Dead**	**TOTAL #**	**pH**	**DO**	**Cond**	**Temp**	**pH**	**DO**	**Cond**	**Temp**
Control DMW	4	4	5		1	1	0		4.3	0.7		7.91	7.01	134.5		7.97	5.32	93.8	
River Water	5	5	4		0	0	1		4.7	0.3		7.53	6.78	228.1		8.11	6.03	192.3	
1.56%	4	3	5		1	2	0		4.0	1.0		7.46	6.63	289		8.17	6.16	284	
3.13%	3	3	1		2	2	4		2.3	2.7		7.48	5.85	362		8.2	6.35	360	
6.25%	4	0	3		1	5	2		2.3	2.7		7.42	6.51	518		8.27	6.28	522	
12.50%	3	4	3		2	1	2		3.3	1.7		7.29	6.29	809		8.24	6.07	797	
25%	1	3	2		4	2	3		2.0	3.0		7.25	6.47	1276		8.16	6.13	1231	
50%	0	0	1		5	5	4		0.3	4.7		6.95	6.24	2298		8.03	6.6	2167	
100%	0	0	0		5	5	5		0.0	5.0		5.29	6.32	4.02ms		7.13	6.29	3.89ms	

Test material: Woods Pt **Date:** 6/11/2012 - 8/11/2012

Test organism: *Ceriodaphnia dubia* **Time:**

	After 48 hours																			
	# Alive				# Dead							Water Quality at 0hr				Water Quality at 48hr			
Concentration	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**Av # Alive**	**Av # Dead**	**TOTAL #**	**pH**	**DO**	**Cond**	**Temp**	**pH**	**DO**	**Cond**	**Temp**
Control DMW	4	4	5		1	1	0		4.3	0.7		7.18	7.04	115.7		7.87	5.98	105.4	
River Water	5	4	5		0	1	0		4.7	0.3		7.41	7.24	214		7.99	5.77	224.9	
1.56%	4	4	5		1	1	0		4.3	0.7		7.49	7.62	297		8.05	5.91	298	
3.13%	4	4	4		1	1	1		4.0	1.0		7.53	7.57	410		8.11	5.83	399	
6.25%	5	4	5		0	1	0		4.7	0.3		7.45	7.22	592		8.11	5.87	584	
12.50%	5	3	4		0	2	1		4.0	1.0		7.34	6.83	875		8.14	5.8	925	
25%	4	4	4		1	1	1		4.0	1.0		7.19	7.21	1407		8.09	6.15	1602	
50%	4	5	4		1	0	1		4.3	0.7		6.91	7.51	2469		8.01	6.03	2794	
100%	4	5	4		1	0	1		4.3	0.7		6.5	7.15	4.82ms		7.74	6.01	4960	

Test material: Mobilong **Date:** 13/11/2012 - 15/11/2012

Test organism: *Ceriodaphnia dubia* **Time:**

	After 48 hours																			
	# Alive				# Dead							Water Quality at 0hr				Water Quality at 48hr			
Concentration	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**Av # Alive**	**Av # Dead**	**TOTAL #**	**pH**	**DO**	**Cond**	**Temp**	**pH**	**DO**	**Cond**	**Temp**
Control DMW	4	5	4		1	0	1		4.3	0.7		6.8	6.91	90.5		7.46	6.41	51.7	
River Water	5	4	4		0	1	1		4.3	0.7		6.94	7.46	208.7		7.56	5.86	214.6	
0.15%	4	5	4		1	0	1		4.3	0.7		7.2	7.32	217		7.66	5.67	240.8	
0.38%	2	3	5		3	2	0		3.3	1.7		7.34	6.84	259		7.73	6.08	274	
0.63%	5	5	4		0	0	1		4.7	0.3		7.44	6.93	314		7.77	5.72	342	
1.25%	3	5	4		2	0	1		4.0	1.0		7.41	6.87	500		7.79	5.66	545	
2.50%	5	2	3		0	3	2		3.3	1.7		7.31	6.64	896		7.74	6.26	899	
5%	2	4	3		3	1	2		3.0	2.0		7.05	6.9	1799		7.62	6.31	1898	
10%	0	0	0		5	5	5		0.0	5.0		6.51	7.21	3320		7.45	6.23	3.47ms	

Ceriodaphnia Survival and Reproduction Test-48 Hr Survival

Start Date:	10/24/2012	Test ID:	Toora	Sample ID:	
End Date:	10/26/2012	Lab ID:	CLW Adelaide	Sample Type:	acidic drainage water
Sample Date:		Protocol:	EPAF 91-EPA Freshwater	Test Species:	CD-Ceriodaphnia dubia
Comments:					

Conc-%	1	2	3
Control	1.0000	1.0000	1.0000
Murray Rv	1.0000	0.8000	1.0000
0.38	1.0000	0.8000	1.0000
0.63	1.0000	0.8000	0.8000
1.3	1.0000	1.0000	1.0000
2.5	1.0000	0.8000	0.8000
5	1.0000	0.4000	0.4000
10	0.4000	0.4000	0.6000
20	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
Pooled	0.9667	1.0000	1.3056	1.1071	1.3453	7.446	6				1	30
0.38	0.9333	0.9655	1.2659	1.1071	1.3453	10.861	3	0.335	2.655	0.3150	1	15
0.63	0.8667	0.8966	1.1865	1.1071	1.3453	11.587	3	1.004	2.655	0.3150	2	15
1.3	1.0000	1.0345	1.3453	1.3453	1.3453	0.000	3	-0.335	2.655	0.3150	0	15
2.5	0.8667	0.8966	1.1865	1.1071	1.3453	11.587	3	1.004	2.655	0.3150	2	15
*5	0.6000	0.6207	0.9049	0.6847	1.3453	42.145	3	3.377	2.655	0.3150	6	15
*10	0.4667	0.4828	0.7518	0.6847	0.8861	15.463	3	4.667	2.655	0.3150	8	15
20	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	3				15	15

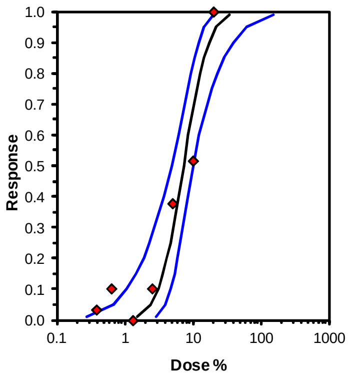
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.91801	0.884	0.96965	2.61347
Equality of variance cannot be confirmed				
The control means are not significantly different (p = 0.37)	1	2.77645		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test	2.5	5	3.53553	40	0.23182	0.24892	0.16046	0.02815	0.0021	6, 17

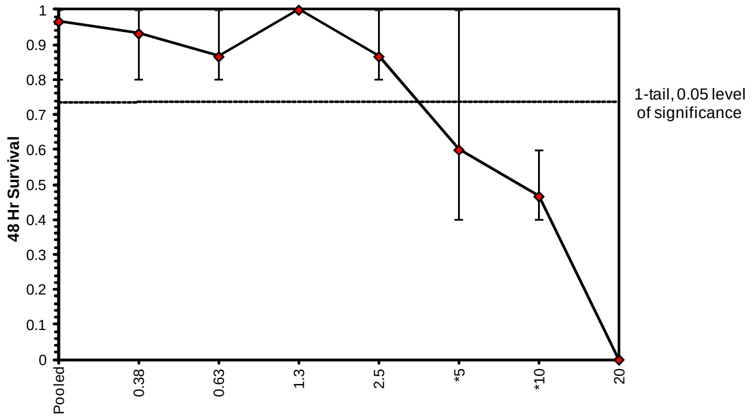
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	3.44663	0.87315	1.73525	5.15801	0.03333	6.01437	11.0705	0.3	0.86677	0.29014	5
Intercept	2.01256	0.82202	0.4014	3.62371							
TSCR	0.05695	0.02713	0.00377	0.11012							

Point	Probits	%	95% Fiducial Limits	
EC01	2.674	1.55528	0.28109	2.86243
EC05	3.355	2.45209	0.682	3.95046
EC10	3.718	3.12568	1.08759	4.71815
EC15	3.964	3.68182	1.48391	5.34084
EC20	4.158	4.19358	1.8926	5.91553
EC25	4.326	4.68896	2.32319	6.48163
EC40	4.747	6.2125	3.79668	8.36977
EC50	5.000	7.35821	4.95531	10.0499
EC60	5.253	8.71521	6.26	12.4675
EC75	5.674	11.547	8.56865	19.2215
EC80	5.842	12.911	9.51766	23.2749
EC85	6.036	14.7056	10.6616	29.3515
EC90	6.282	17.3221	12.184	39.6683
EC95	6.645	22.0804	14.6755	62.7252
EC99	7.326	34.8125	20.4085	151.037



Dose-Response Plot



Ceriodaphnia Survival and Reproduction Test-48 Hr Survival

Start Date:	10/30/2012	Test ID:	Wellington	Sample ID:	
End Date:	11/1/2012	Lab ID:	CLW Adelaide	Sample Type:	acidic drainage water
Sample Date:		Protocol:	EPAF 91-EPA Freshwater	Test Species:	CD-Ceriodaphnia dubia
Comments:					

Conc-%	1	2	3
Control	0.8000	0.8000	1.0000
Murray Rv	1.0000	1.0000	0.8000
1.6	0.8000	0.6000	1.0000
3.1	0.6000	0.6000	0.2000
6.3	0.8000	0.0000	0.6000
13	0.6000	0.8000	0.6000
25	0.2000	0.6000	0.4000
50	0.0000	0.0000	0.2000
100	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
Pooled	0.9000	1.0000	1.2262	1.1071	1.3453	10.637	6				3	30
1.6	0.8000	0.8889	1.1128	0.8861	1.3453	20.637	3	0.701	2.655	0.4296	3	15
*3.1	0.4667	0.5185	0.7453	0.4636	0.8861	32.725	3	2.973	2.655	0.4296	8	15
*6.3	0.4667	0.5185	0.7396	0.2255	1.1071	62.023	3	3.008	2.655	0.4296	8	15
13	0.6667	0.7407	0.9598	0.8861	1.1071	13.299	3	1.647	2.655	0.4296	5	15
*25	0.4000	0.4444	0.6781	0.4636	0.8861	31.157	3	3.387	2.655	0.4296	9	15
*50	0.0667	0.0741	0.3049	0.2255	0.4636	45.094	3	5.694	2.655	0.4296	14	15
100	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	3				15	15

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.95293	0.884	-0.588	0.6234
Bartlett's Test indicates equal variances (p = 0.40)	6.18059	16.8119		
The control means are not significantly different (p = 0.52)	0.70711	2.77645		

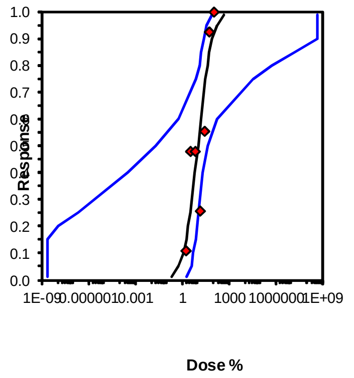
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test	1.6	3.1	2.22711	62.5	0.37463	0.42289	0.3547	0.05235	8.4E-04	6, 17

Maximum Likelihood-Probit

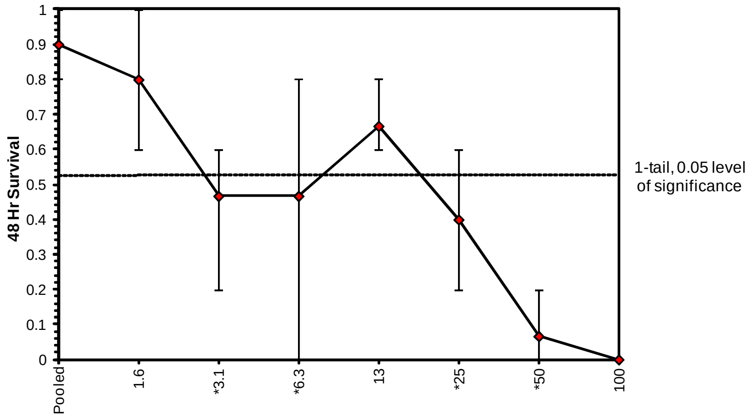
Parameter	Value	SE	95% Fiducial Limits	
Slope	1.3609	0.47978	0.12758	2.59422
Intercept	3.63881	0.60934	2.07247	5.20516
TSCR	0.11027	0.08638	-0.1118	0.33232

Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
0.1	11.7197	11.0705	0.04	1.00021	0.73481	4

Point	Probits	%	95% Fiducial Limits	
EC01	2.674	0.19534	2.1E-09	1.92788
EC05	3.355	0.61881	2.1E-09	3.74854
EC10	3.718	1.14424	2.1E-09	5.45439
EC15	3.964	1.73233	2.1E-09	7.13489
EC20	4.158	2.40869	1.2E-08	8.96434
EC25	4.326	3.19588	2.3E-07	11.0837
EC40	4.747	6.51702	0.00034	21.8777
EC50	5.000	10.0048	0.02047	43.4248
EC60	5.253	15.3593	0.60658	177.526
EC75	5.674	31.3206	8.04481	38891.5
EC80	5.842	41.5566	12.2815	603262
EC85	6.036	57.7816	17.6257	1.7E+07
EC90	6.282	87.479	25.2281	4.9E+08
EC95	6.645	161.756	39.255	4.9E+08
EC99	7.326	512.423	80.3825	4.9E+08



Dose-Response Plot



Ceriodaphnia Survival and Reproduction Test-48 Hr Survival

Start Date:	11/6/2012	Test ID:	Woods Pt	Sample ID:	
End Date:	11/8/2012	Lab ID:	CLW Adelaide	Sample Type:	acidic drainage water
Sample Date:		Protocol:	EPAF 91-EPA Freshwater	Test Species:	CD-Ceriodaphnia dubia
Comments:					

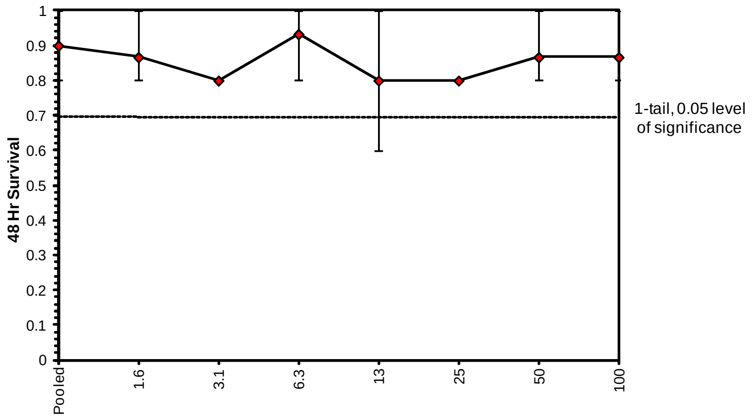
Conc-%	1	2	3
Control	0.8000	0.8000	1.0000
Murray Rv	1.0000	0.8000	1.0000
1.6	0.8000	0.8000	1.0000
3.1	0.8000	0.8000	0.8000
6.3	1.0000	0.8000	1.0000
13	1.0000	0.6000	0.8000
25	0.8000	0.8000	0.8000
50	0.8000	1.0000	0.8000
100	0.8000	1.0000	0.8000

			Transform: Arcsin Square Root						1-Tailed			
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD		
Pooled	0.9000	1.0000	1.2262	1.1071	1.3453	10.637	6				3	30
1.6	0.8667	0.9630	1.1865	1.1071	1.3453	11.587	3	0.419	2.697	0.2558	2	15
3.1	0.8000	0.8889	1.1071	1.1071	1.1071	0.000	3	1.256	2.697	0.2558	3	15
6.3	0.9333	1.0370	1.2659	1.1071	1.3453	10.861	3	-0.419	2.697	0.2558	1	15
13	0.8000	0.8889	1.1128	0.8861	1.3453	20.637	3	1.196	2.697	0.2558	3	15
25	0.8000	0.8889	1.1071	1.1071	1.1071	0.000	3	1.256	2.697	0.2558	3	15
50	0.8667	0.9630	1.1865	1.1071	1.3453	11.587	3	0.419	2.697	0.2558	2	15
100	0.8667	0.9630	1.1865	1.1071	1.3453	11.587	3	0.419	2.697	0.2558	2	15

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.95449	0.894	0.19021	-0.6797
Equality of variance cannot be confirmed				
The control means are not significantly different (p = 0.52)	0.70711	2.77645		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test	100	>100		1	0.20503	0.23144	0.01152	0.01799	0.7175	7, 19

Dose-Response Plot



Ceriodaphnia Survival and Reproduction Test-48 Hr Survival

Start Date:	11/13/2012	Test ID:	Mobilong	Sample ID:	
End Date:	11/15/2012	Lab ID:	CLW Adelaide	Sample Type:	acidic drainage water
Sample Date:		Protocol:	EPAF 91-EPA Freshwater	Test Species:	CD-Ceriodaphnia dubia
Comments:					

Conc-%	1	2	3
Control	0.8000	1.0000	0.8000
Murray Rv	1.0000	0.8000	0.8000
0.15	0.8000	1.0000	0.8000
0.38	0.4000	0.6000	1.0000
0.63	1.0000	1.0000	0.8000
1.3	0.6000	1.0000	0.8000
2.5	1.0000	0.4000	0.6000
5	0.4000	0.8000	0.6000
10	0.0000	0.0000	0.0000

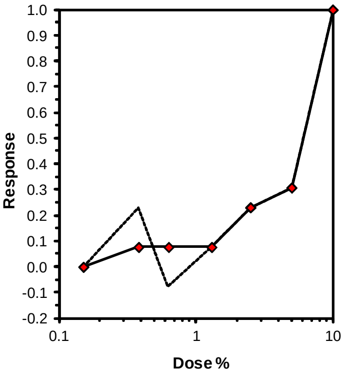
			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
Pooled	0.8667	1.0000	1.1865	1.1071	1.3453	10.364	6				4	30
0.15	0.8667	1.0000	1.1865	1.1071	1.3453	11.587	3	0.000	2.655	0.4084	2	15
0.38	0.6667	0.7692	0.9720	0.6847	1.3453	34.831	3	1.394	2.655	0.4084	5	15
0.63	0.9333	1.0769	1.2659	1.1071	1.3453	10.861	3	-0.516	2.655	0.4084	1	15
1.3	0.8000	0.9231	1.1128	0.8861	1.3453	20.637	3	0.479	2.655	0.4084	3	15
2.5	0.6667	0.7692	0.9720	0.6847	1.3453	34.831	3	1.394	2.655	0.4084	5	15
5	0.6000	0.6923	0.8926	0.6847	1.1071	23.670	3	1.910	2.655	0.4084	6	15
10	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	3				15	15

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.93745	0.884	0.45738	-0.4368
Bartlett's Test indicates equal variances (p = 0.56)	4.88386	16.8119		
The control means are not significantly different (p = 1.00)	0	2.77645		

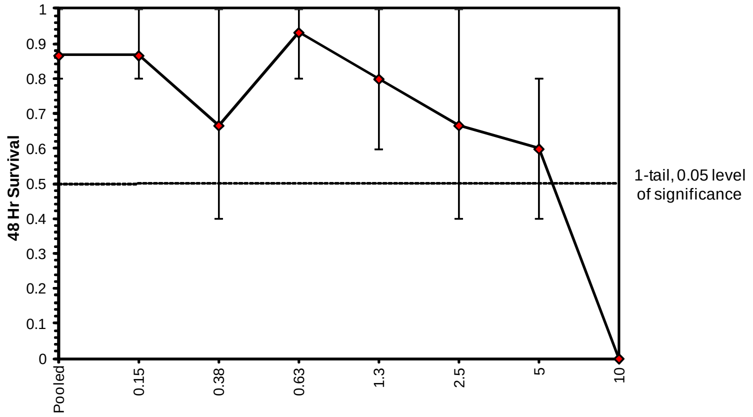
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test	5	10	7.07107	20	0.36674	0.4267	0.06291	0.04732	0.29766	6, 17

Trimmed Spearman-Karber

Trim Level	EC50	95% CL	
0.0%	4.1862	3.1853	5.5016
5.0%	4.7254	3.5224	6.3393
10.0%	5.1393	3.8993	6.7735
20.0%	5.6312	4.1178	7.7008
Auto-0.0%	4.1862	3.1853	5.5016



Dose-Response Plot



Test material: Cu Ref **Date:** 24/10/2012

Test organism: *Ceriodaphnia dubia* **Time:**

After 48 hours

ug/L	# Alive				# Dead							Water Quality at test			
Concentration	A	B	C	D	A	B	C	D	Av # Alive	Av # Dead	TOTAL # Dead	pH	DO (ppm)	Cond (uS/cm)	Temp
0	4	5	4		1	0	1		4.33	0.67	2	8.26	5.02	138.3	25
5	4	4	5		1	1	0		4.33	0.67	2	8.27	4.87	109.7	
10	2	1	1		3	4	4		1.33	3.67	11	8.26	5.06	100.3	
15	0	0	1		5	5	4		0.33	4.67	14	8.24	4.92	101.6	
20	0	0	0		5	5	5		0.00	5.00	15	8.25	5.33	103	

Test material: Cu Ref **Date:** 30/10/2012

Test organism: *Ceriodaphnia dubia* **Time:**

After 48 hours

ug/L	# Alive				# Dead							Water Quality at test			
Concentration	A	B	C	D	A	B	C	D	Av # Alive	Av # Dead	TOTAL # Dead	pH	DO (ppm)	Cond (uS/cm)	Temp
0	5	5	4		0	0	1		4.67	0.33	1	7.7	6.12	159.8	25
5	4	5	5		1	0	0		4.67	0.33	1	7.78	6.11	123.4	
10	2	5	3		3	0	2		3.33	1.67	5	7.89	6.1	130.2	
15	1	0	0		4	5	5		0.33	4.67	14	7.96	6.14	113.3	
20	0	0	0		5	5	5		0.00	5.00	15	8	5.71	101.1	

Test material: Cu Ref **Date:** 6/11/2012

Test organism: *Ceriodaphnia dubia* **Time:**

After 48 hours

ug/L	# Alive				# Dead							Water Quality at test			
Concentration	A	B	C	D	A	B	C	D	Av # Alive	Av # Dead	TOTAL # Dead	pH	DO (ppm)	Cond (uS/cm)	Temp
0	4	5	5		1	0	0		4.67	0.33	1	7.78	6.54	109.6	25
5	5	5	5		0	0	0		5.00	0.00	0	7.75	6.7	106.5	
10	1	3	2		4	2	3		2.00	3.00	9	7.8	6.68	109	
15	0	0	0		5	5	5		0.00	5.00	15	7.83	6.67	110.1	
20	0	0	0		5	5	5		0.00	5.00	15	7.89	6.7	105.3	

Test material: Cu Ref **Date:** 13/11/2012

Test organism: *Ceriodaphnia dubia* **Time:**

After 48 hours

ug/L	# Alive				# Dead							Water Quality at test			
Concentration	A	B	C	D	A	B	C	D	Av # Alive	Av # Dead	TOTAL # Dead	pH	DO (ppm)	Cond (uS/cm)	Temp
0	4	4	4		1	1	1		4.00	1.00	3	7.33	5.95	99.4	25
5	5	4	4		0	1	1		4.33	0.67	2	7.26	6.26	98.7	
10	3	3	4		2	2	1		3.33	1.67	5	7.32	6.44	83.7	
15	0	0	1		5	5	4		0.33	4.67	14	7.41	6.15	81.2	
20	0	0	0		5	5	5		0.00	5.00	15	7.49	5.85	83.1	

Ceriodaphnia Survival and Reproduction Test-48 Hr Survival

Start Date:	10/24/2012	Test ID:	Cu ref	Sample ID:	Copper
End Date:	10/26/2012	Lab ID:		Sample Type:	reference toxicant
Sample Date:		Protocol:		Test Species:	CD-Ceriodaphnia dubia
Comments:					

Conc-ug/L	1	2	3
Control	0.8000	1.0000	0.8000
5	0.8000	0.8000	1.0000
10	0.4000	0.2000	0.2000
15	0.0000	0.0000	0.2000
20	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-ug/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
Control	0.8667	1.0000	1.1865	1.1071	1.3453	11.587	3				2	15
5	0.8667	1.0000	1.1865	1.1071	1.3453	11.587	3	0.000	2.420	0.2669	2	15
*10	0.2667	0.3077	0.5373	0.4636	0.6847	23.753	3	5.886	2.420	0.2669	11	15
*15	0.0667	0.0769	0.3049	0.2255	0.4636	45.094	3	7.993	2.420	0.2669	14	15
20	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	3	9.741	2.470	0.2437	15	15

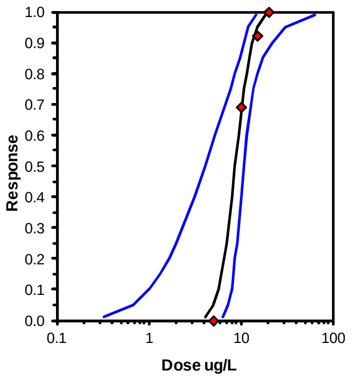
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.62515	0.805	0.81359	-1.6408
Bartlett's Test indicates equal variances (p = 1.00)	0.01338	11.3449		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	5	10	7.07107		0.22687	0.26397	0.61287	0.01825	7.0E-05	3, 8

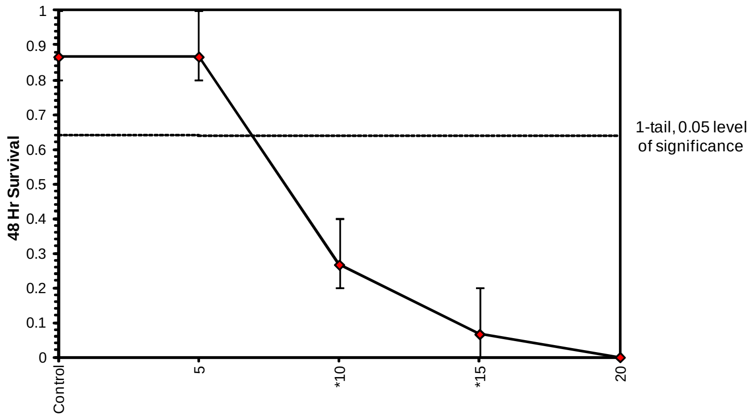
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	7.03361	2.53607	2.06292	12.0043	0.13333	0.41242	5.99146	0.81	0.9413	0.14217	7
Intercept	-1.6208	2.65591	-6.8263	3.58481							
TSCR	0.1185	0.07471	-0.0279	0.26494							

Point	Probits	ug/L	95% Fiducial Limits	
EC01	2.674	4.07903	0.32587	6.4384
EC05	3.355	5.09857	0.69232	7.39005
EC10	3.718	5.7425	1.03274	7.96776
EC15	3.964	6.22229	1.35108	8.39241
EC20	4.158	6.63205	1.67115	8.75419
EC25	4.326	7.00502	2.00374	9.0851
EC40	4.747	8.04054	3.14741	10.0335
EC50	5.000	8.73584	4.10127	10.7251
EC60	5.253	9.49127	5.29052	11.5807
EC75	5.674	10.8943	7.70021	13.8026
EC80	5.842	11.507	8.68149	15.2339
EC85	6.036	12.2648	9.72591	17.5444
EC90	6.282	13.2895	10.8437	21.6835
EC95	6.645	14.9679	12.2148	30.9597
EC99	7.326	18.7091	14.477	63.6984



Dose-Response Plot



Ceriodaphnia Survival and Reproduction Test-48 Hr Survival

Start Date:	10/30/2012	Test ID:	Cu ref	Sample ID:	Copper
End Date:	11/1/2012	Lab ID:		Sample Type:	reference toxicant
Sample Date:		Protocol:		Test Species:	CD-Ceriodaphnia dubia
Comments:					

Conc-ug/L	1	2	3
Control	1.0000	1.0000	0.8000
5	0.8000	1.0000	1.0000
10	0.4000	1.0000	0.6000
15	0.2000	0.0000	0.0000
20	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-ug/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
Control	0.9333	1.0000	1.2659	1.1071	1.3453	10.861	3				1	15
5	0.9333	1.0000	1.2659	1.1071	1.3453	10.861	3	0.000	2.420	0.4089	1	15
10	0.6667	0.7143	0.9720	0.6847	1.3453	34.831	3	1.739	2.420	0.4089	5	15
*15	0.0667	0.0714	0.3049	0.2255	0.4636	45.094	3	5.687	2.420	0.4089	14	15
20	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	3				15	15

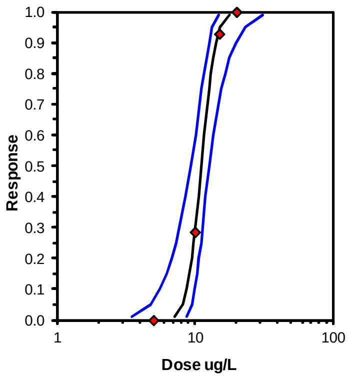
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.94412	0.805	0.48909	0.58181
Bartlett's Test indicates equal variances (p = 0.49)	2.43262	11.3449		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	10	15	12.2474		0.33857	0.3721	0.61622	0.04283	0.00138	3, 8

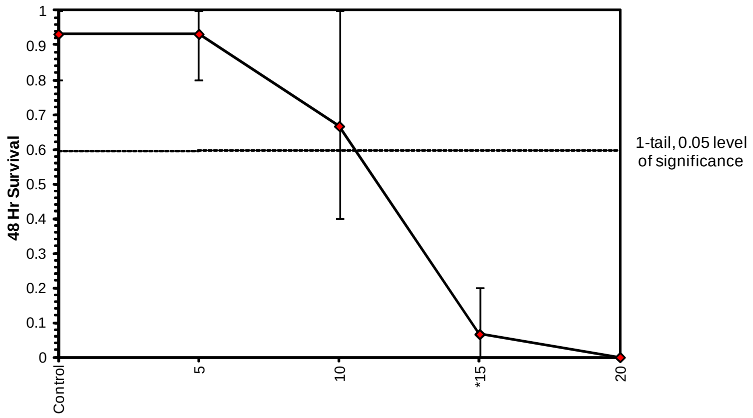
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	11.7515	3.40744	5.07289	18.4301	0.06667	0.02535	5.99146	0.99	1.0488	0.0851	3
Intercept	-7.325	3.65269	-14.484	-0.1657							
TSCR	0.0668	0.04559	-0.0226	0.15615							

Point	Probits	ug/L	95% Fiducial Limits	
EC01	2.674	7.09316	3.55413	8.73305
EC05	3.355	8.10648	4.8037	9.58607
EC10	3.718	8.70458	5.62687	10.099
EC15	3.964	9.13285	6.25013	10.4779
EC20	4.158	9.4882	6.78469	10.8046
EC25	4.326	9.80406	7.26953	11.1083
EC40	4.747	10.6474	8.574	12.0182
EC50	5.000	11.1893	9.37871	12.7223
EC60	5.253	11.7587	10.1529	13.6084
EC75	5.674	12.7702	11.2951	15.609
EC80	5.842	13.1953	11.6967	16.6042
EC85	6.036	13.7088	12.1369	17.912
EC90	6.282	14.3832	12.6616	19.7872
EC95	6.645	15.4444	13.407	23.0606
EC99	7.326	17.6508	14.7862	31.0215



Dose-Response Plot



Ceriodaphnia Survival and Reproduction Test-48 Hr Survival

Start Date:	11/6/2012	Test ID:	Cu ref	Sample ID:	Copper
End Date:	11/08/2012	Lab ID:		Sample Type:	reference toxicant
Sample Date:		Protocol:		Test Species:	CD-Ceriodaphnia dubia
Comments:					

Conc-ug/L	1	2	3
Control	0.8000	1.0000	1.0000
5	1.0000	1.0000	1.0000
10	0.2000	0.6000	0.4000
15	0.0000	0.0000	0.0000
20	0.0000	0.0000	0.0000

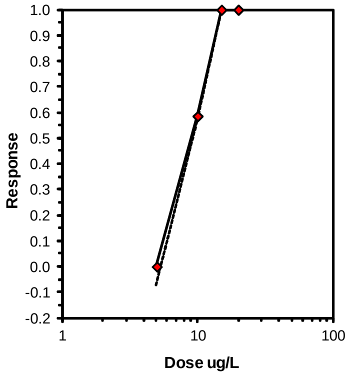
			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-ug/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
Control	0.9333	1.0000	1.2659	1.1071	1.3453	10.861	3				1	15
5	1.0000	1.0714	1.3453	1.3453	1.3453	0.000	3	-0.668	2.340	0.2781	0	15
*10	0.4000	0.4286	0.6781	0.4636	0.8861	31.157	3	4.946	2.340	0.2781	9	15
15	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	3				15	15
20	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	3				15	15

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.92183	0.764	-0.3114	0.41752
Equality of variance cannot be confirmed				

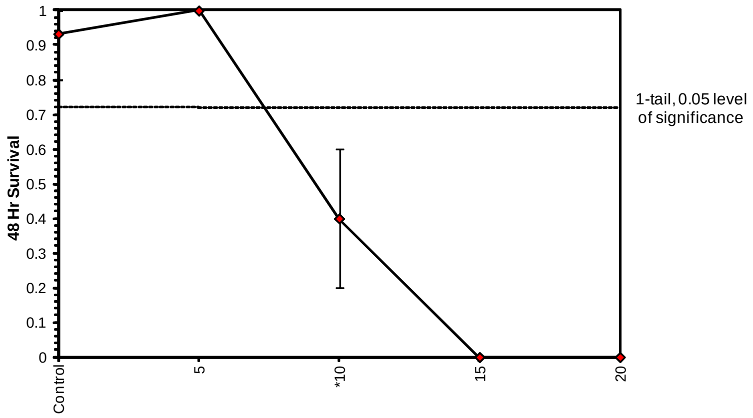
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	5	10	7.07107		0.21294	0.23403	0.39841	0.02118	0.0026	2, 6

Trimmed Spearman-Karber

Trim Level	EC50	95% CL	
0.0%	8.8756	7.7184	10.2064
5.0%	8.8974	7.6191	10.3902
10.0%	8.9190	7.4941	10.6149
20.0%	8.9615	7.1211	11.2774
Auto-0.0%	8.8756	7.7184	10.2064



Dose-Response Plot



Ceriodaphnia Survival and Reproduction Test-48 Hr Survival

Start Date: 11/13/2012 Test ID: Cu ref Sample ID: Copper
End Date: 11/15/2012 Lab ID: Sample Type: reference toxicant
Sample Date: Protocol: Test Species: CD-Ceriodaphnia dubia
Comments:

Conc-ug/L	1	2	3
Control	0.8000	0.8000	0.8000
5	1.0000	0.8000	0.8000
10	0.6000	0.6000	0.8000
15	0.0000	0.0000	0.2000
20	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-ug/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
Control	0.8000	1.0000	1.1071	1.1071	1.1071	0.000	3				3	15
5	0.8667	1.0833	1.1865	1.1071	1.3453	11.587	3	-0.836	2.420	0.2298	2	15
10	0.6667	0.8333	0.9598	0.8861	1.1071	13.299	3	1.552	2.420	0.2298	5	15
*15	0.0667	0.0833	0.3049	0.2255	0.4636	45.094	3	8.449	2.420	0.2298	14	15
20	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	3				15	15

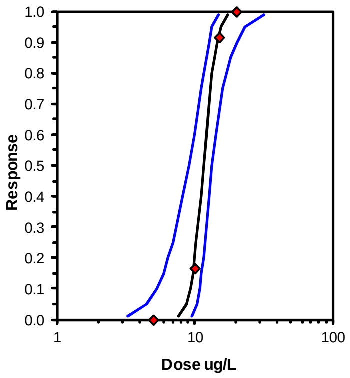
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.74756	0.805	0.93974	-0.8407
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	10	15	12.2474		0.20855	0.26069	0.4823	0.01352	5.6E-05	3, 8

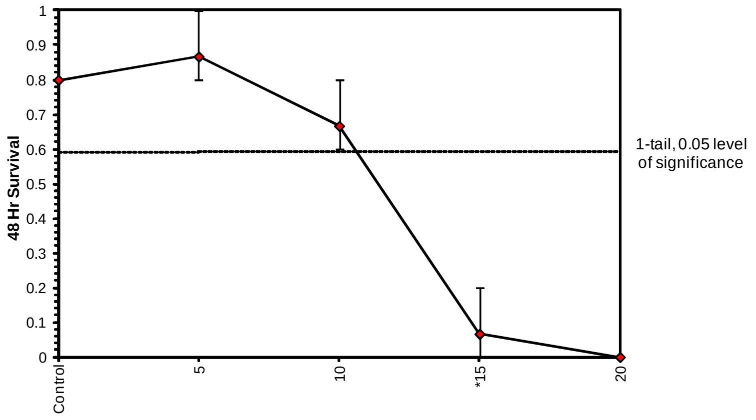
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	12.9467	4.11102	4.88914	21.0043	0.2	0.13939	5.99146	0.93	1.06584	0.07724	3
Intercept	-8.7992	4.50884	-17.636	0.03816							
TSCR	0.16706	0.06804	0.0337	0.30041							

Point	Probits	ug/L	95% Fiducial Limits	
EC01	2.674	7.69405	3.3061	9.5791
EC05	3.355	8.68547	4.51758	10.4128
EC10	3.718	9.26519	5.32301	10.9124
EC15	3.964	9.67804	5.93692	11.2804
EC20	4.158	10.0192	6.46674	11.5963
EC25	4.326	10.3215	6.95049	11.8886
EC40	4.747	11.1243	8.27479	12.752
EC50	5.000	11.637	9.1184	13.4059
EC60	5.253	12.1733	9.95922	14.219
EC75	5.674	13.1201	11.2469	16.0788
EC80	5.842	13.516	11.7	17.031
EC85	6.036	13.9925	12.1876	18.3075
EC90	6.282	14.616	12.7504	20.1757
EC95	6.645	15.5915	13.5149	23.504
EC99	7.326	17.6006	14.85	31.7734



Dose-Response Plot



Raw data from the cladoceran reproduction bioassay

River water (50%)	No. neonates	River water (100%)	Total neonates
Replicate		**Replicate**	
1	21	**1**	19
2	20	**2**	19
3	21	**3**	14
4	19	**4**	13
5	19	**5**	28
6	23	**6**	22
7	8	**7**	8
8	20	**8**	21
9	20	**9**	20
10	22	**10**	dead
Average	19.3	**Average**	18.22

Site	TOORA	Site	TOORA	Site	TOORA	Site	TOORA
Concentration:	0.31%	Concentration:	0.63%	Concentration:	1.25%	Concentration:	2.50%
Replicate	Total	Replicate	Total neonates	Replicate	Total neonates	Replicate	Total neonates
1	14	1	11	1	14	1	3
2	14	2	10	2	8	2	0
3	15	3	10	3	8	3	7
4	11	4	14	4	9	4	4
5	14	5	12	5	7	5	2
6	17	6	11	6	7	6	4
7	14	7	10	7	7	7	3
8	14	8	11	8	12	8	4
9	12	9	6	9	10	9	3
10	7	10	9	10	10	10	3
Average	13.2	Average	10.4	Average	9.2	Average	3.3

Site	Woodspoint	Site	Woodspoint	Site	Woodspoint	Site	Woodspoint
Concentration:	1.56%	Concentration:	3.125%	Concentration:	6.25%	Concentration:	12.50%
Replicate	Total noenates	Replicate	Total neonates	Replicate	Total neonates	Replicate	Total neonates
1	15	1	6	1	3	1	0
2	15	2	4	2	7	2	4
3	16	3	7	3	3	3	5
4	12	4	8	4	4	4	12
5	16	5	12	5	8	5	2
6	7	6	5	6	8	6	5
7	7	7	7	7	4	7	4
8	11	8	8	8	10	8	1
9	19	9	9	9	7	9	3
10	13	10	8	10	4	10	3
Average	13.1	Average	7.4	Average	5.8	Average	3.9

Site	Wellington	Site	Wellington	Site	Wellington	Site	Wellington
Concentration:	0.0970%	Concentration:	0.195%	Concentration:	0.390%	Concentration:	0.78%
Replicate	Total neonates	Replicate	Total neonates	Replicate	Total neonates	Replicate	Total neonates
1	15	1	15	1	14	1	0
2	19	2	20	2	1	2	14
3	21	3	14	3	22	3	11
4	20	4	21	4	15	4	20
5	24	5	22	5	10	5	15
6	17	6	16	6	20	6	17
7	15	7	13	7	14	7	17
8	10	8	15	8	2	8	13
9	19	9	13	9	18	9	12
10	6	10	15	10	14	10	12
Average	16.6	Average	16.4	Average	13	Average	13.1

		27/02/2013			1/03/2013			4/03/2013			6/03/2013			8/03/2013		
		pH	**DO**	**Cond**	**pH**	**DO**	**Cond**	**pH**	**DO**	**Cond**	**pH**	**DO**	**Cond**	**pH**	**DO**	**Cond**
DMW	**100%**	8.00	6.37	191.40	8.42	6.22	221.00	7.09	6.23	248.80	8.55	6.33	187.80	8.14	5.81	214.20
River Water	**50%**	8.06	6.14	258.70	8.63	5.60	290.00	7.43	6.73	287.90	8.45	5.77	201.10	8.36	5.43	241.10
	100%	8.11	6.75	328.00	8.80	5.68	356.00	7.66	6.41	360.00	8.47	5.53	236.30	8.57	5.41	228.40
Wellington	**0.097%**	8.09	7.12	327.00	8.79	5.45	368.00	7.81	6.77	254.50	8.37	6.05	250.30	8.58	5.62	245.10
	0.195%	8.09	6.30	362.00	8.76	5.15	351.00	7.94	6.46	253.00	8.33	6.19	241.20	8.64	5.40	238.20
	0.390%	8.08	6.67	360.00	8.85	5.63	361.00	7.99	6.65	262.00	8.35	6.52	261.90	8.81	6.09	249.40
	0.780%	8.06	6.16	404.00	8.99	6.20	387.00	8.01	6.52	280.20	8.39	6.56	288.20	8.98	6.45	273.70
Mobilong	**0.097%**	7.90	6.22	411.00	8.91	5.47	384.00	7.95	6.65	402.00	8.47	5.88	307.00	8.94	5.97	270.80
	0.195%	7.94	6.23	425.00	8.94	6.29	424.00	8.00	6.78	387.00	8.42	5.69	330.00	8.85	5.62	298.10
	0.390%	7.93	6.32	494.00	8.99	6.83	497.00	8.09	6.41	468.00	8.73	5.44	400.00	8.93	6.58	377.00
	0.780%	7.87	6.59	617.00	8.99	6.70	586.00	8.35	6.97	564.00	8.64	5.98	541.00	8.94	6.54	500.00

		13/03/2013			15/03/2013			18/03/2013			20/03/2013			22/03/2013		
		pH	**DO**	**Cond**	**pH**	**DO**	**Cond**	**pH**	**DO**	**Cond**	**pH**	**DO**	**Cond**	**pH**	**DO**	**Cond**
DMW	**100%**	7.62	6.17	195.50	8.10	7.37	199.70	8.58	6.77	198.20	8.29	6.83	194.60	7.44	6.18	200.10
River Water	**50%**	7.74	6.27	198.10	8.32	6.65	170.80	8.77	6.92	217.20	8.65	6.59	216.90	7.95	6.08	219.60
	100%	7.82	6.05	198.20	8.30	6.93	208.70	8.79	6.33	214.10	8.79	6.25	213.70	8.30	6.15	232.30
Toora	**0.31%**	7.83	6.32	275.00	8.28	6.98	255.80	8.81	6.81	262.30	8.79	7.03	272.00	8.24	6.20	293.00
	0.62%	7.81	6.57	308.00	8.42	6.42	306.00	8.40	6.83	312.10	8.37	6.82	358.00	8.26	6.24	334.00
	1.25%	7.78	6.37	432.00	8.66	6.92	402.00	8.49	6.78	452.00	8.57	6.67	425.00	8.29	6.91	455.00
	2.50%	7.74	6.23	628.00	8.61	6.85	644.00	8.52	6.62	652.00	8.60	6.68	639.00	8.38	6.66	704.00
Wood's Point	**12.5%**	7.62	6.33	857.00	8.43	6.59	912.00	8.54	6.66	822.00	8.90	6.26	940.00	8.62	6.40	1033.00
	25.0%	7.51	6.60	1469.00	8.60	6.55	1484.00	8.56	7.45	1517.00	9.09	6.32	1634.00	8.60	6.00	1531.00
	50.0%	7.27	6.12	2649.00	8.63	6.74	2694.00	8.72	7.69	2551.00	All Dead					
	100.0%	6.81	6.31	4600.00	8.31	6.93	5040.00	8.53	6.97	4800.00						

		20/03/2013			22/03/2013			25/03/2013			27/03/2013			29/03/2013		
		pH	**DO**	**Cond**	**pH**	**DO**	**Cond**	**pH**	**DO**	**Cond**	**pH**	**DO**	**Cond**	**pH**	**DO**	**Cond**
Wood's Point	**1.560%**	7.90	6.70	322.00	8.48	6.26	341.00	8.31	6.06	314.00	7.44	5.33	323.00	7.88	6.79	333.00
	3.125%	7.89	7.01	428.00	8.69	6.80	434.00	8.62	6.10	413.00	7.88	5.83	453.00	7.80	7.17	414.00
	6.250%	7.80	6.45	544.00	8.68	6.16	654.00	8.67	6.01	541.00	8.18	5.80	645.00	7.71	7.20	623.00

Ceriodaphnia Survival and Reproduction Test-Reproduction

Start Date:	Test ID: Mobilong	Sample ID:	
End Date:	Lab ID:	Sample Type:	
Sample Date:	Protocol:	Test Species:	CD-Ceriodaphnia dubia

Comments: reproduction over 9 days

Conc-%	1	2	3	4	5	6	7	8	9	10
RM water	19.000	19.000	14.000	13.000	28.000	22.000	8.000	21.000	20.000	
RMW 50%	21.000	20.000	21.000	19.000	19.000	23.000	8.000	20.000	20.000	22.000
0.097	12.000	17.000	16.000	13.000	14.000	13.000	12.000	18.000	15.000	10.000
0.2	10.000	11.000	13.000	13.000	17.000	8.000	3.000	22.000	13.000	12.000
0.39	11.000	13.000	7.000	10.000	6.000	6.000	8.000	13.000	9.000	9.000
0.78	8.000	9.000	5.000	6.000	12.000	13.000	10.000	15.000	15.000	13.000

			Transform: Untransformed						1-Tailed		Isotonic	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
Pooled	18.789	1.0000	18.789	8.000	28.000	26.108	19				18.789	1.0000
*0.097	14.000	0.7451	14.000	10.000	18.000	17.817	10	3.015	2.306	3.663	14.000	0.7451
*0.2	12.200	0.6493	12.200	3.000	22.000	41.400	10	4.148	2.306	3.663	12.200	0.6493
*0.39	9.200	0.4896	9.200	6.000	13.000	27.971	10	6.037	2.306	3.663	9.900	0.5269
*0.78	10.600	0.5641	10.600	5.000	15.000	33.635	10	5.155	2.306	3.663	9.900	0.5269

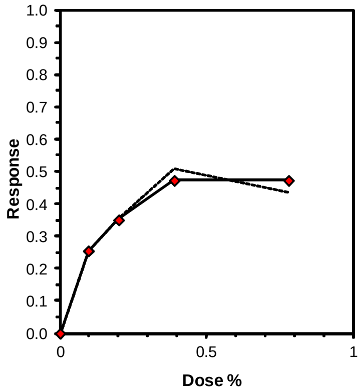
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates normal distribution (p > 0.01)	0.83102	1.035	-0.5036	1.47277
Bartlett's Test indicates equal variances (p = 0.08)	8.39651	13.2767		
The control means are not significantly different (p = 0.65)	0.46768	2.10982		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test	<0.097	0.097			3.66253	0.19492	203.217	16.5326	3.6E-07	4, 54

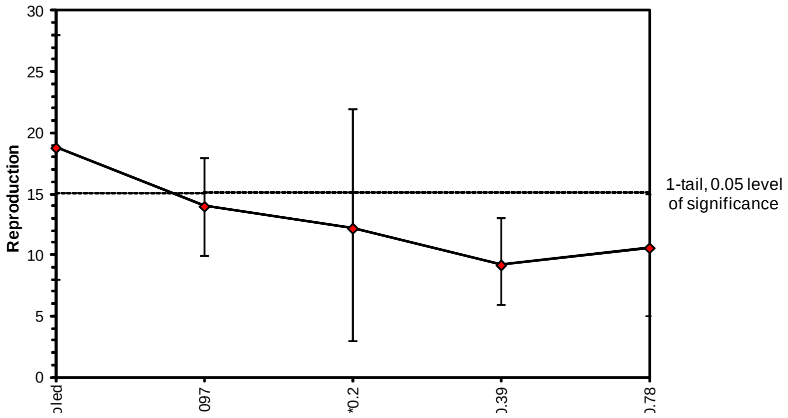
Linear Interpolation (200 Resamples)

Point	%	SD	95% CL		Skew
IC05*	0.0190	0.0097	0.0140	0.0416	5.4995
IC10*	0.0381	0.0160	0.0280	0.0832	3.0908
IC15*	0.0571	0.0241	0.0420	0.1135	3.3244
IC20*	0.0761	0.0359	0.0560	0.2082	2.4029
IC25*	0.0951	0.0506	0.0699	0.2476	1.5732
IC40	0.2765				
IC50	>0.78				

* indicates IC estimate less than the lowest concentration



Dose-Response Plot



Ceriodaphnia Survival and Reproduction Test-Reproduction

Start Date:		Test ID:	Toora	Sample ID:	
End Date:		Lab ID:		Sample Type:	
Sample Date:		Protocol:		Test Species:	CD-Ceriodaphnia dubia
Comments:	reproduction over 9 days				

Conc-%	1	2	3	4	5	6	7	8	9	10
RM water	19.000	19.000	14.000	13.000	28.000	22.000	8.000	21.000	20.000	
RMW 50%	21.000	20.000	21.000	19.000	19.000	23.000	8.000	20.000	20.000	22.000
0.31	14.000	14.000	15.000	11.000	14.000	17.000	14.000	14.000	12.000	7.000
0.63	11.000	10.000	10.000	14.000	12.000	11.000	10.000	11.000	6.000	9.000
1.25	14.000	8.000	8.000	9.000	7.000	7.000	7.000	12.000	10.000	10.000
2.5	3.000	0.000	7.000	4.000	2.000	4.000	3.000	4.000	3.000	3.000

			Transform: Untransformed					Rank	1-Tailed	Isotonic	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Mean	N-Mean
Pooled	18.789	1.0000	18.7895	8.0000	28.0000	26.108	19			18.789	1.0000
*0.31	13.200	0.7025	13.2000	7.0000	17.0000	20.453	10	84.50	101.00	13.200	0.7025
*0.63	10.400	0.5535	10.4000	6.0000	14.0000	19.861	10	74.50	101.00	10.400	0.5535
*1.25	9.200	0.4896	9.2000	7.0000	14.0000	25.517	10	68.50	101.00	9.200	0.4896
*2.5	3.300	0.1756	3.3000	0.0000	7.0000	53.545	10	55.00	101.00	3.300	0.1756

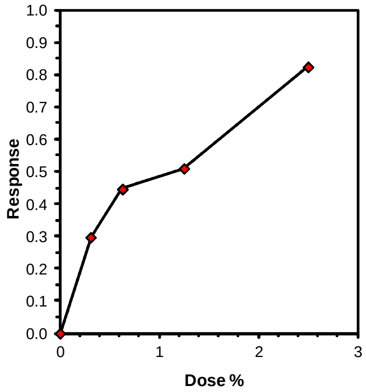
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates non-normal distribution (p <= 0.01)	1.40014	1.035	-1.0169	3.6122
Bartlett's Test indicates unequal variances (p = 2.04E-03)	16.8772	13.2767		
The control means are not significantly different (p = 0.65)	0.46768	2.10982		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Wilcoxon Rank Sum Test	<0.31	0.31		

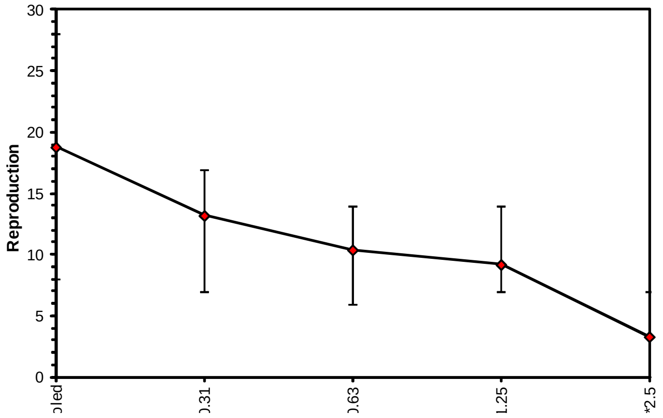
Linear Interpolation (200 Resamples)

Point	%	SD	95% CL		Skew
IC05*	0.0521	0.0154	0.0389	0.0927	2.5543
IC10*	0.1042	0.0308	0.0778	0.1855	2.5543
IC15*	0.1563	0.0423	0.1166	0.2782	1.8302
IC20*	0.2084	0.0527	0.1555	0.3687	1.4031
IC25*	0.2605	0.0641	0.1944	0.4605	1.1795
IC40	0.5302	0.1622	0.3186	1.0741	2.0849
IC50	1.1494	0.2626	0.5901	1.5082	-0.2819

* indicates IC estimate less than the lowest concentration



Dose-Response Plot



Ceriodaphnia Survival and Reproduction Test-Reproduction

Start Date:	Test ID: Wellington	Sample ID:
End Date:	Lab ID:	Sample Type:
Sample Date:	Protocol:	Test Species: CD-Ceriodaphnia dubia
Comments: reproduction over 9 days		

Conc-%	1	2	3	4	5	6	7	8	9	10
RM water	19.000	19.000	14.000	13.000	28.000	22.000	8.000	21.000	20.000	
RMW 50%	21.000	20.000	21.000	19.000	19.000	23.000	8.000	20.000	20.000	22.000
0.097	15.000	19.000	21.000	20.000	24.000	17.000	15.000	10.000	19.000	6.000
0.2	15.000	20.000	14.000	21.000	22.000	16.000	13.000	15.000	13.000	15.000
0.39	14.000	1.000	22.000	15.000	10.000	20.000	14.000	2.000	18.000	14.000
0.78	0.000	14.000	11.000	20.000	15.000	17.000	17.000	13.000	12.000	12.000

			Transform: Untransformed					Rank	1-Tailed	Isotonic	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Mean	N-Mean
Pooled	18.789	1.0000	18.789	8.000	28.000	26.108	19			18.789	1.0000
0.097	16.600	0.8835	16.600	6.000	24.000	32.279	10	122.50	101.00	16.600	0.8835
0.2	16.400	0.8728	16.400	13.000	22.000	20.366	10	119.00	101.00	16.400	0.8728
0.39	13.000	0.6919	13.000	1.000	22.000	53.540	10	101.50	101.00	13.050	0.6945
*0.78	13.100	0.6972	13.100	0.000	20.000	41.100	10	89.00	101.00	13.050	0.6945

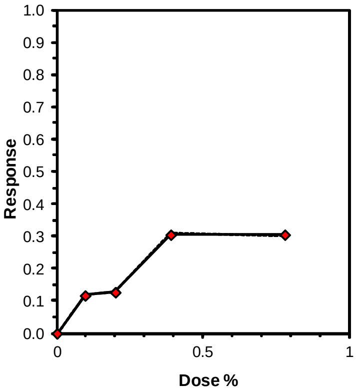
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates non-normal distribution (p <= 0.01)	1.08004	1.035	-0.8573	0.72255
Bartlett's Test indicates equal variances (p = 0.34)	4.52257	13.2767		
The control means are not significantly different (p = 0.65)	0.46768	2.10982		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Wilcoxon Rank Sum Test	0.39	0.78	0.55154	256.41

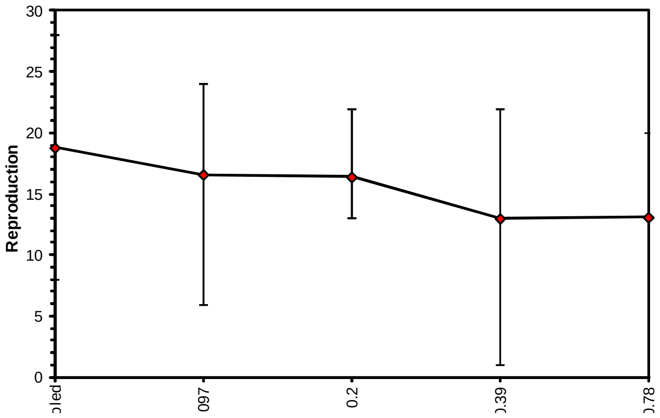
Linear Interpolation (200 Resamples)

Point	%	SD	95% CL		Skew
IC05*	0.0416	0.0678	0.0201	0.2452	1.6824
IC10*	0.0832	0.0858	0.0402	0.3296	1.1413
IC15	0.2243				
IC20	0.2776				
IC25	0.3309				
IC40	>0.78				
IC50	>0.78				

* indicates IC estimate less than the lowest concentration



Dose-Response Plot



Ceriodaphnia Survival and Reproduction Test-Reproduction

Start Date:		Test ID:	Woods Pt	Sample ID:	
End Date:		Lab ID:		Sample Type:	
Sample Date:		Protocol:		Test Species:	CD-Ceriodaphnia dubia
Comments:	reproduction over 9 days				

Conc-%	1	2	3	4	5	6	7	8	9	10
RM water	19.000	19.000	14.000	13.000	28.000	22.000	8.000	21.000	20.000	
RMW 50%	21.000	20.000	21.000	19.000	19.000	23.000	8.000	20.000	20.000	22.000
1.56	15.000	15.000	16.000	12.000	16.000	7.000	7.000	11.000	19.000	13.000
3.13	6.000	4.000	7.000	8.000	12.000	5.000	7.000	8.000	9.000	8.000
6.25	3.000	7.000	3.000	4.000	8.000	8.000	4.000	10.000	7.000	4.000
12.5	0.000	4.000	5.000	12.000	2.000	5.000	4.000	1.000	3.000	3.000
25	0.000	0.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000

			Transform: Untransformed					Rank	1-Tailed	Isotonic	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Mean	N-Mean
Pooled	18.789	1.0000	18.7895	8.0000	28.0000	26.108	19			18.789	1.0000
*1.56	13.100	0.6972	13.1000	7.0000	19.0000	29.989	10	83.50	99.00	13.100	0.6972
*3.13	7.400	0.3938	7.4000	4.0000	12.0000	30.015	10	62.00	99.00	7.400	0.3938
*6.25	5.800	0.3087	5.8000	3.0000	10.0000	42.854	10	59.00	99.00	5.800	0.3087
*12.5	3.900	0.2076	3.9000	0.0000	12.0000	84.135	10	57.00	99.00	3.900	0.2076
*25	0.200	0.0106	0.2000	0.0000	1.0000	210.819	10	55.00	99.00	0.200	0.0106

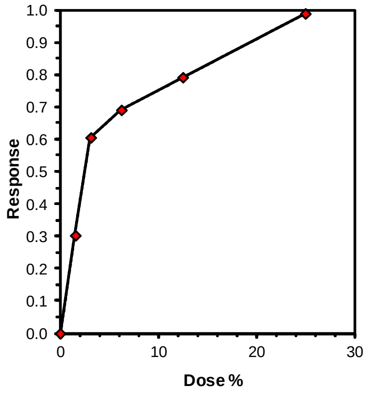
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates non-normal distribution (p <= 0.01)	1.24606	1.035	-0.6052	2.67888
Bartlett's Test indicates unequal variances (p = 4.19E-07)	37.774	15.0863		
The control means are not significantly different (p = 0.65)	0.46768	2.10982		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Wilcoxon Rank Sum Test	<1.56	1.56		

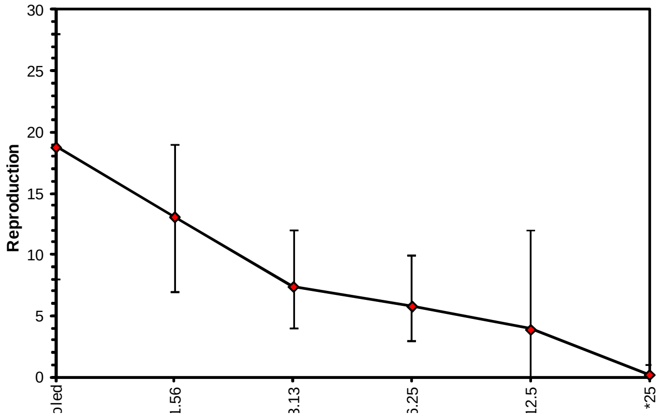
Linear Interpolation (200 Resamples)

Point	%	SD	95% CL		Skew
IC05*	0.2576	0.0750	0.1752	0.4732	1.6332
IC10*	0.5152	0.1499	0.3504	0.9464	1.6332
IC15*	0.7728	0.2174	0.5255	1.4196	1.3315
IC20*	1.0304	0.2591	0.7007	1.6988	0.8256
IC25*	1.2880	0.2787	0.8759	1.8996	0.4041
IC40	2.0630	0.3067	1.4015	2.5164	-0.2762
IC50	2.5806	0.2893	2.0815	3.1265	0.4715

* indicates IC estimate less than the lowest concentration



Dose-Response Plot



Ceriodaphnia Survival and Reproduction Test-48 Hr Survival

Start Date:	3/27/2013	Test ID:	Cerio	Sample ID:	reference toxicamt
End Date:	3/29/2013	Lab ID:		Sample Type:	copper
Sample Date:		Protocol:		Test Species:	CD-Ceriodaphnia dubia

Comments: Copper reference toxicant test for Chronic Ceriodaphnia dubia tests

Conc-ug/L	1	2	3	4
Control	1.0000	0.8000	1.0000	1.0000
5	1.0000	0.8000	0.8000	0.8000
10	0.6000	0.6000	0.0000	0.0000
15	0.0000	0.4000	0.0000	0.4000
20	0.0000	0.0000	0.0000	0.0000

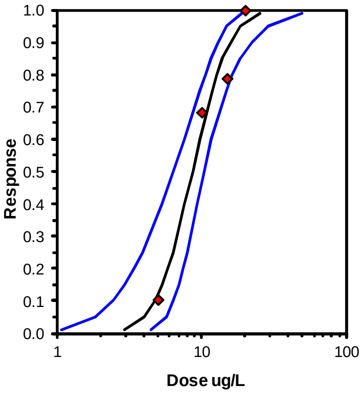
			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-ug/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
Control	0.9500	1.0000	1.2857	1.1071	1.3453	9.261	4				1	20
5	0.8500	0.8947	1.1667	1.1071	1.3453	10.206	4	0.682	2.290	0.4000	3	20
*10	0.3000	0.3158	0.5558	0.2255	0.8861	68.618	4	4.179	2.290	0.4000	14	20
*15	0.2000	0.2105	0.4551	0.2255	0.6847	58.254	4	4.755	2.290	0.4000	16	20
20	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	4				20	20

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.93886	0.844	-6E-16	-1.1659
Bartlett's Test indicates equal variances (p = 0.17)	5.02942	11.3449		

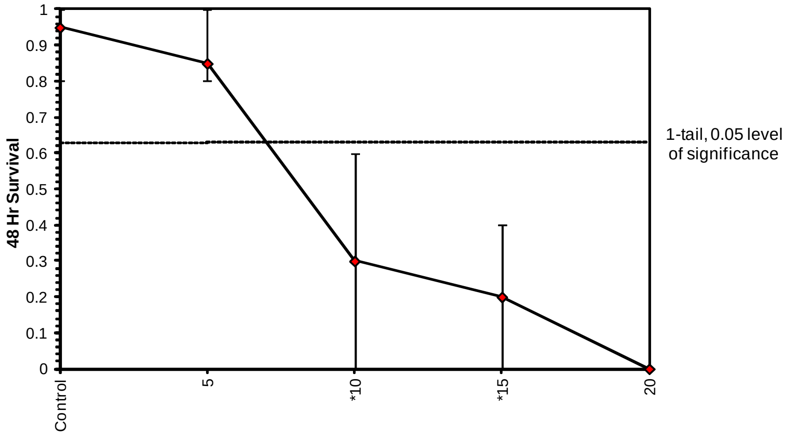
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	5	10	7.07107		0.32125	0.34884	0.70887	0.06102	7.3E-04	3, 12

Maximum Likelihood-Probit											
Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	4.94245	1.06148	2.86194	7.02295	0.05	2.52344	5.99146	0.28	0.93851	0.20233	4
Intercept	0.36148	1.10881	-1.8118	2.53476							
TSCR	0.04848	0.0474	-0.0444	0.14137							

Point	Probits	ug/L	95% Fiducial Limits	
EC01	2.674	2.93643	1.07654	4.49945
EC05	3.355	4.03371	1.84646	5.6756
EC10	3.718	4.77762	2.45598	6.43874
EC15	3.964	5.35557	2.97262	7.0216
EC20	4.158	5.86438	3.45528	7.53184
EC25	4.326	6.33925	3.92657	8.00878
EC40	4.747	7.71342	5.37752	9.42145
EC50	5.000	8.67974	6.44131	10.4791
EC60	5.253	9.76711	7.63311	11.7813
EC75	5.674	11.8843	9.78035	14.8135
EC80	5.842	12.8467	10.6503	16.4378
EC85	6.036	14.0672	11.6671	18.7092
EC90	6.282	15.7689	12.9605	22.2304
EC95	6.645	18.677	14.9522	29.0761
EC99	7.326	25.6563	19.1475	49.1237



Dose-Response Plot



Ceriodaphnia Survival and Reproduction Test-48 Hr Survival

Start Date:	4/13/2013	Test ID:	Cerio	Sample ID:	reference toxicamt
End Date:	4/15/2013	Lab ID:		Sample Type:	copper
Sample Date:		Protocol:		Test Species:	CD-Ceriodaphnia dubia

Comments: Copper reference toxicant test for Chronic Ceriodaphnia dubia tests

Conc-ug/L	1	2	3	4
Control	0.8000	1.0000	1.0000	0.8000
5	0.8000	0.8000	0.8000	0.8000
10	0.2000	0.0000	0.4000	0.4000
15	0.0000	0.2000	0.2000	0.0000
20	0.0000	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-ug/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
Control	0.9000	1.0000	1.2262	1.1071	1.3453	11.212	4			2	20
5	0.8000	0.8889	1.1071	1.1071	1.1071	0.000	4	14.00	10.00	4	20
*10	0.2500	0.2778	0.5146	0.2255	0.6847	42.578	4	10.00	10.00	15	20
*15	0.1000	0.1111	0.3446	0.2255	0.4636	39.900	4	10.00	10.00	18	20
20	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	4			20	20

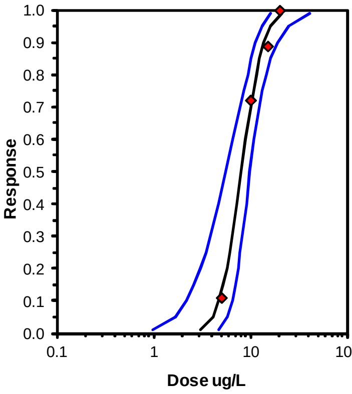
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.91384	0.844	-0.4901	-0.2333
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	5	10	7.07107	

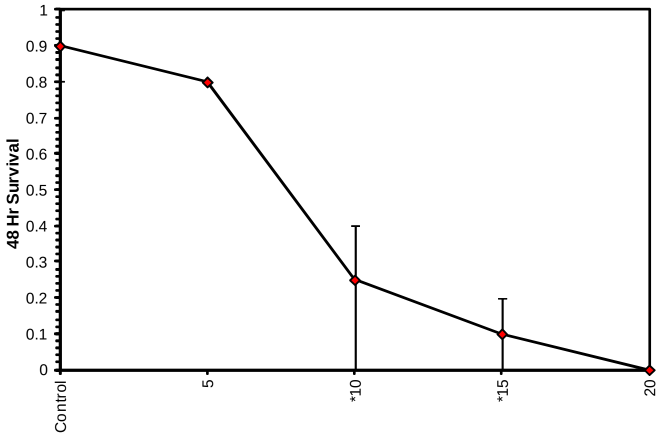
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	5.53415	1.32305	2.94097	8.12732	0.1	0.81146	5.99146	0.67	0.91042	0.1807	3
Intercept	-0.0384	1.35798	-2.7	2.62326							
TSCR	0.09817	0.06509	-0.0294	0.22575							

Point	Probits	ug/L	95% Fiducial Limits	
EC01	2.674	3.09068	0.97958	4.77842
EC05	3.355	4.1039	1.65606	5.84552
EC10	3.718	4.77358	2.18638	6.52239
EC15	3.964	5.28611	2.63358	7.03221
EC20	4.158	5.73242	3.05007	7.47375
EC25	4.326	6.14523	3.45601	7.88254
EC40	4.747	7.32213	4.70542	9.07108
EC50	5.000	8.1361	5.62661	9.93865
EC60	5.253	9.04055	6.67089	10.9826
EC75	5.674	10.7719	8.59329	13.3582
EC80	5.842	11.5477	9.37753	14.6289
EC85	6.036	12.5226	10.2834	16.42
EC90	6.282	13.8672	11.4057	19.2262
EC95	6.645	16.13	13.0627	24.7296
EC99	7.326	21.418	16.3553	40.8476



Dose-Response Plot



Ceriodaphnia Survival and Reproduction Test-48 Hr Survival

Start Date:	4/20/2013	Test ID:	Cerio	Sample ID:	reference toxicant
End Date:	4/22/2013	Lab ID:		Sample Type:	copper
Sample Date:		Protocol:		Test Species:	CD-Ceriodaphnia dubia

Comments: reference toxiant test for chronic tests with Ceriodaphnia dubia

Conc-ug/L	1	2	3	4
Control	1.0000	1.0000	1.0000	0.8000
5	0.8000	0.8000	0.6000	0.8000
10	0.2000	0.0000	0.2000	0.2000
15	0.2000	0.0000	0.2000	0.0000
20	0.0000	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-ug/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
Control	0.9500	1.0000	1.2857	1.1071	1.3453	9.261	4			1	20
5	0.7500	0.7895	1.0519	0.8861	1.1071	10.508	4	11.50	10.00	5	20
*10	0.1500	0.1579	0.4041	0.2255	0.4636	29.464	4	10.00	10.00	17	20
*15	0.1000	0.1053	0.3446	0.2255	0.4636	39.900	4	10.00	10.00	18	20
20	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	4			20	20

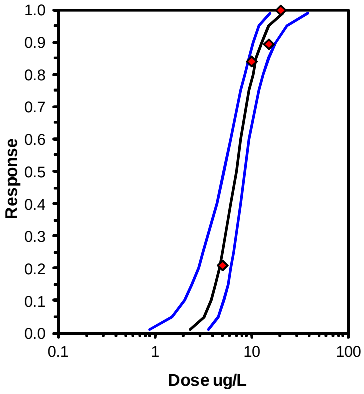
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.75274	0.844	-0.8328	-1.1047
Bartlett's Test indicates equal variances (p = 0.99)	0.13583	11.3449		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	5	10	7.07107	

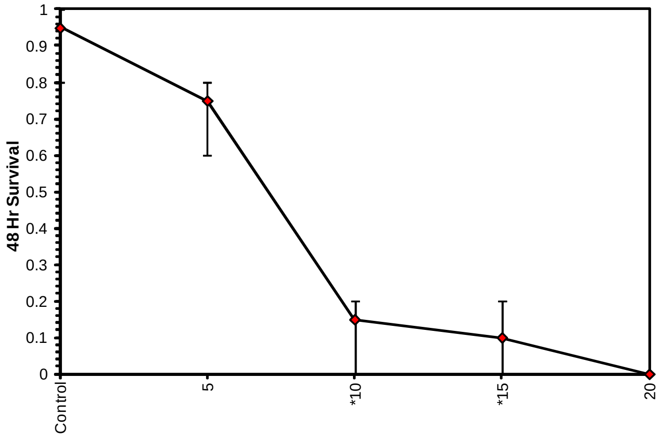
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	4.91819	1.02608	2.90707	6.92931	0.05	1.83925	5.99146	0.4	0.84613	0.20333	3
Intercept	0.83857	0.99269	-1.1071	2.78425							
TSCR	0.04814	0.04777	-0.0455	0.14176							

Point	Probits	ug/L	95% Fiducial Limits	
EC01	2.674	2.36113	0.89923	3.60996
EC05	3.355	3.24853	1.52783	4.57164
EC10	3.718	3.85084	2.02156	5.19832
EC15	3.964	4.31911	2.43792	5.67834
EC20	4.158	4.73157	2.82536	6.0995
EC25	4.326	5.11667	3.20239	6.4939
EC40	4.747	6.23185	4.35633	7.66485
EC50	5.000	7.01664	5.19761	8.54144
EC60	5.253	7.90026	6.13871	9.61542
EC75	5.674	9.62212	7.84675	12.0782
EC80	5.842	10.4053	8.54808	13.3793
EC85	6.036	11.3989	9.37471	15.187
EC90	6.282	12.7851	10.4341	17.9748
EC95	6.645	15.1556	12.0745	23.3697
EC99	7.326	20.8515	15.5416	39.0665



Dose-Response Plot



Raw data from the shrimp survival bioassay

Test material: Toora 96 hr test **Date:**
Test organism: Shrimp **Time:**

	48hr		96hr											
	# Alive		# Alive		96 h	Water Quality at 0hr			Water Quality at 48hr			Water Quality at 96hr		
Concentration	A	B	A	B	Av # Alive	pH	DO	Cond	pH	DO	Cond	pH	DO	Cond
MHW Control	8	9	8	7	7.5	7.24	6.56	229.3	7.83	6.24	332	7.73	5.44	286
River Water	8	10	8	10	9	7.45	6.48	205	7.86	6.39	247.2	7.85	6	311
1.56%	10	10	10	10	10	7.31	6.65	608	7.72	6	463	7.8	5.25	619
3.12%	10	10	10	10	10	7.38	6.17	724	7.77	5.54	713	7.79	6.21	787
6.25%	9	10	8	10	9	7.33	6.4	1176	7.73	6.45	1228	7.83	5.87	1315
12.50%	10	10	10	10	10	7.24	6.62	1883	7.69	6.21	2119	7.8	6.93	2015
25%	10	10	10	10	10	6.98	6.18	3.98ms	7.61	6.01	3.68ms	7.66	5.46	4.07ms
50%	10	10	10	10	10	6.68	6.07	6.98ms	7.27	5.29	7.51ms	7.33	5.5	6.60ms
100%	4	5	4	5	4.5	4.91	6.16	13.56ms	3.69	5.33	14.45ms	4	5.61	14.56ms

Test material: Wellington 96 hr test **Date:**
Test organism: Shrimp **Time:**

	48hr		96hr											
	# Alive		# Alive		96 h	Water Quality at 0hr			Water Quality at 48hr			Water Quality at 96hr		
Concentration	A	B	A	B	Av # Alive	pH	DO	Cond	pH	DO	Cond	pH	DO	Cond
MHW Control	8	9	8	7	7.5	7.24	6.56	229.3	7.83	6.24	332	7.73	5.44	286
River Water	8	10	8	10	9	7.45	6.48	205	7.86	6.39	247.2	7.85	6	311
1.56%	10	10	9	10	9.5	7.48	7	264	6.46	6.4	274	6.66	5.48	293
3.12%	10	10	10	7	8.5	7.51	6.84	345	6.81	5.97	341	7.08	6.15	390
6.25%	10	10	10	10	10	7.48	6.94	476	7.09	5.55	468	7.24	5.96	514
12.50%	10	9	10	8	9	7.31	6.93	898	7.2	6.15	839	7.5	6.13	1002
25%	9	8	9	6	7.5	7.15	6.58	1291	7.2	5.18	1436	7.49	5.28	1415
50%	8	10	8	10	9	6.82	6.48	2353	7.24	5.99	2224	7.41	5.73	2483
100%	10	10	10	10	10	5.66	6.73	3.96ms	6.84	5.81	4.00ms	6.74	5.52	4.39ms

Test material: Woods Pt 96 hr test **Date:**
Test organism: Shrimp **Time:**

	48hr		96hr											
	# Alive		# Alive		96 h	Water Quality at 0hr			Water Quality at 48hr			Water Quality at 96hr		
Concentration	A	B	A	B	Av # Alive	pH	DO	Cond	pH	DO	Cond	pH	DO	Cond
MHW Control	10	10	9	10	9.5	7.91	6.82	205.9	7.32	6.5	337	7.43	6.48	310
River Water	10	10	10	10	10	8.04	6.51	263	7.6	6.9	275	7.69	6.52	273
1.56%	10	10	100	10	55	7.94	7.29	306	7.7	6.29	322	7.71	6.47	332
3.12%	10	10	10	10	10	7.78	7.28	573	7.87	6.77	410	7.74	6.93	451
6.25%	10	10	9	10	9.5	7.89	6.56	409	7.83	6.44	587	7.75	6.39	642
12.50%	10	10	10	10	10	7.66	6.08	948	7.81	6.46	1039	7.77	6.47	987
25%	10	10	10	10	10	7.43	6.46	1520	7.79	6.37	1652	7.61	6.32	1559
50%	7	9	7	9	8	7.13	6.43	2644	7.73	6.22	2783	7.59	6.68	2781
100%	9	9	9	9	9	6.62	6.73	4.61ms	7.66	6.26	4.85ms	7.72	6.41	5.3ms

Test material: Mobilong 96 hr test **Date:**
Test organism: Shrimp **Time:**

	48hr		96hr											
	# Alive		# Alive		96 h	Water Quality at 0hr			Water Quality at 48hr			Water Quality at 96hr		
Concentration	A	B	A	B	Av # Alive	pH	DO	Cond	pH	DO	Cond	pH	DO	Cond
MHW Control	10	10	9	10	9.5	7.91	6.82	205.9	7.32	6.5	337	7.43	6.48	310
River Water	10	10	10	10	10	8.04	6.51	263	7.6	6.9	275	7.69	6.52	273
1.56%	10	10	10	9	9.5	7.45	7.07	690	7.99	6.09	731	8.15	6.29	811
3.12%	10	10	10	10	10	7.28	6.39	1235	7.93	6.23	1238	8.07	6.16	1229
6.25%	10	10	10	9	9.5	7.08	6.38	2094	7.83	6.44	2099	7.87	6.06	2441
12.50%	10	9	10	9	9.5	6.51	6.48	3.68ms	7.59	6.68	4.02ms	7.59	6.28	4.87ms
25%	10	9	7	9	8	4.5	6.51	7.6ms	5.42	6.57	7.85ms	5.25	6.45	8.97ms
50%	0	0	-	-	0	3.07	6.78	13.37ms	3.3	6.15	13.17ms			
100%	0	0	-	-	0	2.73	5.12	27.04ms	2.68	6.22	26.81ms			

Acute Fish Test-96 Hr Survival

Start Date:	10/29/2012	Test ID:	Toora	Sample ID:	
End Date:	11/2/2012	Lab ID:	CLW Adelaide	Sample Type:	acidic drainage water
Sample Date:		Protocol:		Test Species:	PA-Paratya australiensis
Comments:					

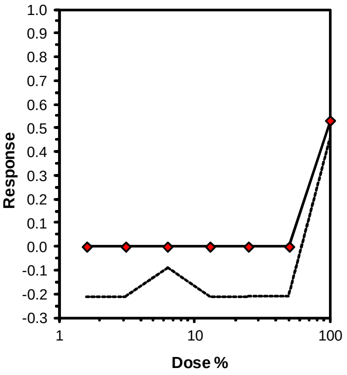
Conc-%	1	2
Control	0.8000	0.7000
Murray Rv	0.8000	1.0000
1.6	1.0000	1.0000
3.1	1.0000	1.0000
6.3	0.8000	1.0000
13	1.0000	1.0000
25	1.0000	1.0000
50	1.0000	1.0000
100	0.4000	0.5000

			Transform: Arcsin Square Root					Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Resp	Number
Pooled	0.8250	1.0000	1.1544	0.9912	1.4120	15.615	4	7	40
1.6	1.0000	1.2121	1.4120	1.4120	1.4120	0.000	2	0	20
3.1	1.0000	1.2121	1.4120	1.4120	1.4120	0.000	2	0	20
6.3	0.9000	1.0909	1.2596	1.1071	1.4120	17.115	2	2	20
13	1.0000	1.2121	1.4120	1.4120	1.4120	0.000	2	0	20
25	1.0000	1.2121	1.4120	1.4120	1.4120	0.000	2	0	20
50	1.0000	1.2121	1.4120	1.4120	1.4120	0.000	2	0	20
100	0.4500	0.5455	0.7351	0.6847	0.7854	9.685	2	11	20

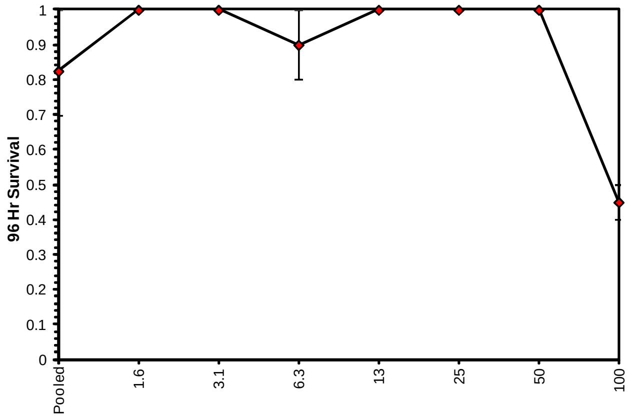
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				
The control means are not significantly different (p = 0.33)	1.29024	4.30265		

Trimmed Spearman-Karber

Trim Level	EC50	95% CL	
0.0%			
5.0%			
10.0%			
20.0%			
Auto-46.8%	95.964	72.988	126.172



Dose-Response Plot



Acute Fish Test-96 Hr Survival

Start Date:	10/29/2012	Test ID:	Wellington	Sample ID:	
End Date:	11/2/2012	Lab ID:	CLW Adelaide	Sample Type:	acidic drainage water
Sample Date:		Protocol:		Test Species:	PA-Paratya australiensis
Comments:					

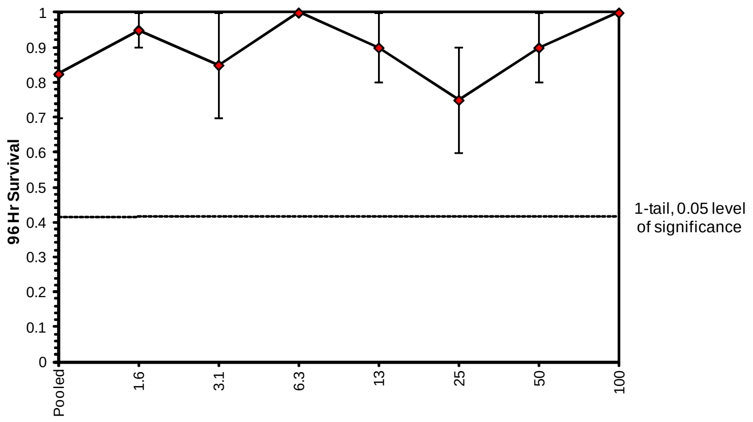
Conc-%	1	2
Control	0.8000	0.7000
Murray Rv	0.8000	1.0000
1.6	0.9000	1.0000
3.1	1.0000	0.7000
6.3	1.0000	1.0000
13	1.0000	0.8000
25	0.9000	0.6000
50	0.8000	1.0000
100	1.0000	1.0000

			Transform: Arcsin Square Root						1-Tailed			
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD		
Pooled	0.8250	1.0000	1.1544	0.9912	1.4120	15.615	4				7	40
1.6	0.9500	1.1515	1.3305	1.2490	1.4120	8.661	2	-1.075	2.700	0.4425	1	20
3.1	0.8500	1.0303	1.2016	0.9912	1.4120	24.767	2	-0.288	2.700	0.4425	3	20
6.3	1.0000	1.2121	1.4120	1.4120	1.4120	0.000	2	-1.572	2.700	0.4425	0	20
13	0.9000	1.0909	1.2596	1.1071	1.4120	17.115	2	-0.642	2.700	0.4425	2	20
25	0.7500	0.9091	1.0676	0.8861	1.2490	24.041	2	0.530	2.700	0.4425	5	20
50	0.9000	1.0909	1.2596	1.1071	1.4120	17.115	2	-0.642	2.700	0.4425	2	20
100	1.0000	1.2121	1.4120	1.4120	1.4120	0.000	2	-1.572	2.700	0.4425	0	20

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				
The control means are not significantly different (p = 0.33)	1.29024	4.30265		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	100	>100		1	0.40965	0.48979	0.03231	0.03581	0.54035	7, 10

Dose-Response Plot



Acute Fish Test-96 Hr Survival

Start Date:	10/29/2012	Test ID:	Woods Pt	Sample ID:	
End Date:	11/2/2012	Lab ID:	CLW Adelaide	Sample Type:	acidic drainage water
Sample Date:		Protocol:		Test Species:	PA-Paratya australiensis
Comments:					

Conc-%	1	2
Control	0.9000	1.0000
Murray Rv	1.0000	1.0000
1.6	1.0000	1.0000
3.1	1.0000	1.0000
6.3	0.9000	1.0000
13	1.0000	1.0000
25	1.0000	1.0000
50	0.7000	0.9000
100	0.9000	0.9000

			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
Pooled	0.9750	1.0000	1.3713	1.2490	1.4120	5.942	4				1	40
1.6	1.0000	1.0256	1.4120	1.4120	1.4120	0.000	2	-0.577	2.700	0.1906	0	20
3.1	1.0000	1.0256	1.4120	1.4120	1.4120	0.000	2	-0.577	2.700	0.1906	0	20
6.3	0.9500	0.9744	1.3305	1.2490	1.4120	8.661	2	0.577	2.700	0.1906	1	20
13	1.0000	1.0256	1.4120	1.4120	1.4120	0.000	2	-0.577	2.700	0.1906	0	20
25	1.0000	1.0256	1.4120	1.4120	1.4120	0.000	2	-0.577	2.700	0.1906	0	20
*50	0.8000	0.8205	1.1201	0.9912	1.2490	16.280	2	3.558	2.700	0.1906	4	20
100	0.9000	0.9231	1.2490	1.2490	1.2490	0.000	2	1.731	2.700	0.1906	2	20

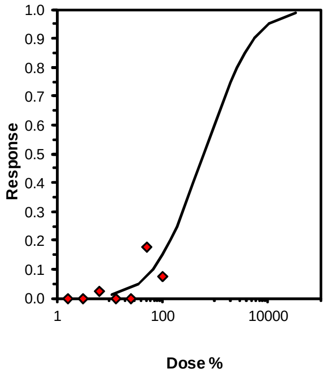
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				
The control means are not significantly different (p = 0.42)	1	4.30265		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	25	50	35.3553	4	0.10535	0.10966	0.02266	0.00665	0.03919	7, 10

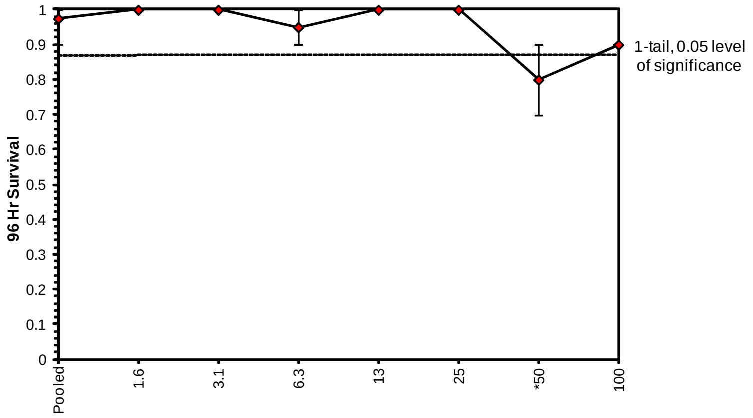
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	1.32561	0.9953	-0.6252	3.27639	0.025	6.93933	11.0705	0.23	2.79087	0.75437	8
Intercept	1.3004	1.7742	-2.177	4.77783							
TSCR	0.01464	0.01368	-0.0122	0.04145							

Point	Probits	%	95% Fiducial Limits
EC01	2.674	10.8628	
EC05	3.355	35.4846	
EC10	3.718	66.6967	
EC15	3.964	102.097	
EC20	4.158	143.21	
EC25	4.326	191.449	
EC40	4.747	397.879	
EC50	5.000	617.829	
EC60	5.253	959.37	
EC75	5.674	1993.81	
EC80	5.842	2665.4	
EC85	6.036	3738.72	
EC90	6.282	5723.12	
EC95	6.645	10757.1	
EC99	7.326	35139.6	



Dose-Response Plot



Acute Fish Test-96 Hr Survival

Start Date: 10/29/2012 | Test ID: Mobilong | Sample ID:
End Date: 11/2/2012 | Lab ID: CLW Adelaide | Sample Type: acidic drainage water
Sample Date: | Protocol: | Test Species: PA-Paratya australiensis
Comments:

Conc-%	1	2
Control	0.9000	1.0000
Murray Rv	1.0000	1.0000
1.6	1.0000	0.9000
3.1	1.0000	1.0000
6.3	1.0000	0.9000
13	1.0000	0.9000
25	0.7000	0.9000
50	0.0000	0.0000
100	0.0000	0.0000

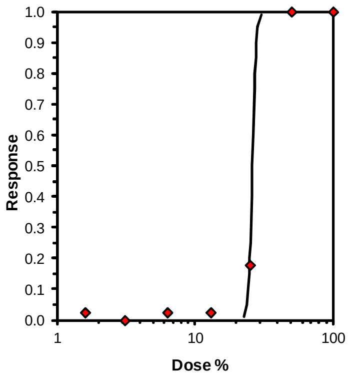
			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
Pooled	0.9750	1.0000	1.3713	1.2490	1.4120	5.942	4				1	40
1.6	0.9500	0.9744	1.3305	1.2490	1.4120	8.661	2	0.436	2.660	0.2484	1	20
3.1	1.0000	1.0256	1.4120	1.4120	1.4120	0.000	2	-0.436	2.660	0.2484	0	20
6.3	0.9500	0.9744	1.3305	1.2490	1.4120	8.661	2	0.436	2.660	0.2484	1	20
13	0.9500	0.9744	1.3305	1.2490	1.4120	8.661	2	0.436	2.660	0.2484	1	20
*25	0.8000	0.8205	1.1201	0.9912	1.2490	16.280	2	2.690	2.660	0.2484	4	20
50	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20
100	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				
The control means are not significantly different (p = 0.42)	1	4.30265		

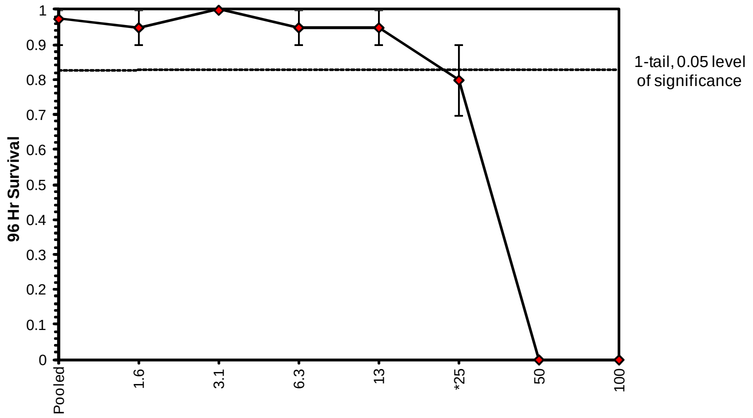
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	13	25	18.0278	7.69231	0.14828	0.15434	0.02157	0.01163	0.20839	5, 8

			Maximum Likelihood-Logit								
Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	74.9745	188.854	-295.18	445.128	0.025	1.2069	11.0705	0.94			50
Intercept	-106.38	264.008	-623.83	411.077							
TSCR	0.03333	0.01639	0.00122	0.06545							

Point	Logits	%	95% Fiducial Limits
EC01	-4.595	22.781	
EC05	-2.944	23.9657	
EC10	-2.197	24.522	
EC15	-1.735	24.8729	
EC20	-1.386	25.1404	
EC25	-1.099	25.3635	
EC40	-0.405	25.9092	
EC50	0.000	26.2338	
EC60	0.405	26.5626	
EC75	1.099	27.1341	
EC80	1.386	27.3749	
EC85	1.735	27.6693	
EC90	2.197	28.0652	
EC95	2.944	28.7167	
EC99	4.595	30.21	



Dose-Response Plot



Acute Fish Test-96 Hr Survival

Start Date:	10/29/2012	Test ID:	Mobilong	Sample ID:	
End Date:	11/2/2012	Lab ID:	CLW Adelaide	Sample Type:	acidic drainage water
Sample Date:		Protocol:		Test Species:	PA-Paratya australiensis
Comments:					

Conc-%	1	2
Control	0.9000	1.0000
Murray Rv	1.0000	1.0000
1.6	1.0000	0.9000
3.1	1.0000	1.0000
6.3	1.0000	0.9000
13	1.0000	0.9000
25	0.7000	0.9000
50	0.0000	0.0000
100	0.0000	0.0000

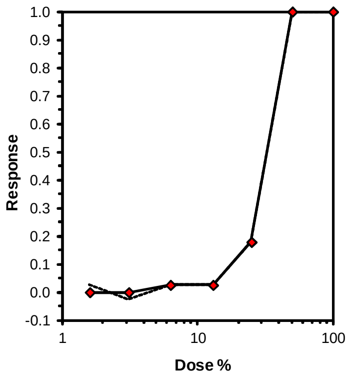
			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
Pooled	0.9750	1.0000	1.3713	1.2490	1.4120	5.942	4				1	40
1.6	0.9500	0.9744	1.3305	1.2490	1.4120	8.661	2	0.436	2.660	0.2484	1	20
3.1	1.0000	1.0256	1.4120	1.4120	1.4120	0.000	2	-0.436	2.660	0.2484	0	20
6.3	0.9500	0.9744	1.3305	1.2490	1.4120	8.661	2	0.436	2.660	0.2484	1	20
13	0.9500	0.9744	1.3305	1.2490	1.4120	8.661	2	0.436	2.660	0.2484	1	20
*25	0.8000	0.8205	1.1201	0.9912	1.2490	16.280	2	2.690	2.660	0.2484	4	20
50	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20
100	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				
The control means are not significantly different (p = 0.42)	1	4.30265		

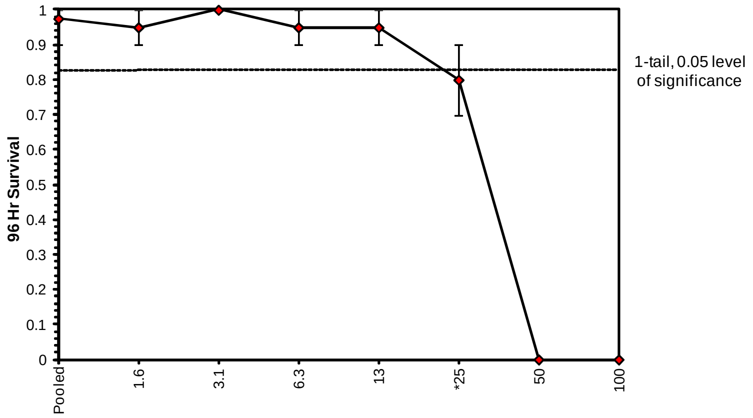
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	13	25	18.0278	7.69231	0.14828	0.15434	0.02157	0.01163	0.20839	5, 8

Trimmed Spearman-Karber

Trim Level	EC50	95% CL	
0.0%	30.220	26.396	34.598
5.0%	31.751	27.852	36.196
10.0%	32.336	27.556	37.945
20.0%	32.774	30.003	35.801
Auto-0.0%	30.220	26.396	34.598



Dose-Response Plot



Raw data from the fish survival bioassay

Test material: Toora 7 day test
Date: 27/10/12 - 31-10/12 - 2/11/12
Test organism: Fish Fry- Murray Cod
Time:

	48hr						96hr						144hr							
	# Alive			# Dead			# Alive			# Dead			# Alive			# Dead			144 h	
Concentration	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	Av # Alive	Av # Dead
MFS Control	10	10	10	0	0	0	9	10	9	1	0	1	9	10	9	1	0	1	9.3	0.7
River Water 100%A	10	10	10	0	0	0	9	10	10	1	0	0	9	10	9	1	0	1	9.3	0.7
River Water 100%B	10	10	10	0	0	0	10	10	10	0	0	0	10	10	10	0	0	0	10.0	0.0
River Water 50%	10	10	10	0	0	0	10	10	10	0	0	0	10	10	10	0	0	0	10.0	0.0
0.25%	10	9	10	0	1	0	10	9	9	0	1	1	10	8	7	0	2	3	8.3	1.7
0.50%	10	10	10	0	0	0	10	10	10	0	0	0	9	9	8	1	1	2	8.7	1.3
1%	10	9	10	0	1	0	6	8	10	4	2	0	5	8	9	5	2	1	7.3	2.7
2.00%	10	10	10	0	0	0	10	9	10	0	1	0	9	8	8	1	2	2	8.3	1.7
3.12%	0	0	0	10	10	10														
6.25%	0	0	0	10	10	10														
12.50%	0	0	0	10	10	10														
25%	0	0	0	10	10	10														
50%	0	0	0	10	10	9														
100%	0	0	0	9	10	10														

	Water Quality at 0hr				Water Quality at 48hr				Water Quality at 96hr				Water Quality at 144hr			
Concentration	pH	DO	Cond	Temp	pH	DO	Cond	Temp	pH	DO	Cond	Temp	pH	DO	Cond	Temp
MFS Control					7.99	4.93	1324		7.82	4.28	1243		7.95	4.48	1257	
River Water 100%A	7.45	6.51	210		8.16	5	410		8.11	4.63	402		8.09	4.77	339	
River Water 100%B	7.28	6.29	252		7.89	5.21	321		8.07	4.37	300		7.69	5.7	353	
River Water 50%	7.31	6.34	589		7.84	5.01	689		7.99	4.74	691		7.73	6.04	7.07	
0.25%					7.82	4.35	281		7.94	4.45	275		7.65	4.99	295	
0.50%					7.83	5.11	337		7.95	4.92	345		7.71	5.34	358	
1%					7.71	4.52	453		7.91	4.07	460		7.66	4.72	446	
2.00%					7.78	4.62	625		7.89	4.25	611		7.63	4.8	630	
3.12%																
6.25%																
12.50%																
25%																
50%																
0%																
0																

Acute Fish Test-7 Day

Start Date:	10/27/2012	Test ID:	Toora	Sample ID:	
End Date:	11/2/2012	Lab ID:	CLW Adelaide	Sample Type:	acidic drainage water
Sample Date:		Protocol:		Test Species:	MC-Murray Cod
Comments:					

Conc-%	1	2	3	4	5	6
Control	0.9000	1.0000	0.9000			
Murray Rv	0.9000	1.0000	0.9000	1.0000	1.0000	1.0000
0.25	1.0000	0.8000	0.7000			
0.5	0.9000	0.9000	0.8000			
1	0.5000	0.8000	0.9000			
2	0.9000	0.8000	0.8000			
3.1	0.0000	0.0000	0.0000			
6.3	0.0000	0.0000	0.0000			
13	0.0000	0.0000	0.0000			
25	0.0000	0.0000	0.0000			
50	0.0000	0.0000	0.0000			
100	0.0000	0.0000	0.0000			

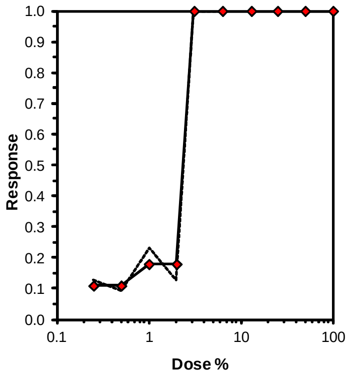
			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
Pooled	0.9556	1.0000	1.3396	1.2490	1.4120	6.412	9				4	90
0.25	0.8333	0.8721	1.1701	0.9912	1.4120	18.578	3	1.878	2.340	0.2112	5	30
0.5	0.8667	0.9070	1.2017	1.1071	1.2490	6.817	3	1.527	2.340	0.2112	4	30
*1	0.7333	0.7674	1.0472	0.7854	1.2490	22.686	3	3.240	2.340	0.2112	8	30
2	0.8333	0.8721	1.1544	1.1071	1.2490	7.096	3	2.051	2.340	0.2112	5	30
3.1	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	3				30	30
6.3	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	3				30	30
13	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	3				30	30
25	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	3				30	30
50	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	3				30	30
100	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	3				30	30

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.94495	0.873	-0.0746	0.07999
Bartlett's Test indicates equal variances (p = 0.19)	6.10536	13.2767		
The control means are not significantly different (p = 0.41)	0.88192	2.36462		

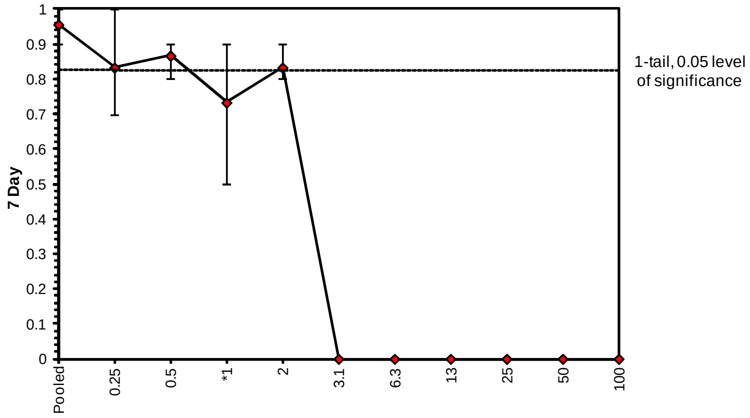
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	0.5	1	0.70711	200	0.13077	0.13802	0.05962	0.01833	0.03927	4, 16

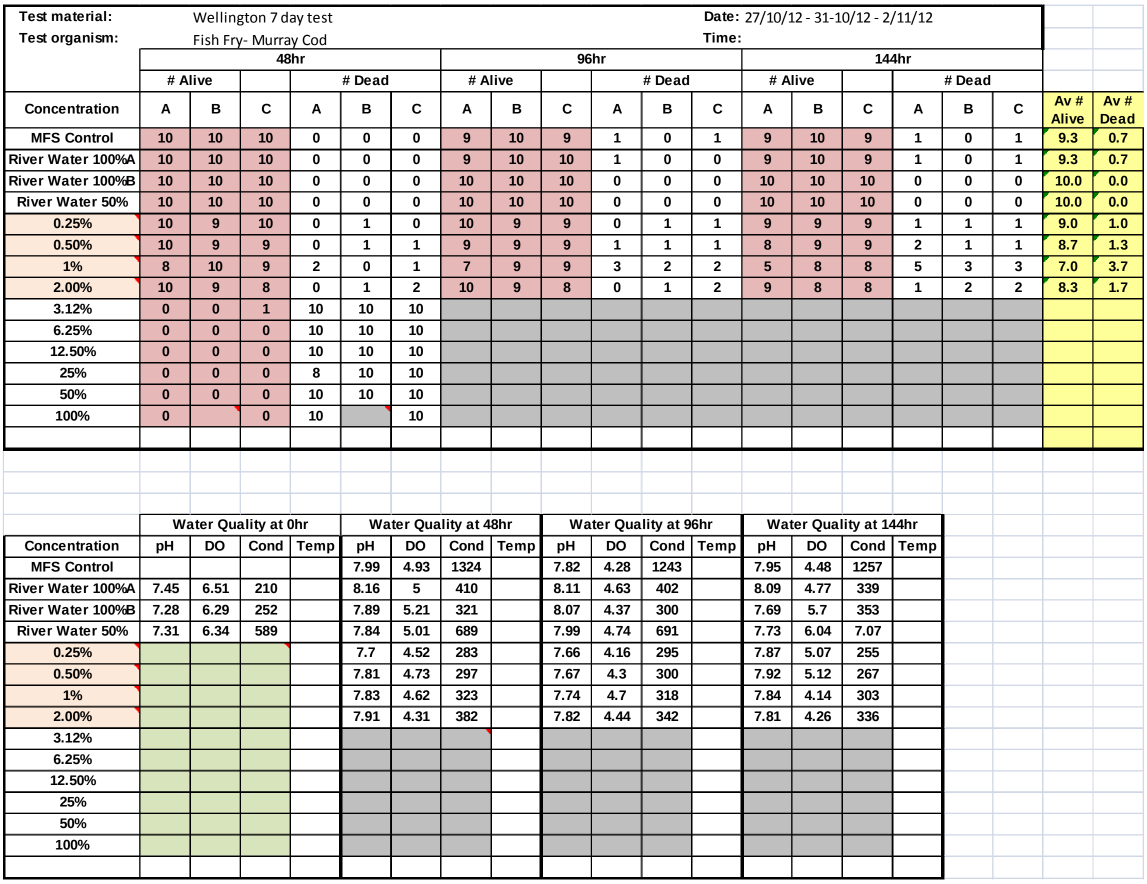
Trimmed Spearman-Karber

Trim Level	EC50	95% CL	
0.0%			
5.0%			
10.0%			
20.0%	2.3729	2.2667	2.4840
Auto-11.0%	2.1655	1.8295	2.5632



Dose-Response Plot





Test material: Wellington 7 day test **Date:** 27/10/12 - 31-10/12 - 2/11/12
Test organism: Fish Fry- Murray Cod **Time:**

	48hr						96hr						144hr							
	# Alive			# Dead			# Alive			# Dead			# Alive			# Dead				
Concentration	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	Av # Alive	Av # Dead
MFS Control	10	10	10	0	0	0	9	10	9	1	0	1	9	10	9	1	0	1	9.3	0.7
River Water 100%A	10	10	10	0	0	0	9	10	10	1	0	0	9	10	9	1	0	1	9.3	0.7
River Water 100%B	10	10	10	0	0	0	10	10	10	0	0	0	10	10	10	0	0	0	10.0	0.0
River Water 50%	10	10	10	0	0	0	10	10	10	0	0	0	10	10	10	0	0	0	10.0	0.0
0.25%	10	9	10	0	1	0	10	9	9	0	1	1	9	9	9	1	1	1	9.0	1.0
0.50%	10	9	9	0	1	1	9	9	9	1	1	1	8	9	9	2	1	1	8.7	1.3
1%	8	10	9	2	0	1	7	9	9	3	2	2	5	8	8	5	3	3	7.0	3.7
2.00%	10	9	8	0	1	2	10	9	8	0	1	2	9	8	8	1	2	2	8.3	1.7
3.12%	0	0	1	10	10	10														
6.25%	0	0	0	10	10	10														
12.50%	0	0	0	10	10	10														
25%	0	0	0	8	10	10														
50%	0	0	0	10	10	10														
100%	0		0	10		10														

	Water Quality at 0hr				Water Quality at 48hr				Water Quality at 96hr				Water Quality at 144hr			
Concentration	pH	DO	Cond	Temp	pH	DO	Cond	Temp	pH	DO	Cond	Temp	pH	DO	Cond	Temp
MFS Control					7.99	4.93	1324		7.82	4.28	1243		7.95	4.48	1257	
River Water 100%A	7.45	6.51	210		8.16	5	410		8.11	4.63	402		8.09	4.77	339	
River Water 100%B	7.28	6.29	252		7.89	5.21	321		8.07	4.37	300		7.69	5.7	353	
River Water 50%	7.31	6.34	589		7.84	5.01	689		7.99	4.74	691		7.73	6.04	7.07	
0.25%					7.7	4.52	283		7.66	4.16	295		7.87	5.07	255	
0.50%					7.81	4.73	297		7.67	4.3	300		7.92	5.12	267	
1%					7.83	4.62	323		7.74	4.7	318		7.84	4.14	303	
2.00%					7.91	4.31	382		7.82	4.44	342		7.81	4.26	336	
3.12%																
6.25%																
12.50%																
25%																
50%																
100%																

Acute Fish Test-7 Day

Start Date:	10/27/2012	Test ID:	Toora	Sample ID:	
End Date:	11/2/2012	Lab ID:	CLW Adelaide	Sample Type:	acidic drainage water
Sample Date:		Protocol:		Test Species:	MC-Murray Cod
Comments:					

Conc-%	1	2	3	4	5	6
Control	0.9000	1.0000	0.9000			
Murray Rv	0.9000	1.0000	0.9000	1.0000	1.0000	1.0000
0.25	1.0000	0.8000	0.7000			
0.5	0.9000	0.9000	0.8000			
1	0.5000	0.8000	0.9000			
2	0.9000	0.8000	0.8000			
3.1	0.0000	0.0000	0.0000			
6.3	0.0000	0.0000	0.0000			
13	0.0000	0.0000	0.0000			
25	0.0000	0.0000	0.0000			
50	0.0000	0.0000	0.0000			
100	0.0000	0.0000	0.0000			

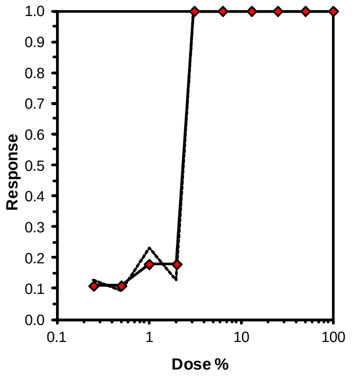
			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
Pooled	0.9556	1.0000	1.3396	1.2490	1.4120	6.412	9				4	90
0.25	0.8333	0.8721	1.1701	0.9912	1.4120	18.578	3	1.878	2.340	0.2112	5	30
0.5	0.8667	0.9070	1.2017	1.1071	1.2490	6.817	3	1.527	2.340	0.2112	4	30
*1	0.7333	0.7674	1.0472	0.7854	1.2490	22.686	3	3.240	2.340	0.2112	8	30
2	0.8333	0.8721	1.1544	1.1071	1.2490	7.096	3	2.051	2.340	0.2112	5	30
3.1	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	3				30	30
6.3	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	3				30	30
13	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	3				30	30
25	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	3				30	30
50	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	3				30	30
100	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	3				30	30

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.94495	0.873	-0.0746	0.07999
Bartlett's Test indicates equal variances (p = 0.19)	6.10536	13.2767		
The control means are not significantly different (p = 0.41)	0.88192	2.36462		

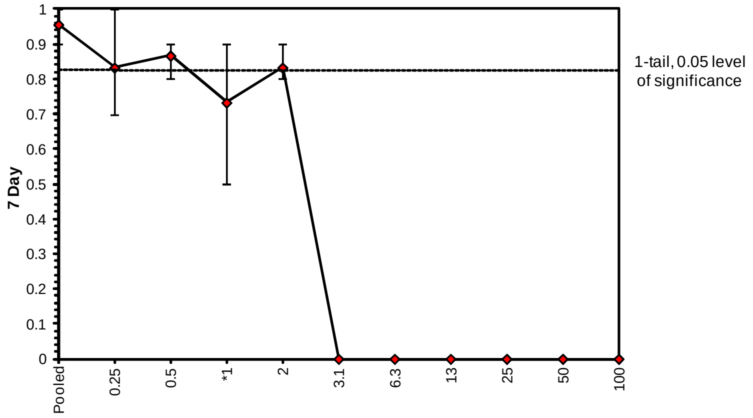
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	0.5	1	0.70711	200	0.13077	0.13802	0.05962	0.01833	0.03927	4, 16

Trimmed Spearman-Karber

Trim Level	EC50	95% CL	
0.0%			
5.0%			
10.0%			
20.0%	2.3729	2.2667	2.4840
Auto-11.0%	2.1655	1.8295	2.5632



Dose-Response Plot



Test material: Woods Point 7 day test
Test organism: Fish Fry- Murray Cod
Date: 27/10/12 - 29-10/12 - 31/10/12- 2/11/12
Time:

	48hr						96hr						144hr							
	# Alive			# Dead			# Alive			# Dead			# Alive			# Dead				
Concentration	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	Av # Alive	Av # Dead
MFS Control	10	10	10	0	0	0	9	10	9	1	0	1	9	10	9	1	0	1	9.3	0.7
River Water 100%A	10	10	10	0	0	0	9	10	10	1	0	0	9	10	9	1	0	1	9.3	0.7
River Water 100%B	10	10	10	0	0	0	10	10	10	0	0	0	10	10	10	0	0	0	10.0	0.0
River Water 50%	10	10	10	0	0	0	10	10	10	0	0	0	10	10	10	0	0	0	10.0	0.0
0.78%	10	10	10	0	0	0	9	10	10	1	0	0	9	9	6	1	1	4	8.0	2.0
1.50%	10	10	9	0	0	1	10	10	9	0	0	1	9	9	8	1	1	2	8.7	1.3
3.12%	10	10	10	0	0	0	10	9	10	0	1	0	9	9	9	1	1	1	9.0	1.0
6.25%	10	10	10	0	0	0	9	10	10	1	0	0	8	10	10	2	0	0	9.3	0.7
12.50%	9	10	10	1	0	0	9	10	10	1	0	0	9	10	9	1	0	1	9.3	0.7
25%	10	9	10	0	1	0	10	8	10	0	2	0	9	7	10	1	3	0	8.7	1.3
50%	9	10	10	1	0	0	9	10	10	1	0	0	9	10	9	1	0	1	9.3	0.7
100%	10	10	10	0	0	0	10	10	10	0	0	0	10	10	10	0	0	0	10.0	0.0
																			######	######

	Water Quality at 0hr				Water Quality at 48hr				Water Quality at 96hr				Water Quality at 144hr			
Concentration	pH	DO	Cond	Temp	pH	DO	Cond	Temp	pH	DO	Cond	Temp	pH	DO	Cond	Temp
MFS Control					7.99	4.93	1324		7.82	4.28	1243		7.95	4.48	1257	
River Water 100%A	7.45	6.51	210		8.16	5	410		8.11	4.63	402		8.09	4.77	339	
River Water 100%B	7.28	6.29	252		7.89	5.21	321		8.07	4.37	300		7.69	5.7	353	
River Water 50%	7.31	6.34	589		7.84	5.01	689		7.99	4.74	691		7.73	6.04	7.07	
0.78%	6.23	5.89	248.7		7.21	5.21	302		7.9	4.88	299		7.72	5.49	300	
1.56%	6.76	5.91	303		7.34	5.01	351		7.92	4.85	247		7.76	5.37	344	
3.12%	6.91	6.84	396		8.09	4.98	476		8.01	5.12	432		7.95	4.66	449	
6.25%	6.96	6.32	576		8.1	5.41	690		8.04	5.05	612		7.93	4.79	626	
12.50%	6.97	6.52	892		8.14	5.53	1071		8.07	5.13	981		7.83	4.27	1000	
25%	6.95	5.84	1488		8.07	4.95	1662		7.98	5.14	1536		7.75	4.16	1673	
50%	6.83	6.42	2516		7.69	4.63	3170		7.78	5.21	2.99ms		7.68	4.36	2.92ms	
100%	6.53	6.97	4.77ms		7.6	4.26	4980		7.68	5.02	4.82ms		7.56	4.18	5.33ms	

Acute Fish Test-7 Day

Start Date:	10/27/2012	Test ID:	Woods Pt	Sample ID:	
End Date:	11/2/2012	Lab ID:	CLW Adelaide	Sample Type:	acidic drainage water
Sample Date:		Protocol:		Test Species:	MC-Murray Cod
Comments:					

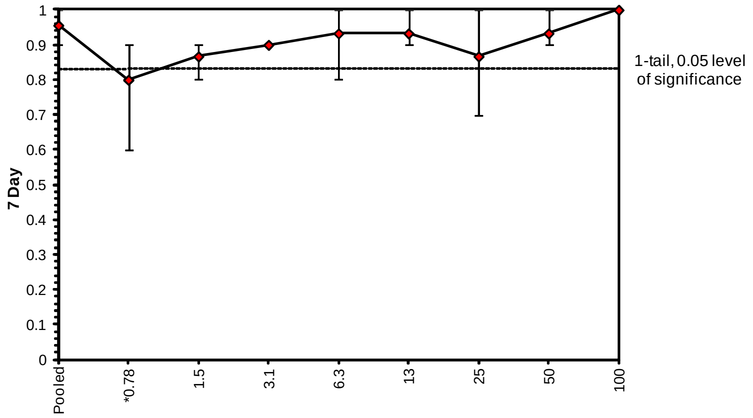
Conc-%	1	2	3	4	5	6
Control	0.9000	1.0000	0.9000			
Murray Rv	0.9000	1.0000	0.9000	1.0000	1.0000	1.0000
0.78	0.9000	0.9000	0.6000			
1.5	0.9000	0.9000	0.8000			
3.1	0.9000	0.9000	0.9000			
6.3	0.8000	1.0000	1.0000			
13	0.9000	1.0000	0.9000			
25	0.9000	0.7000	1.0000			
50	0.9000	1.0000	0.9000			
100	1.0000	1.0000	1.0000			

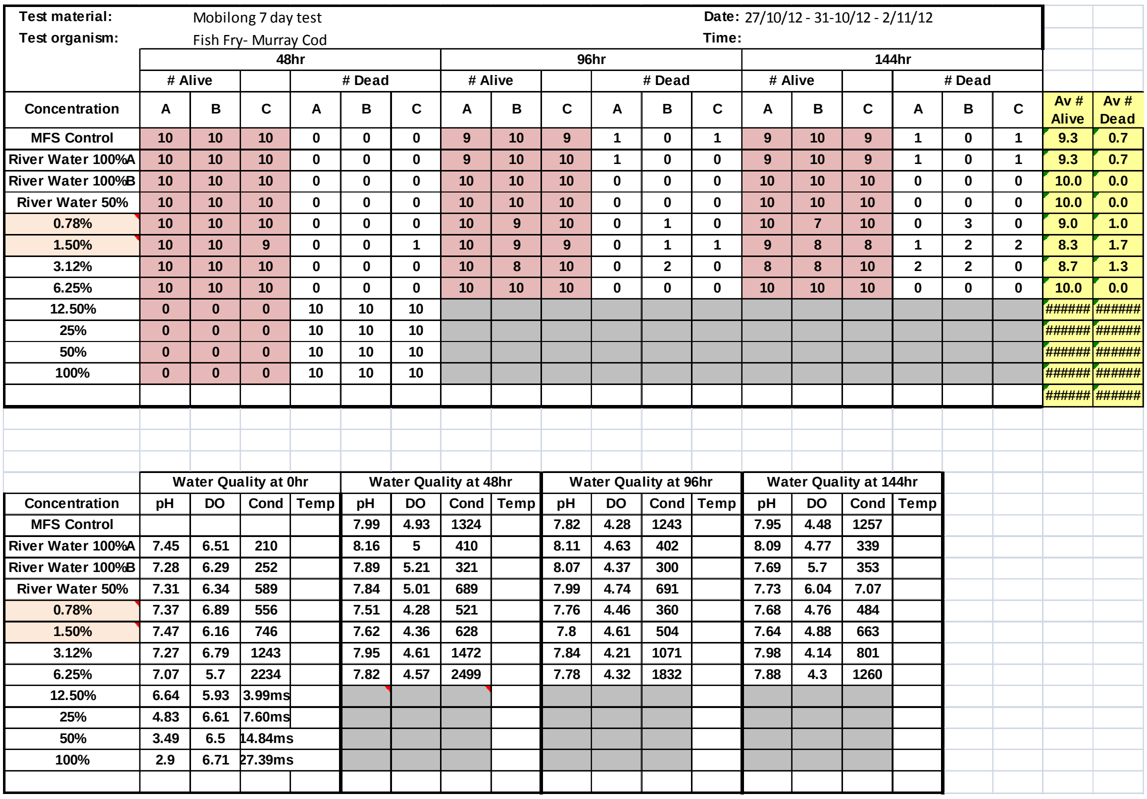
			Transform: Arcsin Square Root						1-Tailed	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD
Pooled	0.9556	1.0000	1.3396	1.2490	1.4120	6.412	9			
*0.78	0.8000	0.8372	1.1281	0.8861	1.2490	18.577	3	2.636	2.530	0.2030
1.5	0.8667	0.9070	1.2017	1.1071	1.2490	6.817	3	1.718	2.530	0.2030
3.1	0.9000	0.9419	1.2490	1.2490	1.2490	0.000	3	1.128	2.530	0.2030
6.3	0.9333	0.9767	1.3104	1.1071	1.4120	13.432	3	0.364	2.530	0.2030
13	0.9333	0.9767	1.3034	1.2490	1.4120	7.219	3	0.451	2.530	0.2030
25	0.8667	0.9070	1.2174	0.9912	1.4120	17.431	3	1.523	2.530	0.2030
50	0.9333	0.9767	1.3034	1.2490	1.4120	7.219	3	0.451	2.530	0.2030
100	1.0000	1.0465	1.4120	1.4120	1.4120	0.000	3	-0.903	2.530	0.2030

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.94402	0.906	-0.6133	0.09507
Equality of variance cannot be confirmed				
The control means are not significantly different (p = 0.41)	0.88192	2.36462		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	100	>100		1	0.12449	0.13138	0.02393	0.01449	0.16256	8, 24

Dose-Response Plot





Test material: Mobilong 7 day test **Date:** 27/10/12 - 31-10/12 - 2/11/12

Test organism: Fish Fry- Murray Cod **Time:**

	48hr						96hr						144hr							
	# Alive			# Dead			# Alive			# Dead			# Alive			# Dead				
Concentration	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	Av # Alive	Av # Dead
MFS Control	10	10	10	0	0	0	9	10	9	1	0	1	9	10	9	1	0	1	9.3	0.7
River Water 100%A	10	10	10	0	0	0	9	10	10	1	0	0	9	10	9	1	0	1	9.3	0.7
River Water 100%B	10	10	10	0	0	0	10	10	10	0	0	0	10	10	10	0	0	0	10.0	0.0
River Water 50%	10	10	10	0	0	0	10	10	10	0	0	0	10	10	10	0	0	0	10.0	0.0
0.78%	10	10	10	0	0	0	10	9	10	0	1	0	10	7	10	0	3	0	9.0	1.0
1.50%	10	10	9	0	0	1	10	9	9	0	1	1	9	8	8	1	2	2	8.3	1.7
3.12%	10	10	10	0	0	0	10	8	10	0	2	0	8	8	10	2	2	0	8.7	1.3
6.25%	10	10	10	0	0	0	10	10	10	0	0	0	10	10	10	0	0	0	10.0	0.0
12.50%	0	0	0	10	10	10													######	######
25%	0	0	0	10	10	10													######	######
50%	0	0	0	10	10	10													######	######
100%	0	0	0	10	10	10													######	######
																			######	######

	Water Quality at 0hr				Water Quality at 48hr				Water Quality at 96hr				Water Quality at 144hr			
Concentration	pH	DO	Cond	Temp	pH	DO	Cond	Temp	pH	DO	Cond	Temp	pH	DO	Cond	Temp
MFS Control					7.99	4.93	1324		7.82	4.28	1243		7.95	4.48	1257	
River Water 100%A	7.45	6.51	210		8.16	5	410		8.11	4.63	402		8.09	4.77	339	
River Water 100%B	7.28	6.29	252		7.89	5.21	321		8.07	4.37	300		7.69	5.7	353	
River Water 50%	7.31	6.34	589		7.84	5.01	689		7.99	4.74	691		7.73	6.04	7.07	
0.78%	7.37	6.89	556		7.51	4.28	521		7.76	4.46	360		7.68	4.76	484	
1.50%	7.47	6.16	746		7.62	4.36	628		7.8	4.61	504		7.64	4.88	663	
3.12%	7.27	6.79	1243		7.95	4.61	1472		7.84	4.21	1071		7.98	4.14	801	
6.25%	7.07	5.7	2234		7.82	4.57	2499		7.78	4.32	1832		7.88	4.3	1260	
12.50%	6.64	5.93	3.99ms													
25%	4.83	6.61	7.60ms													
50%	3.49	6.5	14.84ms													
100%	2.9	6.71	27.39ms													

Acute Fish Test-7 Day

Start Date:	10/27/2012	Test ID:	Mobilong	Sample ID:	
End Date:	11/2/2012	Lab ID:	CLW Adelaide	Sample Type:	acidic drainage water
Sample Date:		Protocol:		Test Species:	MC-Murray Cod
Comments:					

Conc-%	1	2	3	4	5	6
Control	0.9000	1.0000	0.9000			
Murray Rv	0.9000	1.0000	0.9000	1.0000	1.0000	1.0000
0.78	1.0000	0.7000	1.0000			
1.5	0.9000	0.8000	0.8000			
3.1	0.8000	0.8000	1.0000			
6.3	1.0000	1.0000	1.0000			
13	0.0000	0.0000	0.0000			
25	0.0000	0.0000	0.0000			
50	0.0000	0.0000	0.0000			
100	0.0000	0.0000	0.0000			

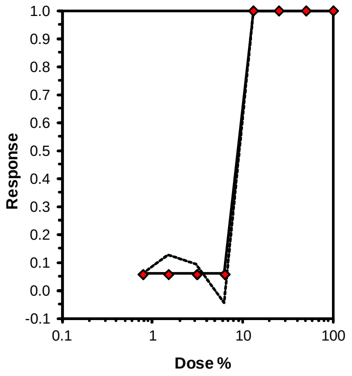
			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
Pooled	0.9556	1.0000	1.3396	1.2490	1.4120	6.412	9				4	90
0.78	0.9000	0.9419	1.2717	0.9912	1.4120	19.107	3	0.810	2.340	0.1960	3	30
1.5	0.8333	0.8721	1.1544	1.1071	1.2490	7.096	3	2.211	2.340	0.1960	5	30
3.1	0.8667	0.9070	1.2088	1.1071	1.4120	14.562	3	1.562	2.340	0.1960	4	30
6.3	1.0000	1.0465	1.4120	1.4120	1.4120	0.000	3	-0.865	2.340	0.1960	0	30
13	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	3				30	30
25	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	3				30	30
50	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	3				30	30
100	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	3				30	30

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.94372	0.873	-0.417	0.46981
Equality of variance cannot be confirmed				
The control means are not significantly different (p = 0.41)	0.88192	2.36462		

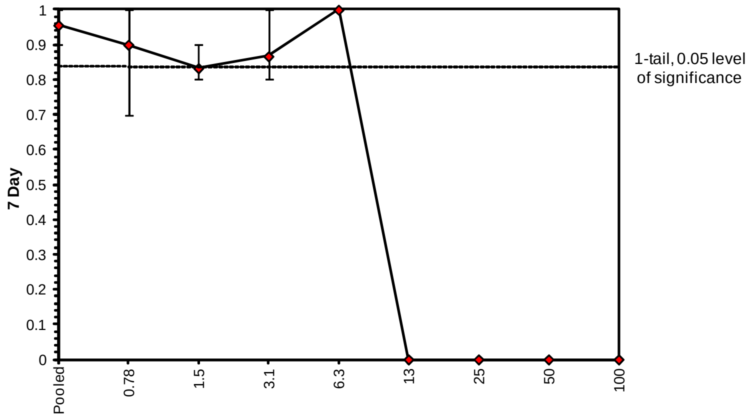
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	6.3	13	9.04986	15.873	0.11914	0.12574	0.03554	0.01578	0.10896	4, 16

Trimmed Spearman-Karber

Trim Level	EC50	95% CL	
0.0%			
5.0%			
10.0%	8.8498	8.5463	9.1640
20.0%	8.8498	8.5463	9.1640
Auto-5.8%	8.8498	8.5463	9.1640



Dose-Response Plot



Murray cod larvae lengths after 7-day exposure to LMIRA drainage water and their dilutions

Sample	Treatment (% dilution)	Mean Length (mm)	Std Deviation
MFS (synthetic water)	100%	12.31	0.20
River Water-1	100%	12.52	0.21
***River Water-2**	100%	13.01	0.20
River Water	50%	12.98	0.10
		0.00	0.00
Woods Point	100	12.69	0.18
Woods Point	50	12.78	0.18
Woods Point	25	12.87	0.23
Woods Point	12.5	12.89	0.14
Woods Point	6.25	12.75	0.13
Woods Point	3.125	12.73	0.17
Mobilong	6.25	12.74	0.09
Mobilong	3.125	12.68	0.23
Mobilong	1.56	13.13	0.11
Mobilong	0.78	12.97	0.19
Wellington	2	13.07	0.22
Wellington	1	12.96	0.22
Wellington	0.5	12.86	0.25
Wellington	0.25	12.72	0.51
Toora	2	13.08	0.26
Toora	1	12.77	0.16
Toora	0.5	12.97	0.10

*Controls for Toora and Wellington samples

Shrimp oxidative stress data-LMIRA project

Values are Mean ± STD, n=4/treatment

Glutathione Reductase (mU/mg protein)

	Toora mean	STD	Mobilong mean	STD	Wellington mean	STD	Woods Point mean	STD
MHW	5.51	0.98	6.98	0.90	5.30	0.59	5.14	0.87
RW	5.21	0.18	7.37	0.64	5.48	0.40	5.05	1.02
1.56%	5.70	1.42	6.95	1.27	6.09	0.59	4.97	0.68
3.12%	6.11	0.70	6.42	0.51	6.05	1.03	4.85	1.09
6.25%	4.77	0.21	8.07	2.36	4.91	1.93	4.26	0.84
12.50%	4.78	0.75	6.40	2.02	5.22	0.56	4.38	0.12
25%	4.73	0.68	6.95	0.39	5.29	1.10	4.45	0.83
50%	4.59	0.25			6.10	0.33	4.72	1.32
100%	4.31	0.30			4.68	0.20	4.61	0.69

GST (mU/mg protein)

	Toora mean	STD	Mobilong mean	STD	Wellington mean	STD	Woods Point mean	STD
MHW	26.14	1.49	19.41	3.51	25.00	3.57	16.78	2.63
RW	28.12	2.52	17.90	4.56	23.07	3.75	16.01	2.47
1.56%	27.62	3.15	17.32	2.16	27.84	3.48	18.51	4.42
3.12%	30.15	5.80	21.91	5.96	33.77	5.70	16.95	3.63
6.25%	23.91	5.11	21.91	3.09	19.17	4.48	18.29	2.14
12.50%	17.60	3.15	15.80	3.75	18.50	2.37	17.12	1.49
25%	24.60	3.72	19.59	1.94	19.90	2.85	19.90	2.85
50%	30.09	3.88			28.48	5.56	18.43	4.33
100%	23.40	6.57			22.42	4.61	17.72	5.99

Appendix C Hardness Algorithms for Adjustment of Metal Trigger Values

Table D1. Hardness algorithms for selected metals

Metal	Algorithm
Cadmium	$TV(H/30)^{0.89}$
Chromium(III)	$TV(H/30)^{0.82}$
Copper	$TV(H/30)^{0.85}$
Lead	$TV(H/30)^{1.27}$
Nickel	$TV(H/30)^{0.85}$
Zinc	$TV(H/30)^{0.85}$

TV = trigger value at 30 mg/L hardness

H = hardness (mg/L)

Note that the correction factor only applies to chromium(III) and not chromium(VI)

Table D2. Approximate factors to apply to the default trigger values (i.e. multiplying factors)

Hardness Category (mg/L as $CaCO_3$)	Water Hardness (mg/L as $CaCO_3$)	Cd	Cr(III)	Cu	Pb	Ni	Zn
Soft (0-59)	30	TV	TV	TV	TV	TV	TV
Moderate (60-119)	60	1.9	1.8	1.8	2.4	1.8	1.8
Moderate (60-119)	90	2.7	2.5	2.5	4.0	2.5	2.5
Hard (120-179)	150	4.2	3.7	3.9	7.6	3.9	3.9
Very Hard (180-240)	210	5.7	4.9	5.2	11.8	5.2	5.2
Extremely Hard (>241)	400	10.0	8.4	9.0	26.7	9.0	9.0

Water hardness can be estimated from measured Ca and Mg concentrations using the equation: $4.11 \times [Mg] + 2.47 \times [Ca]$

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FOR FURTHER INFORMATION

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