Intertek GEOTECH
GEOTECHNICAL SERVICES PTY LTD

TOXICITY ASSESSMENT FOR A SIMULATED DESALINATION BRINE EFFLUENT

- Prepared for GHD Pty Ltd -

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EXECUTIVE SUMMARY

A toxicity assessment was performed for one desalination brine effluent to identify any adverse biological effects potentially resulting from a continuous discharge of reverse osmosis reject water into the marine environment at the proposed Cape Riche seawater desalination plant in Western Australia. Given this assessment was performed in fulfilment of pre-commissioning requirements, the simulated desalination brine was prepared from site-specific seawater to the chemical specifications of the expected effluent composition.

The simulated desalination brine was chemically characterised to identify any contaminants of potential concern exceeding the ANZECC/ARMCANZ (2000) water quality guidelines relevant to south Western Australia. The potential for adverse biological effects resulting from the simulated brine were assessed using both acute and chronic ecotoxicity tests for six representative marine species including the microalga *Isochrysis galbana*, the macroalga *Ecklonia radiata*, the sea urchin *Heliocidaris erythrogramma*, the bivalve *Mytilis edulis*, the copepod *Gladioferens imparipes* and the fish *Pagrus auratus*.

Based on the chemical characterisation of the simulated brine, a dilution factor of >40 would be required to lower all contaminants of potential concern (including salinity) to levels below the recommended marine water quality guideline trigger values. The chronic dilution factors calculated from the 99 and 95% levels of species protection (i.e. dilution factors of 53 and 40, respectively) would be suitable to achieve this level of dilution, as well as a high to moderate level of species protection. Good agreement between the chemical characterisation and ecotoxicity testing suggested that a minimum safe dilution factor of 40 should be applied when discharging a reverse osmosis reject water with similar physico-chemical characteristics to the simulated brine assessed in this study.

However it is noted that the derived safe dilution factors will be less representative where marine water quality guideline exceedances are greater and/or other contaminants not addressed in this study are identified in the commissioned Cape Riche desalination plant reject water. For this reason, it is recommended that the toxicity of the brine effluent be reassessed upon plant commissioning to confirm that an appropriate level of species protection is being achieved.

DISCLAIMER

One simulated desalination brine effluent was prepared and tested by Intertek-Geotech for GHD Pty Ltd to assess the potential for toxicity to marine organisms on discharge into the Cape Riche receiving environment. The toxicity assessment was performed in accordance with principles outlined in ANZECC/ARMCANZ (2000) and similar peer reviewed marine toxicity assessments for simulated brine effluents.

Intertek-Geotech has endeavoured to achieve high accuracy results and information using certified techniques and equipment. However, Intertek-Geotech shall not be held responsible or liable for the results of any actions taken on the basis of the information contained in this document. Moreover, this report should not be the sole reference when considering issues that may have commercial implications.

All data and information will remain proprietary to GHD Pty Ltd and is regarded as strictly confidential by all Intertek-Geotech personnel. Any queries related to this report may be directed to David Strom at Intertek-Geotech.

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	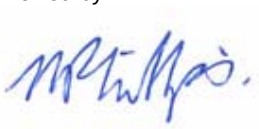
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1 INTRODUCTION

In April 2011, a toxicity assessment was performed for one desalination brine effluent to identify any adverse biological effects potentially resulting from a continuous discharge into the marine environment at the proposed Cape Riche seawater desalination plant in Western Australia. Considering this toxicity assessment was initiated prior to commissioning of the Cape Riche seawater desalination plant, the desalination brine was simulated to the chemical specifications of the expected effluent composition.

The simulated brine effluent was chemically characterised (including measurements of pH, dissolved oxygen, salinity, conductivity, turbidity, total suspended solids, nutrients, dissolved metals and surfactants) and compared to the ANZECC/ARMCANZ (2000) marine water quality guidelines to identify any contaminants of potential concern above background levels in the site-specific seawater. The toxicity of the simulated desalination brine effluent was assessed using local marine test organisms representative of the south Western Australian temperate waters of Cape Riche. Both acute and chronic endpoints for several species from different trophic levels were measured. For this assessment, acute bioassay endpoints were defined as lethal or sub-lethal effects occurring over a short-term of exposure using a less sensitive life stage of the test organism. The chronic bioassay endpoints were defined as sub-lethal effects occurring over a longer term of exposure using a more sensitive life stage of the test organism.

Acute and chronic toxicity tests were performed with six species including the microalga *Isochrysis galbana*, the macroalga *Ecklonia radiata*, the sea urchin *Heliocidaris erythrogramma*, the bivalve *Mytilis edulis*, the copepod *Gladioferens imparipes* and the fish *Pagrus auratus*. In accordance with ANZECC/ARMCANZ (2000), results from the toxicity testing were used to derive both acute and chronic species protection trigger values for 99, 95, 90 and 80% levels of species protection. The derived acute and chronic species protection trigger values for the simulated brine effluent are presented as the safe dilution factors required to achieve an adequate level of species protection in the mixing zone of the Cape Riche marine environment.

2 METHODS

2.1 Simulated Brine Effluent

The high salinity of reverse osmosis reject water is a common characteristic of seawater desalination plant effluents. In addition, several chemical additives are dosed throughout the desalination process to facilitate specific functions including chlorination, pH adjustment, coagulation, flocculation, dechlorination, antiscaling and membrane cleaning. Common chemical additives including sodium hypochlorite, sulphuric acid, sodium hydroxide, ferric chloride, surfactants (e.g. sodium dodecylsulphate), polymers (e.g. polyDADMAC or polyacrylamide), sodium metabisulphite, antiscalants (e.g. polyacrylate or polyphosphates) and biocides (e.g. DBNPA) would generally be utilised in the process specific functions.

Similar studies performed in Western Australia have identified the elevated salinity and residual levels of antiscalant in the reverse osmosis reject water to be of most significance when assessing the toxicity of desalination plant effluent discharges (Geotechnical Services Report ENV05-214). In keeping with previous studies, a high salinity brine effluent was simulated to include a residual concentration of antiscalant considered to be representative of levels expected for discharge from the Cape Riche desalination plant.

The reject water is expected to contain a maximum salinity of 70‰ and a residual antiscalant concentration of 1.4 mg/L (GHD Pty Ltd *pers comm*). Based on this information, a representative sample of site-specific seawater was collected offshore from Cape Riche and delivered to Geotechnical Services Pty Ltd on the 6th April, 2011 by GHD Pty Ltd. The seawater was passed through a reverse osmosis membrane (Citor 30TS System) to simulate the desalination process. The processed reject water was then collected and the antiscalant 'Nalco Permatreat PC-1020' was dosed into the brine at a nominal concentration of 1.4 mg/L. This simulated brine effluent was considered to be representative of the reverse osmosis reject water to be discharged into the marine receiving environment from the proposed Cape Riche desalination plant.

2.2 Diluent Seawater and Concentration Series

All toxicity tests were performed using filtered seawater as the diluent. Seawater was collected from Cape Riche (WA) and filtered immediately though a 0.45 µm membrane filter. The seawater was stored in acid-washed (10% HNO_3) polyethylene containers at 4°C.

For all toxicity tests, seven concentrations of the simulated brine effluent were prepared using a 1:2 dilution series (*v/v*) with filtered seawater. The concentration series included a Control (0) with brine concentrations of 1.6, 3.1, 6.3, 12.5, 25, 50 and 100%. The physico-chemistry of the simulated brine effluent was not adjusted prior to testing to ensure the toxicity would be representative of *in situ* release and exposure.

2.3 Chemical Characterisation

Physico-chemical measurements including pH, dissolved oxygen, salinity, conductivity, turbidity, total suspended solids, nutrients, metals and surfactants were measured for both the site-specific seawater diluent and simulated desalination brine effluent. Chemical measurements were then compared to the ANZECC/ARMCANZ (2000) marine water quality guideline trigger values to identify any adverse changes in water quality and contaminants of potential concern.

2.4 Physico-chemical Analyses

Physico-chemical parameters for each concentration were measured at the start and end of each toxicity test. Measurements of pH, dissolved oxygen and salinity were reported with the toxicity test results.

2.5 Ecotoxicity Testing

2.5.1 Definition of acute and chronic endpoints

For the purposes of this simulated brine toxicity assessment, the 'acute' bioassay endpoints are defined herein as a lethal or sub-lethal effect occurring over a short-term of exposure using a less sensitive life stage of the test organism. The 'chronic' bioassay endpoints are defined as a sub-lethal effect occurring over a longer term of exposure using a more sensitive life stage of the test organism.

For all bioassays, the chronic endpoints were always more sensitive than the acute endpoints of the same species. Test endpoints have been appropriately classified as acute or chronic and are summarised in Table 1.

Table 1: Classification of acute and chronic bioassay endpoints for selected test species

Bioassay	Test Species	Acute Endpoint	Chronic Endpoint
Microalgae *	*Isochrysis galbana*	72-hour cell division	72-hour cell division
Macroalgae	*Ecklonia radiata*	48-hour germination	72-hour tube development
Echinoderm	*Heliocidaris erythrogramma*	1-hour fertilisation	72-hour larval development
Mollusc	*Mytilis edulis*	96-hour survival	72-hour larval development
Crustacean	*Gladioferens imparipes*	48-hour survival	21-day reproduction
Fish	*Pagrus auratus*	96-hour survival	7-day larval growth

* May be considered both acute and chronic under the ANZECC/ARMCANZ (2000) definition.

2.5.2 Microalgal growth inhibition bioassay

The effect of the simulated brine on microalgal growth was tested using the marine dinoflagellate *Isochrysis galbana*. This test determines the inhibition of cell division over 72-hours and is based on the OECD Test Guideline 201 (1984) and the protocol of Stauber et al. (1994). The test protocol is summarised in Table 2.

Microalgal stock culture

The marine microalga *I. galbana* was cultured in f_2 nutrient medium (Guillard and Ryther, 1962). The in-house culture was maintained axenically on a 12-h light:12-h dark cycle at 21°C. Cells in log phase growth were washed and centrifuged three times to remove culturing medium prior to use in the microalgal bioassay.

Microalgal bioassay

A 20 mL aliquot of each control, concentration, reference toxicant and matrix matched blank solution was dispensed into 120 mL acid washed polycarbonate vials in triplicate. To each vial, 0.2 mL of both 25 mM sodium nitrate and 1.6 mM potassium dihydrogen phosphate were added as nutrients. Each flask was inoculated to 3×10^4 cells/mL of the prewashed *I. galbana* suspension. Vials were incubated at 21°C on a 12-h light:12-h dark photoperiod at 150 µmol photons s^{-1} m^{-2}. The initial and final algal cell density was determined using an automated microplate reader. Microalgal growth inhibition was reported as a percentage of the control.

Quality assurance

A spiked copper reference bioassay was performed concurrently to ensure *I. galbana* was responding as expected to a known toxicant. The spiked copper concentration series consisted of a Control (0), 5, 10, 20 and 40 µg/L treatments. Test acceptability was achieved when (i) the cell division rate in the control treatments was 1.4±0.4 replications per day and (ii) microalgal growth inhibition from the reference toxicant was within the expected response range.

Table 2: Summary of the test protocol for the *Isochrysis galbana* growth inhibition bioassay

Test Parameter	Specification
Organism	*Isochrysis galbana*
Test Type	Static
Test Duration	72-h
Temperature	21 ± 1°C
Light Quality	Daylight Fluorescent Lighting
Light Intensity	150 µmol photons s^{-1} m^{-2}
Photoperiod	12 hour light : 12 hour dark
Test Chamber Size	120 mL
Test Solution Volume	20 mL
Renewal of Test Solutions	None
Age of Test Organisms	4-6 days
Initial Cell Density	3×10^4 cells/mL
No. of Replicates	3
Shaking Rate	Twice daily by hand
Dilution Water	0.45 µm filtered seawater
Concentration Series	Control (0), 1.6, 3.1, 6.3, 12.5, 25, 50 and 100%
Dilution Factor	1:2
Endpoint	Algal Growth Inhibition
Test Acceptability	Control Cell Division Rate 1.4 ± 0.4 Replications per day and Copper Reference EC50 within 8 – 17 µg/L

2.5.3 Macroalgal germination and tube growth bioassays

The effect of the simulated brine on macroalgal zygote germination and germination tube growth was tested using the marine kelp *Ecklonia radiata*. This test determines the inhibition of germination over 48-hours and germination tube development over 72-hours. Both endpoints are based on methods described by Burridge et al. (1999) and are summarised in Table 3.

Macroalgal stock culture

Fertile blades of *E. radiata* were collected offshore from Fremantle, WA. On delivery to the laboratory, the blades were rinsed with deionised water and air dried under ambient laboratory conditions. Once dried, the sori were dissected and placed into seawater to initiate the release of zoospores.

Macroalgal bioassay

A 25 mL aliquot of each control, concentration and reference toxicant was dispensed into 30 mL acid washed borosilicate glass beakers in triplicate. A microscope cover slip was placed into each beaker to allow zygotes to settle and germinate. Each beaker was then inoculated with 0.5 mL of the zoospore solution and incubated at 21°C under ambient light conditions. After 48-hours, the cover slips were removed, mounted on a slide and the proportion of germinated zygotes (indicated by the presence or absence of a germination tube) were determined on visual inspection using a microscope (acute endpoint). Similarly, after 72-hours the germination tube length was measured using a microscope mounted digital camera with Digimizer® imaging software package (chronic endpoint). Acute germination and chronic germination tube length was reported as a percentage of the control.

Quality assurance

A spiked copper reference bioassay was performed concurrently to ensure *E. radiata* was responding as expected to a known toxicant. The spiked copper concentration series consisted of a Control (0), 20, 40, 80 and 160 µg/L treatments. Test acceptability was achieved when (i) germination in the control treatments was >80% and (ii) germination / tube development inhibition from the reference toxicant was within the expected response range.

Table 3: Summary of the test protocol for the acute and chronic *Ecklonia radiata* bioassays

Test Parameter	Acute Specification	Chronic Specification
Organism	*Ecklonia radiata*	*Ecklonia radiata*
Test Type	Static	Static
Test Duration	48-h	72-h
Temperature	21 ± 1°C	21 ± 1°C
Light Quality	Ambient illumination	Ambient illumination
Test Chamber Size	30 mL	30 mL
Test Solution Volume	25 mL	25 mL
Renewal of Test Solutions	None	None
Age of Test Organisms	< 1 hour	< 1 hour
No. of Replicates	3	3
Dilution Water	0.45 µm filtered seawater	0.45 µm filtered seawater
Concentration Series	Control (0), 1.6, 3.1, 6.3, 12.5, 25, 50 and 100%	Control (0), 1.6, 3.1, 6.3, 12.5, 25, 50 and 100%
Dilution Factor	1:2	1:2
Endpoint	Inhibition of germination	Inhibition of germination tube
Test Acceptability	>80% germination in controls and Copper Reference EC50 within 63 – 87 µg/L	>80% germination in controls and Copper Reference EC50 within 16 – 37 µg/L

2.5.4 Sea urchin fertilisation and development bioassays

The effect of the simulated brine on sea urchin fertilisation and larval development was tested using the marine echinoderm *Heliocidaris erythrogramma*. The acute bioassay assesses fertilisation success after sperm are exposed to a sample for 1-hour followed by incubation with viable eggs. The chronic bioassay assesses the inhibition of sea urchin zygote development into pluteus larvae over 72-hours post fertilisation. Both methods are based on Simon and Laginestra (1997) and test protocols are summarised in Table 4.

Sea urchin stock culture

Gametes from adult *H. erythrogramma* were obtained from sea urchins collected in the Abrolhos Islands, Geraldton, WA. Mature adults were spawned by injecting potassium chloride through the peristomal membrane on the oral surface. Eggs were screened for viability prior to testing.

Sea urchin bioassay

A 5 mL aliquot of each control, concentration and reference toxicant solution was dispensed into 10 mL acid washed borosilicate glass vials in triplicate. For the acute sea urchin bioassay, each vial is inoculated with 0.1 mL of sperm solution and then incubated at 21°C under ambient light conditions. Following the 1-hour exposure duration, 1 mL of the egg suspension was added and the proportion of fertilised eggs counted after 20 minutes. Fertilisation was indicated by the presence of an elevated fertilisation membrane. For the chronic sea urchin bioassay, the test duration was extended to 72-hours (without sperm pre-exposure) followed by determination of any undeveloped or abnormal larvae indicated by incomplete pluteus development and asymmetrical development. Acute fertilisation and chronic development was reported as a percentage of the control.

Quality assurance

A spiked copper reference bioassay was performed concurrently to ensure *H. erythrogramma* bioassays were responding as expected to a known toxicant. The spiked copper concentration series consisted of a Control (0), 5, 10, 20, 40, 80 and 120 µg/L treatments. Test acceptability was achieved when (i) fertilisation / larval development in the control treatments is >80% and (ii) inhibition of fertilisation / larval development from the reference toxicant was within the expected response range.

Table 4: Summary of the test protocol for the acute and chronic *Heliocidaris erythrogramma* bioassays

Test Parameter	Acute Specification	Chronic Specification
Organism	*Heliocidaris erythrogramma*	*Heliocidaris erythrogramma*
Test Type	Static	Static
Test Duration	1-h	72-h
Temperature	21 ± 1°C	21 ± 1°C
Light Quality	Ambient illumination	Ambient illumination
Test Chamber Size	10 mL	10 mL
Test Solution Volume	5 mL	5 mL
Renewal of Test Solutions	None	None
Age of Test Organisms	< 1 hour	< 1 hour
No. of Replicates	3	3
Dilution Water	0.45 µm filtered seawater	0.45 µm filtered seawater
Concentration Series	Control (0), 1.6, 3.1, 6.3, 12.5, 25, 50 and 100%	Control (0), 1.6, 3.1, 6.3, 12.5, 25, 50 and 100%
Dilution Factor	1:2	1:2
Endpoint	Inhibition of fertilisation	Inhibition of larval development
Test Acceptability	>80% fertilisation in controls and Copper Reference EC50 within 37 – 61 µg/L	>80% development in controls and Copper Reference EC50 within 18 – 33 µg/L

2.5.5 Bivalve survival and larval development bioassays

The effect of the simulated brine on bivalve survival and larval development was assessed using the blue mussel *Mytilis edulis*. The acute bioassay assesses survival of juvenile mussels after a 96-hour static-renewal exposure. Similar to the sea urchin, the chronic bivalve endpoint assesses the development of fertilised larvae over 48-hours. The methods are based on ASTM E724-98 and both protocols are summarised in Table 5.

Mussel stock culture

Both juvenile (<10 mm in length) and adult (>50 mm in length) mussels were obtained from Cockburn Sound, WA. For the chronic bioassay, mature adult mussels were placed into temperature controlled trays at 15°C. The temperature was gradually increased to 21°C to induce spawning. Eggs and sperm were collected, mixed and inoculated within 1 hour of spawning.

Mussel bioassay

For the acute bioassay, juvenile mussels (<10 mm in length) were added into 1 L beakers containing 900 mL of test solution. Test solutions were aerated and completely renewed on a daily basis over the 96-hour exposure duration. All mortalities were removed from the test solution during daily renewals and recorded. After the 96-hour exposure duration, surviving mussels were counted (indicated by a closed shell and unassisted movement) and reported as a percentage of the control.

For the chronic bioassay, eggs and sperm were collected, mixed and inoculated within 1-hour of spawning. A 3 mL aliquot of each control, concentration and reference toxicant solution was dispensed into 5 mL acid washed borosilicate glass vials in triplicate. Each vial was inoculated with 0.2 mL of the fertilised egg suspension to a final concentration of 30 eggs/mL. Vials were then incubated at 21°C under ambient light conditions. After the 48-h exposure duration, the proportion of normally developed larvae (D-veliger) were counted and reported as a percentage of the control.

Quality assurance

A spiked copper reference bioassay was performed concurrently to ensure the juvenile mussels and mussel larvae were responding as expected to a known toxicant. The spiked copper concentration series consisted of a Control (0), 5, 10, 20, 40, 80 and 120 µg/L treatments. Test acceptability was achieved when (i) juvenile survival / normal development in the control treatments is >80% and (ii) juvenile survival / inhibition of larval development from the reference toxicant was within the expected response range.

Table 5: Summary of the test protocol for the acute and chronic *Mytilis edulis* bioassays

Test Parameter	Acute Specification	Chronic Specification
Organism	*Mytilis edulis*	*Mytilis edulis*
Test Type	Static - Renewal	Static
Test Duration	96-h	48-h
Temperature	21 ± 1°C	21 ± 1°C
Light Quality	Ambient illumination	Ambient illumination
Photoperiod	12 hour light : 12 hour dark	12 hour light : 12 hour dark
Test Chamber Size	1000 mL	5 mL
Test Solution Volume	900 mL	3 mL
Renewal of Test Solutions	Daily	None
Age of Test Organisms	Juvenile (< 10 mm)	< 1 hour
Density	10 per replicate	30 eggs/mL
No. of Replicates	3	3
Dilution Water	0.45 µm filtered seawater	0.45 µm filtered seawater
Concentration Series	Control (0), 1.6, 3.1, 6.3, 12.5, 25, 50 and 100%	Control (0), 1.6, 3.1, 6.3, 12.5, 25, 50 and 100%
Dilution Factor	1:2	1:2
Endpoint	Survival	Inhibition of larval development
Test Acceptability	>80% survival in controls and Copper Reference EC50 within 54 – 81 µg/L	>80% development in controls and Copper Reference EC50 within 33 – 49 µg/L

2.5.6 Copepod survival and reproduction bioassays

The effect of the simulated brine on copepod survival and reproduction was tested using the estuarine calanoid *Gladioferens imparipes*. The 48-hour acute survival and 21-day chronic reproduction endpoints are based on USEPA Method 1002.0 (USEPA, 2003). Both test protocols are summarised in Table 6.

Copepod stock culture

The copepod *G. imparipes* was cultured in-house under ambient light conditions at 21°C. Culturing water was renewed every second day with 0.45 µm filtered seawater followed by feeding with marine microalgae (*I. galbana*). Prior to testing (<24 hours), females with visible egg clutches were isolated and newly hatched nauplii were collected for test initiation.

Copepod bioassays

A 3 mL aliquot of each control, concentration and reference toxicant solution was dispensed into acid washed polycarbonate 24 well microplates. Four replicates per treatment were dispensed and a microalgae food source was added to final concentration of 2.5×10^4 cells/mL. To each well, 5 newly hatched nauplii were added and incubated at 21°C under ambient light conditions. Survival of the nauplii was determined after 48-h (acute endpoint). Waters were renewed daily until the nauplii reached maturation. At maturation, one paired adult male and female were isolated into each replicate. Every second day the nauplii produced were counted (chronic endpoint), removed and waters renewed for the remainder of the test duration. Copepod survival and reproduction effects were reported as a percentage of the control.

Quality assurance

A spiked copper reference bioassay was performed concurrently to ensure *G. imparipes* was responding as expected to a known toxicant. The spiked copper concentration series consisted of a Control (0), 5, 10, 20, 40, 80, and 120 µg/L treatments. Test acceptability was achieved when (i) copepod survival in the control treatments was >80% and (ii) copepod survival / reproduction effects from the reference toxicant were within the expected response range.

Table 6: Summary of the test protocol for the *Gladioferens imparipes* acute survival and chronic reproduction bioassays

Test Parameter	Acute Specification	Chronic Specification
Organism	*Gladioferens imparipes*	*Gladioferens imparipes*
Test Type	Static	Static Renewal
Test Duration	48-h	21-day
Temperature	21 ± 1°C	21 ± 1°C
Light Quality	Ambient illumination	Ambient illumination
Photoperiod	12 hour light : 12 hour dark	12 hour light : 12 hour dark
Test Chamber Size	3.2 mL	3.2 mL
Test Solution Volume	3 mL	3 mL
Renewal of Test Solutions	Daily	Daily
Age of Test Organisms	Nauplii < 24-h	Nauplii < 24-h
Organisms per Replicate	5	1 Female + 1 Male (adult)
No. of Replicates	4	4
Feeding	On renewal	On renewal
Dilution Water	0.45 µm filtered seawater	0.45 µm filtered seawater
Concentration Series	Control (0), 1.6, 3.1, 6.3, 12.5, 25, 50 and 100%	Control (0), 1.6, 3.1, 6.3, 12.5, 25, 50 and 100%
Dilution Factor	1:2	1:2
Endpoint	Survival	Reproduction
Test Acceptability	>80% survival in controls and Copper Reference EC50 within 52 – 68 µg/L	>80% survival in controls and Copper Reference EC50 within 21 – 36 µg/L

2.5.7 Fish larvae survival and growth bioassays

The effect of simulated brine on the survival and growth of fish larvae was tested using larvae and fertilised eggs of *Pagrus auratus*, respectively. The 96-hour acute survival and 7-day chronic growth endpoints were based on USEPA Method 1004.0 (USEPA, 2003). Both test protocols are summarised in Table 7.

Fish stock culture

The fertilised *P. auratus* eggs were obtained from naturally spawning broodstock maintained in a low density flow through system by the Aquaculture Development Unit of Challenger TAFE, Fremantle, WA. Broodstock were fed a varied diet and only high viability batches of fertilised eggs (>80%) and larvae were accepted for use in the bioassays.

Fish bioassays

A 400 mL aliquot of each control, concentration and reference toxicant solution was dispensed into acid washed 500 mL borosilicate glass beakers. To each of the four replicates per treatment, 20 larvae or fertilised eggs (<24-hours) were added. Hatched larvae were fed a concentrated rotifer stock (0.5 mL) daily after Day 2. The test was incubated at 21°C on a 12-h light:12-h dark photoperiod. Survival of the fish larvae was determined after 96-h (acute endpoint). On Day 7, the remaining fish larvae were removed and the length measured (mm) using a microscope mounted digital camera and Digimizer® imaging software package (chronic endpoint). Fish survival and growth effects were reported as a percentage of the control.

Quality assurance

A spiked copper reference bioassay was performed concurrently to ensure *P. auratus* was responding as expected to a known toxicant. The spiked copper concentration series consisted of a Control (0), 10, 25, 50, 75 and 100 µg/L treatments. Test acceptability was achieved when (i) survival in the control treatments was >80% and (ii) fish larvae survival / growth effects from the reference toxicant were within the expected response range.

Table 7: Summary of the test protocol for the *Pagrus auratus* acute survival and chronic growth bioassays

Test Parameter	Acute Specification	Chronic Specification
Organism	*Pagrus auratus*	*Pagrus auratus*
Test Type	Static	Static
Test Duration	96-h	7-day
Temperature	21 ± 1°C	21 ± 1°C
Light Quality	Ambient illumination	Ambient illumination
Photoperiod	12 hour light : 12 hour dark	12 hour light : 12 hour dark
Test Chamber Size	500 mL	500 mL
Test Solution Volume	400 mL	400 mL
Renewal of Test Solutions	None	None
Age of Test Organisms	2-day hatched larvae	< 24-h fertilised eggs
Organisms per Replicate	15	15
No. of Replicates	4	4
Feeding	Daily	Daily
Dilution Water	0.45 µm filtered seawater	0.45 µm filtered seawater
Concentration Series	Control (0), 1.6, 3.1, 6.3, 12.5, 25, 50 and 100%	Control (0), 1.6, 3.1, 6.3, 12.5, 25, 50 and 100%
Dilution Factor	1:2	1:2
Endpoint	Survival	Growth (length)
Test Acceptability	>80% survival in controls and Copper Reference EC50 within 82 – 114 µg/L	>80% survival in controls and Copper Reference EC50 within 43 – 69 µg/L

2.6 Statistical Analysis

The IC50/EC50 values (i.e. the inhibitory or effective concentration of simulated brine which gave a 50% reduction in cell division, reproduction, growth, development or survival compared to the controls) were calculated using ToxCalc Version 5.0.23 (Tidepool Software). Dunnett's Multiple Comparison Test was used to determine which concentrations were significantly different to the controls in order to estimate the Lowest Observable Effect Concentrations (LOEC) and No Observable Effect Concentrations (NOEC).

It should be noted that NOEC and LOEC values have been reported for historical comparisons only. The LOEC and NOEC values are not statistically robust and should not be used independently. For this reason, the reported IC10/EC10 values have been used in place of the LOEC.

For all bioassays, percentage data were Arcsin transformed and tested for normality and equality of variance. Probit-maximum Likelihood or Linear Interpolation analyses were used to determine the EC50 and EC10 concentrations.

2.7 Calculation of Species Protection Trigger Values

Following the protocol outlined in ANZECC/ARMCANZ (2000), species protection trigger values were calculated using the BurrliOZ statistics program (Campbell et al., 2000) to determine the diffuser configuration that will ensure the desalination plant effluent remains below the required dilution target in the mixing zone. Both acute and chronic species protection trigger values at 99, 95, 90 and 80% species protection levels were derived using the EC10 data from six bioassays (4 different trophic levels). For example, dilution factors calculated for the 99% species protection trigger value based on the EC10 biological effects data will theoretically result in 1% of the exposed species showing a 10% reduction in cell division, reproduction, growth, development or survival if the dilution target is exceeded.

Please note that while a range of trigger values and safe dilution factors are presented, the appropriate level of species protection must be implemented as stipulated by the governing regulatory authority.

3 RESULTS AND DISCUSSION

3.1 Chemical Characterisation

Quality assurance and quality control criteria were within acceptable limits for all chemical analyses. All physico-chemical parameters measured above detection limits in the site-specific seawater diluent were generally higher in the simulated brine effluent (Table 8).

Table 8: Chemical characterisation of seawater diluent and simulated brine effluent

Chemical Parameter	Practical Quantitation Limit (PQL)	Marine Trigger Value*	Measured Concentration	
			Seawater	Simulated Brine
pH (units)	0.1	8.00 – 8.40	8.11	7.70
Dissolved Oxygen (%)	1	90	94	83
Salinity (‰)	0.1	36.1 [a]	35.3	67.8
Conductivity (mS at 25°C)	0.1	N/A	53.2	91.4
Turbidity (NTU)	0.1	1	0.2	9.7
Total Suspended Solids (mg/L)	5	N/A	-	1
Surfactants - MBAS (µg/L)	50	N/A	-	-
Nutrients (µg/L)				
Total Phosphorous	5	20	12	50
Orthophosphate	2	5	2	2
Total Nitrogen	50	230	110	250
Total Oxidised Nitrogen	2	5	-	43
Ammonia	1	5	9	39
Soluble Metals (mg/L)				
Aluminium (Al)	0.001	0.055 [b]	-	-
Arsenic (As)	0.001	0.013 [b]	-	-
Beryllium (Be)	0.0001	N/A	-	-
Boron (B)	0.006	N/A	4.8	9.2
Cadmium (Cd)	0.0006	0.006	-	-
Calcium (Ca)	0.005	N/A	430	780
Chromium (Cr)	0.001	0.004	-	-
Cobalt (Co)	0.001	0.001	-	-
Copper (Cu)	0.001	0.001	-	-
Iron (Fe)	0.002	N/A	-	-
Lead (Pb)	0.001	0.004	-	-
Magnesium (Mg)	0.005	N/A	1300	2900
Manganese (Mn)	0.001	1.900 [b]	-	0.002
Mercury (Hg)	0.0001	0.0004	-	-
Molybdenum (Mo)	0.001	N/A	0.011	0.023
Nickel (Ni)	0.001	0.070	-	-
Potassium (K)	0.05	N/A	440	950
Selenium (Se)	0.001	0.011 [b]	-	-
Silver (Ag)	0.001	0.001	-	-
Sodium (Na)	0.05	N/A	11000	18000
Tin (Sn)	0.001	N/A	-	-
Vanadium (V)	0.002	0.100	-	-
Zinc (Zn)	0.005	0.015	-	-

* Default ANZECC/ARMCANZ (2000) trigger value for protection of moderately disturbed marine ecosystems in south Western Australia

\- Denotes the concentration as being below the Practical Quantitation Limit (PQL)

a Limited to 0.8‰ above background level of salinity (ANZECC/ARMCANZ, 2000)

b Trigger value for freshwaters (where marine trigger value not available)

N/A Marine water quality guideline not available for reference

Measurements of pH, dissolved oxygen, salinity, turbidity, nutrients (including total phosphorous, total nitrogen, nitrogen oxides and ammonia) exceeded the marine water quality guideline trigger values for slightly to moderately disturbed ecosystems in south Western Australia (ANZECC/ARMCANZ, 2000). Although marine water quality guideline trigger values were not available for conductivity, total suspended solids and certain metals (including boron, calcium, magnesium, manganese, molybdenum, potassium and sodium), elevated concentrations above background levels in the Cape Riche seawater diluent were noted.

3.2 Ecotoxicity Testing

3.2.1 Concentration series

The concentration series for the simulated brine effluent were prepared using a 1:2 dilution series (*v/v*) with the filtered Cape Riche seawater diluent. The pH was approximately 0.4 units lower than the site collected seawater and decreased as the salinity increased. The physico-chemistry of the simulated brine effluent was not adjusted prior to testing to ensure the toxicity would be representative of *in situ* release and exposure.

Table 9: Physico-chemical parameters measured for the simulated brine concentration series

Simulated Brine (%)	Physico-chemical Parameters		
	pH	D.O. (%)	Salinity (ppt)
Control	8.11	94	35
1.6	8.11	92	35
3.1	8.09	91	36
6.3	8.08	92	37
12.5	8.06	91	40
25.0	7.95	86	43
50.0	7.82	86	52
100.0	7.70	83	68

3.2.2 Microalgal growth inhibition bioassay

All raw data and statistical calculations for the *I. galbana* growth inhibition bioassay are attached in Appendix A.

Quality assurance

Control growth rates were 1.17 ± 0.11 replications per day and were within the normal range of 1.4 ± 0.4 replications per day. The reference toxicant copper gave a 72-h IC50 of 9 µg/L and was within the acceptable limits of 8 – 17 µg/L. With the exception of pH and salinity, all physico-chemical parameters were within the optimal range for microalgal growth and are shown in Table 10.

Toxicity test

The simulated brine did not appear to inhibit microalgal growth, with a 72-h IC50 value >100%. However, significantly enhanced microalgal growth was detected at simulated brine concentrations of 12.5% and higher (Appendix A). It was likely that elevated concentrations of nutrients in the simulated brine were enhancing microalgal growth.

Table 10: Physico-chemical parameters measured at the end of the microalgal cell division bioassay

Simulated Brine (%)	72-hour Cell Division		
	pH	D.O. (%)	Salinity (ppt)
Control	8.34	91	35
1.6	8.36	93	35
3.1	8.35	89	36
6.3	8.32	94	37
12.5	8.29	94	40
25.0	8.26	89	43
50.0	8.14	90	52
100.0	7.99	92	68

3.2.3 Macroalgal germination and tube growth bioassays

All raw data and statistical calculations for the *E. radiata* acute germination and chronic development bioassays are attached in Appendix B.

Quality assurance

The percentage germination of zygotes in the control treatments for both the acute and chronic tests was 100±0% and 100±8% (respectively) which was above the minimum acceptable limit of >80%. The reference toxicant copper gave an acute 48-h EC50 of 74 µg/L and a chronic 72-hour EC50 of 22 µg/L. The acute and chronic copper reference test results were within the acceptable limits of 63 – 87 µg/L and 16 – 37 µg/L, respectively. With the exception of pH and salinity, all physico-chemical parameters were within the optimal range for macroalgal germination and tube development (Table 11).

Toxicity test

For the acute test, the simulated brine was toxic to germination of zygotes with a 48-h EC50 value of 56.6% (95% Confidence Intervals of 53.4 – 60.1%). Statistically significant effects on germination were detected at a 48-h EC10 value of 25.6%.

For the chronic test, the simulated brine inhibited tube growth with a 72-h EC50 value of 19.6% (95% Confidence Intervals of 14.7 – 25.5%). Statistically significant effects on tube growth were detected at a 72-h EC10 value of 4.4%.

Table 11: Physico-chemical parameters measured at the end of the macroalgal acute germination and chronic tube growth bioassay

Simulated Brine (%)	48-hour Germination			72-hour Tube Growth		
	pH	D.O. (%)	Salinity (ppt)	pH	D.O. (%)	Salinity (ppt)
Control	8.21	92	35	8.23	91	35
1.6	8.18	89	35	8.20	94	35
3.1	8.18	89	36	8.19	93	36
6.3	8.17	91	37	8.18	96	37
12.5	8.03	91	40	8.12	92	40
25.0	7.98	92	43	8.02	91	43
50.0	7.87	89	52	7.88	95	52
100.0	7.79	88	68	7.82	90	68

3.2.4 Sea urchin fertilisation and larval development bioassays

All raw data and statistical calculations for the *H. erythrogramma* acute fertilisation and chronic larval development bioassays are attached in Appendix C.

Quality assurance

The percentage fertilisation and larvae development in the control treatments for both the acute and chronic tests was 100±0% and 101±9% (respectively) which was above the minimum acceptable limit of >80%. The reference toxicant copper gave an acute 1-h EC50 of 41 µg/L and a chronic 72-hour EC50 of 22 µg/L. The acute and chronic copper reference test results were within the acceptable limits of 37 – 61 µg/L and 18 – 33 µg/L, respectively. With the exception of pH and salinity, all physico-chemical parameters were within the optimal range for sea urchin fertilisation and larval development (Table 12).

Toxicity test

For the acute test, the simulated brine inhibited fertilisation with a 1-h EC50 value of 51.2% (95% Confidence Intervals of 45.7 – 51.0%). Statistically significant effects on fertilisation were detected at a 1-h EC10 value of 19.6%.

For the chronic test, the simulated brine inhibited larval development with a 72-h EC50 value of 12.9% (95% Confidence Intervals of 11.7 – 14.1%). Statistically significant effects on larval development were detected at a 72-h EC10 value of 5.9%.

Table 12: Physico-chemical parameters measured at the end of the sea urchin acute fertilisation and chronic larval development bioassay

Simulated Brine (%)	1-hour Fertilisation			72-hour Larval Development		
	pH	D.O. (%)	Salinity (ppt)	pH	D.O. (%)	Salinity (ppt)
Control	8.14	93	35	8.19	89	35
1.6	8.14	91	35	8.21	88	35
3.1	8.13	92	36	8.20	91	36
6.3	8.11	94	37	8.13	90	37
12.5	8.06	93	40	8.09	96	40
25.0	7.92	95	43	7.99	92	43
50.0	7.80	95	52	7.86	87	52
100.0	7.73	91	68	7.79	89	68

3.2.5 Bivalve survival and larval development bioassays

All raw data and statistical calculations for the *M. edulis* acute survival and chronic larval development bioassays are attached in Appendix D.

Quality assurance

The percentage survival and larvae development in the control treatments for both the acute and chronic tests was 100±0% and 100±0% (respectively) which was above the minimum acceptable limit of >80%. The reference toxicant copper gave an acute 96-h EC50 of 72 µg/L and a chronic 72-hour EC50 of 48 µg/L. The acute and chronic copper reference test results were within the acceptable limits of 54 – 81 µg/L and 33 – 49 µg/L, respectively. With the exception of pH and salinity, all physico-chemical parameters were within the optimal range for mussel survival and larval development (Table 13).

Toxicity test

For the acute test, the simulated brine inhibited survival with a 96-h EC50 value of 43.2% (95% Confidence Intervals of 40.6 – 46.2%). Statistically significant effects on survival were detected at a 96-h EC10 value of 16.3%.

For the chronic test, the simulated brine inhibited larval development with a 72-h EC50 value of 13.9% (95% Confidence Intervals of 9.3 – 20.9%). Statistically significant effects on larval development were detected at a 72-h EC10 value of 3.5%.

Table 13: Physico-chemical parameters measured at the end of the bivalve acute survival and chronic larval development bioassay

Simulated Brine (%)	96-hour Survival			72-hour Larval Development		
	pH	D.O. (%)	Salinity (ppt)	pH	D.O. (%)	Salinity (ppt)
Control	8.17	95	35	8.12	88	35
1.6	8.18	92	35	8.09	86	35
3.1	8.16	94	36	8.02	86	36
6.3	8.15	89	37	7.99	90	37
12.5	8.03	91	40	7.95	87	40
25.0	7.96	94	43	7.91	86	43
50.0	7.82	88	52	7.87	83	52
100.0	7.71	88	68	7.82	83	68

3.2.6 Copepod survival and reproduction bioassays

All raw data and statistical calculations for the *G. imparipes* acute survival and chronic reproduction bioassays are attached in Appendix E.

Quality assurance

The percentage survival of copepods in the control treatments for both the acute and chronic tests was 94±7% and 96±9% (respectively) which was above the minimum acceptable limit of 80%. The reference toxicant copper gave an acute 48-h EC50 of 58 µg/L and a chronic 21-day EC50 of 25 µg/L. The acute and chronic reference test results were within the acceptable limits of 52 – 68 µg/L and 21 – 36 µg/L, respectively.

All physico-chemical parameters for copepod survival and reproduction bioassays are shown in Table 14. Given the water was renewed every second day in reproduction bioassay, the mean physico-chemistry (±1 Standard Deviation) is shown. With the exception of pH and salinity, all physico-chemical parameters were within the optimal range for copepod survival and reproduction.

Toxicity test

For the acute test, the simulated brine inhibited survival with a 48-h EC50 value of 30.5% (95% Confidence Intervals of 25.8 – 35.3%). Statistically significant effects on survival were detected at a 48-h EC10 value of 12.7%.

For the chronic test, the simulated brine inhibited reproduction with a 21-day EC50 value of 14.4% (95% Confidence Intervals of 10.9 – 18.3%). Statistically significant effects on survival were detected at a 21-day EC10 value of 3.4%.

Table 14: Physico-chemical parameters measured at the end of the copepod acute survival and chronic reproduction bioassay

Simulated Brine (%)	48-hour Survival			21-day Reproduction		
	pH	D.O. (%)	Salinity (ppt)	pH	D.O. (%)	Salinity (ppt)
Control	8.16	91	35	8.14 ± 0.05	91 ± 5	35 ± 0
1.6	8.13	90	35	8.15 ± 0.03	89 ± 3	35 ± 1
3.1	8.11	89	36	8.11 ± 0.07	92 ± 5	36 ± 1
6.3	8.04	88	37	8.08 ± 0.06	96 ± 6	37 ± 1
12.5	8.01	85	40	8.02 ± 0.04	93 ± 4	40 ± 0
25.0	7.90	87	43	7.96 ± 0.02	89 ± 5	43 ± 0
50.0	7.81	88	52	7.89 ± 0.03	95 ± 2	52 ± 1
100.0	7.73	89	68	7.81 ± 0.07	92 ± 7	68 ± 1

3.2.7 Fish larvae survival and growth bioassays

All raw data and statistical calculations for the *P. auratus* acute survival and chronic growth bioassays are attached in Appendix F.

Quality assurance

The percentage survival of fish larvae in the control treatments for both the acute and chronic tests was 93±6% and 100±11% (respectively) which was above the minimum acceptable limit of >80%. The reference toxicant copper gave an acute 96-h EC50 of 97 µg/L and a chronic 7-day EC50 of 48 µg/L. The acute and chronic copper reference test results were within the acceptable limits of 82 – 114 µg/L and 43 – 69 µg/L, respectively. With the exception of pH and salinity, all physico-chemical parameters were within the optimal range for fish larvae survival and growth (Table 15).

Toxicity test

For the acute test, the simulated brine was inhibited survival of fish larvae with a 96-h EC50 value of 39.8% (95% Confidence Intervals of 29.2 – 51.5%). Statistically significant effects on survival were detected at a 96-h EC10 value of 16.3%.

For the chronic test, the simulated brine inhibited larval growth with a 7-day EC50 value of 19.4% (95% Confidence Intervals of 13.5 – 26.6%). Statistically significant effects on growth were detected at a 7-day EC10 value of 5.8%.

Table 15: Physico-chemical parameters measured at the end of the fish acute survival and chronic growth bioassay

Simulated Brine (%)	96-hour Survival			7-day Growth		
	pH	D.O. (%)	Salinity (ppt)	pH	D.O. (%)	Salinity (ppt)
Control	8.15	89	35	8.08	83	35
1.6	8.12	85	35	8.04	85	35
3.1	8.09	84	36	8.02	87	36
6.3	8.11	91	37	7.97	86	37
12.5	8.03	87	40	8.00	82	40
25.0	7.98	86	43	7.96	84	43
50.0	7.84	82	52	7.88	85	52
100.0	7.73	83	68	7.79	81	68

3.2.8 Species protection trigger values

Species protection trigger values were calculated to determine the diffuser configuration that will ensure the desalination brine effluent remains below the required dilution target in the mixing zone. For example, dilution factors calculated for the 99% species protection trigger value based on the EC10 biological effects data will theoretically result in 1% of the exposed species showing a 10% reduction in acute or chronic effects if the dilution target is exceeded.

Based on the EC10 effects data from the six ‘acute’ marine bioassays (Table 16), the safe dilution factors required to achieve 99, 95, 90 or 80% species protection in seawater were determined to be 10, 9, 8 and 7, respectively (Figure 1, Table 17). Based on the EC10 data from the six ‘chronic’ marine bioassays (Table 16), the safe dilution factors required to achieve 99, 95, 90 or 80% species protection in seawater were determined to be 53, 40, 35 and 28, respectively (Figure 2, Table 17). Therefore, a minimum safe dilution factor of 10 or 53 in the mixing zone will be sufficient to achieve a 99% level of species protection based on either acute or chronic effects, respectively.

Table 16: Summary of acute and chronic statistical effects data

Effect Concentration	Acute Effects (% Brine)				Chronic Effects (% Brine)			
	EC50	EC10	LOEC	NOEC	EC50	EC10	LOEC	NOEC
Microalgae	>100	>100	>100	100	>100	>100	>100	100
Macroalgae	56.6	25.6	25.0	12.5	19.6	4.4	6.3	3.1
Echinoderm	51.2	19.6	25.0	12.5	12.9	5.9	6.3	3.1
Mollusc	43.2	16.3	25.0	12.5	13.9	3.5	6.3	3.1
Crustacean	30.5	12.7	25.0	12.5	14.4	3.4	6.3	3.1
Fish	39.8	16.3	25.0	12.5	19.4	5.8	6.3	3.1

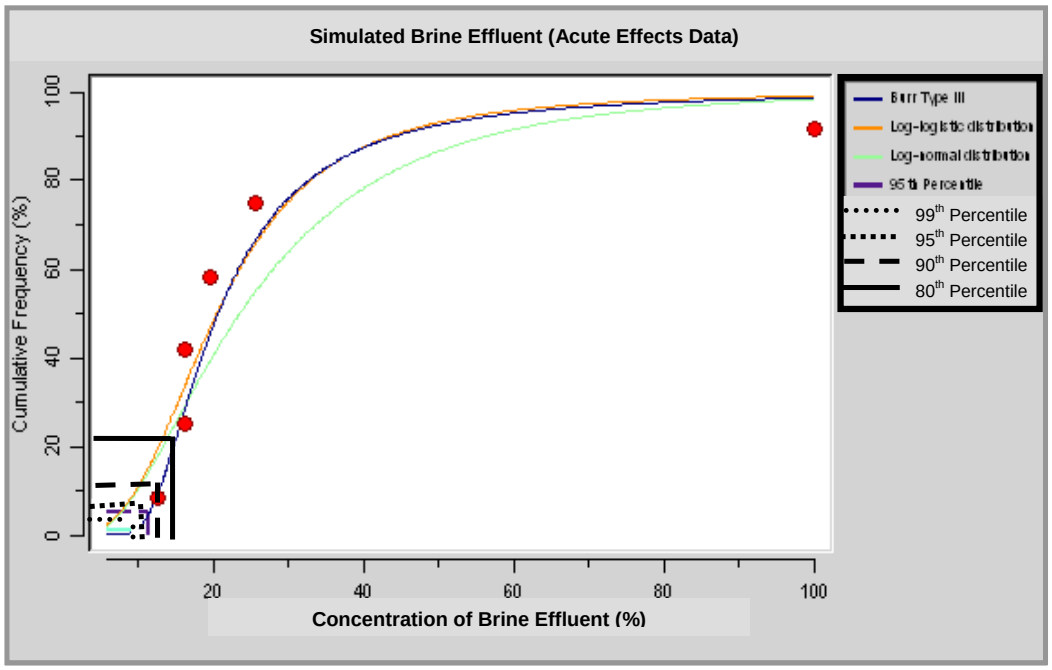


Figure 1: Species sensitivity distribution based on acute EC10 effects data

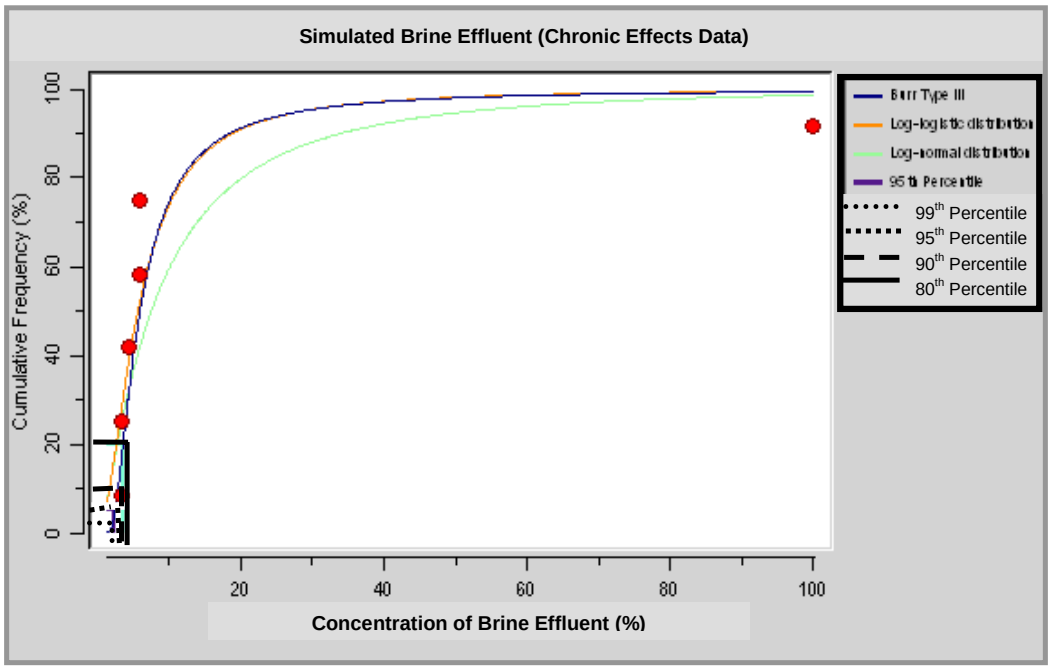


Figure 2: Species sensitivity distribution based on chronic EC10 effects data

Table 17: Calculated safe dilution factors required to achieve 99, 95, 90 and 80% levels of acute or chronic species protection

Level of Species Protection	Brine Effluent (%)		Dilution Factor Required	
	Acute	Chronic	Acute	Chronic
99%	9.6	1.9	10	53
95%	11.4	2.5	9	40
90%	12.7	2.9	8	35
80%	14.7	3.6	7	28

3.3 Summary of Chemical Characterisation and Ecotoxicity Testing

Based on chemical characterisation of the simulated brine, a dilution factor of >40 would be required to lower all contaminants of potential concern (including salinity) to levels below the recommended marine water quality guideline trigger values (ANZECC/ARMCANZ, 2000). The 'chronic' dilution factors calculated for the 99 and 95% species protection trigger values (i.e. dilution factors of 53 and 40, respectively) would be suitable to achieve this level of dilution, as well as a high to moderate to level of species protection. Good agreement between the chemical characterisation and ecotoxicity testing suggested that a minimum safe dilution factor of 40 should be applied when discharging a reverse osmosis reject water with similar chemical characteristics to the simulated brine assessed in this study.

4 CONCLUSIONS

The simulated brine effluent was considered to be representative of the reject water expected to be discharged into the marine environment from the Cape Riche desalination plant. The simulated brine effluent caused both acute and chronic toxicity to all species tested, though acute effects for the same species were observed to occur at higher concentrations than the chronic effects. For example, the safe dilution factor required to achieve a 99% level of species protection was approximately 5 times lower for the acute exposures (dilution factor of 10) compared to the chronic exposures (dilution factor of 53).

The acute safe dilution factors are more useful for predicting toxicity from a short-term exposure to the brine effluent in the mixing zone, (e.g. adult copepod temporarily passing through the plume), compared to the chronic safe dilution factors which are more useful for predicting toxicity from a continuous exposure (e.g. a sea urchin larvae settling in the mixing zone for further development). The acute or chronic safe dilution factors may be selected where detailed information for the Cape Riche marine ecology is available. The chronic safe dilution factor is preferred over the acute safe dilution factors considering the discharge will be continuous and a more conservative level of environmental protection would be achieved (ANZECC/ARMCANZ, 2000).

It was not possible to identify the cause(s) of toxicity in this testing program. Individual or additive combinations of the physico-chemistry, simulated brine effluent or antiscalant may have contributed to the observed toxicity. However, based on chemical characterisation of the simulated brine, a dilution factor of >40 would be required to lower all contaminants of potential concern (including salinity) to levels below the recommended marine water quality guideline trigger values. Only the 'chronic' dilution factors calculated for the 99 and 95% species protection trigger values (i.e. dilution factors of 53 and 40, respectively) would be suitable to achieve this level of dilution. This result was comparable to the Perth Seawater Desalination Plant (WA) which currently implements a safe dilution factor of 45 to achieve a moderate level of species protection in the mixing zone.

While varying degrees of toxicity were observed for all species and test endpoints, enhanced growth was observed for the microalga *I. galbana*, even at the highest concentrations of the simulated brine tested. Elevated nutrient loadings were likely responsible for the enhanced microalgal growth with total phosphorous, total nitrogen, nitrogen oxides and ammonia present at concentrations exceeding the ANZECC/ARMCANZ (2000) marine water quality guideline trigger values. Nutrient classed contaminants are known to influence aquatic photosynthetic primary producers such as microalgae. While the increased nutrient loading may not be directly toxic, the potential for enhanced microalgal growth and indirect biological effects such as eutrophication is acknowledged. It remains unclear as to whether the nutrient loadings masked toxic effects from other contaminants of potential concern in the simulated brine to *I. galbana*, however the nutrient loadings would be

diluted below trigger levels specific to south Western Australia where the minimum safe dilution factor of >40 is applied.

It is also acknowledged that results from this toxicity assessment are specific to the physico-chemistry and of the simulated brine. For example, the lowest pH in the simulated brine dilution series was pH 7.70. Indirect physico-chemical effects on the bioavailability of certain contaminants (such as pH controlled speciation of metals or ammonia) should not be discounted when assessing the cause of toxicity. The influence of physico-chemical changes on the speciation and bioavailability of brine associated contaminants may be of greater importance once the many processing phases of a large scale reverse osmosis desalination plant are considered (e.g. chlorination, pH adjustment, coagulation, flocculation, dechlorination, antiscaling and membrane cleaning).

Furthermore, the concentration and combination of chemical additives tested in this simulated brine assessment should be regarded as the upper limit for discharge, unless further testing suggests otherwise. For example, the derived safe dilution factors will be less representative where water quality guideline exceedances are greater and/or other contaminants not assessed in this study are identified in the Cape Riche desalination plant reject water (e.g. where an alternative antiscalant to Nalco Permatreat PC-1020 is used in the commissioned desalination plant). For this reason, it is recommended that the toxicity of the brine effluent be reassessed upon plant commissioning to confirm an appropriate level of species protection is being achieved.

5 REFERENCES

ANZECC/ARMCANZ (2000). Australian 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.

ASTM E724-98 Standard (2004). 'Standard Guide for Conducting Static Acute Toxicity Tests Starting with Embryos of Four Species of Saltwater Bivalve Molluscs'. ASTM International, West Conshohocken, PA, 2004, DOI: 10.1520/E0724-98R04.

Burridge, T.R., M. Karistianos and J.R. Bidwell (1999). The use of aquatic macrophyte ecotoxicological assays in monitoring coastal effluent discharges in Southern Australia. Marine Pollution Bulletin, 39:89-96.

Campbell, E., Palmer, M.J., Shao, Q., Warne, M. StJ. and Wilson, D. (2000). BurrliOZ: A computer program for calculating toxicant trigger values for the ANZECC and ARMCANZ water quality guidelines. In: ANZECC and ARMCANZ (2000) Australian and New Zealand Guidelines for Fresh and Marine Water Quality, Australian and New Zealand Environment and Conservation Council and Agriculture and Resource Management Council of Australia and New Zealand, Canberra.

Geotechnical Services Report ECX05-214 (2005). The provision of water quality monitoring services for Cockburn Sound (simulated brine toxicity assessment). Prepared for the Water Corporation of Western Australia.

Guillard, R.R.L. and Ryther, J.H. (1962). Studies of marine planktonic diatoms. *I. Cyclotella nana* and *Detonula confervaceae* Hustedt (Cleve) Gran. Canadian Journal of Microbiology, 8, 229-339.

OECD (1984). Guidelines for testing of chemicals. Algal growth inhibition test. Test Guideline No. 201. Organisation for Economic Cooperation and Development.

Simon, J. and Laginestra, E. (1997) Bioassay for testing sublethal toxicity in effluents using gametes of the sea urchin *Heliocidaris tuberculata*. National Pulp Mills Research Program Technical Report Series No. 20, Canberra: CSIRO, pp. 13-18.

Stauber, J.L., Tsai, J., Vaughan, G., Peterson, S.M. and Brockbank, C.I. (1994). Algae as indicators of toxicity of BEKM effluents. National Pulp Mills Research Program Technical Report Series No. 3, Canberra: CSIRO, pp. 1-82.

USEPA (2003). Short-term methods for assessing the chronic toxicity of effluents and receiving water to marine and estuarine organisms. US Environmental Protection Authority, Washington DC, USA.

APPENDICES

- APPENDIX A -

Isochrysis galbana Cell Division Bioassay

Raw Data and Statistical Analyses

Intertek GEOTECH GEOTECHNICAL SERVICES PTY LTD
41-45 Furnace Road, Welshpool, Western Australia, 6106
Locked Bag 27, Welshpool DC, Western Australia, 6986
Email: geotech@geotechnical-services.com.au
(24 hours) Telephone: (08) 9458 8877
Facsimile: (08) 9458 8857
ABN 58 050 543 194
ACN 050 543 194

Ecotoxicology Laboratory Test Report

Report Date: 3rd June 2011

Sample Details

Client	GHD Services Pty Ltd	**Lab ID No**	ECX11-0704
Attention	Oliver Glade-Wright GHD House 239 Adelaide Terrace Perth WA 6004	**Sampled By**	GHD Services Pty Ltd
		Date Sampled	06/04/11
		Date Received	07/04/11
Phone No	(08) 6222 8649	**Project Start**	07/04/11
Order No	51629	**Project Finish**	01/06/11

Bioassay Details

Test Performed	72-h Microalgal Cell Division
Test Protocol	WIENV-45
Reference	OECD 201 / Stauber et al., 1994
Test Species	*Isochrysis galbana*
Test Temperature	21°C

Concentration-Response Data

Brine Simulate (%)	Microalgal Cell Division (% Control)
Control	101 ± 5
1.6	100 ± 2
3.1	95 ± 5
6.3	107 ± 2
12.5	117 ± 3
25.0	142 ± 14
50.0	182 ± 12
100.0	115 ± 18

Statistical Effects Data

Effect Concentration	Microalgal Cell Division Inhibition (% Brine)
EC50	>100
EC10	>100
LOEC	>100
NOEC	100

Quality Assurance Limits

Quality Assurance Criteria	Microalgal Cell Division Inhibition
Reference Toxicant	Copper
Cusum Chart Limits	8 – 17 µg/L
Observed EC50	9 µg/L
Coefficient of Variance	16.5%
Control Cell Division	1.17 ± 0.11 replications per day
Test Acceptability	YES

Report Approval

David Strom
Principal Ecotoxicologist & Divisional Manager

NATA ACCREDITED FOR TECHNICAL COMPETENCE

Microalgae - Copper Reference

Start Date:	7/04/2011	Test ID:	ECX11-0704	Sample ID:	GHD Simulated Brine
End Date:	1/06/2011	Lab ID:	FREO-Geotech Fremantle La	Sample Type:	Copper
Sample Date:	7/04/2011	Protocol:	WIENV-45	Test Species:	I. galbana
Comments:					

Conc-ug/L	1	2	3
0	0.9524	1.0000	1.0000
5	0.7143	0.6429	0.6905
10	0.3810	0.4762	0.4286
20	0.3095	0.2857	0.3095
40	0.0476	0.1429	0.0952

			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
0	0.9841	1.0000	1.4641	1.3508	1.5208	6.702	3				5	300
*5	0.6825	0.6935	0.9726	0.9303	1.0069	4.003	3	9.344	2.470	0.1299	96	300
*10	0.4286	0.4355	0.7135	0.6652	0.7616	6.754	3	14.270	2.470	0.1299	171	300
*20	0.3016	0.3065	0.5813	0.5639	0.5900	2.587	3	16.784	2.470	0.1299	209	300
*40	0.0952	0.0968	0.3071	0.2200	0.3876	27.352	3	21.997	2.470	0.1299	271	300

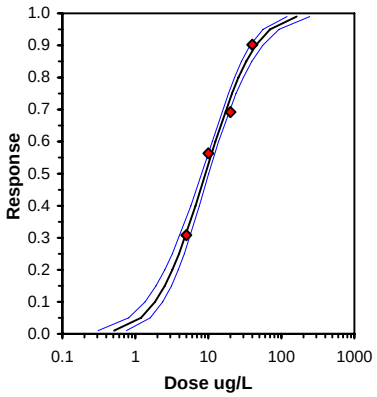
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.94776	0.835	-0.6493	-0.011
Bartlett's Test indicates equal variances (p = 0.27)	5.20836	13.2767		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	<5	5			0.0436	0.0441	0.57661	0.00415	1.0E-08	4, 10

Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	1.85929	0.1268	1.61077	2.10781	0.01667	5.36741	5.99146	0.07	0.95957	0.53784	3
Intercept	3.21588	0.14584	2.93004	3.50172							
TSCR	0.0167	0.0074	0.00221	0.0312							

Point	Probits	ug/L	95% Fiducial Limits	
EC01	2.674	0.51094	0.30704	0.75605
EC05	3.355	1.18823	0.81022	1.5979
EC10	3.718	1.86337	1.35737	2.38427
EC15	3.964	2.52426	1.92086	3.12625
EC20	4.158	3.213	2.52917	3.88069
EC25	4.326	3.95186	3.19963	4.67559
EC40	4.747	6.65745	5.74817	7.52769
EC50	5.000	9.11105	8.10196	10.1176
EC60	5.253	12.4689	11.2677	13.7819
EC75	5.674	21.0056	18.7846	23.9111
EC80	5.842	25.836	22.7878	30.0434
EC85	6.036	32.8854	28.4342	39.353
EC90	6.282	44.5489	37.4334	55.4658
EC95	6.645	69.861	56.0418	92.6141
EC99	7.326	162.469	118.774	243.709



Microalgae - Brine

Start Date:	7/04/2011	Test ID:	ECX11-0704	Sample ID:	GHD Simulated Brine
End Date:	1/06/2011	Lab ID:	FREO-Geotech Fremantle La	Sample Type:	Brine
Sample Date:	7/04/2011	Protocol:	WIENV-45	Test Species:	I. galbana
Comments:					

Conc-%	1	2	3
0	0.9524	1.0000	1.0000
1.6	1.0000	0.9762	1.0000
3.1	0.9524	1.0000	0.9048
6.3	1.0000	1.0000	1.0000
12.5	1.0000	1.0000	1.0000
25	1.0000	1.0000	1.0000
50	1.0000	1.0000	1.0000
100	1.0000	1.0000	0.9524

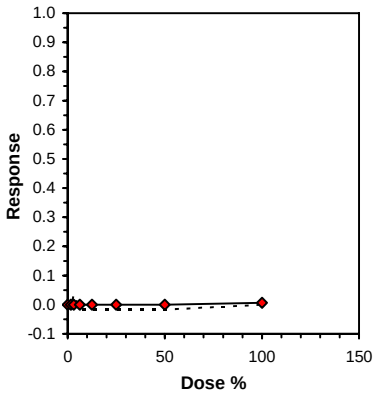
			Transform: Arcsin Square Root					2-Tailed			Isotonic	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
0	0.9841	1.0000	1.4641	1.3508	1.5208	6.702	3				0.9895	1.0000
1.6	0.9921	1.0081	1.4858	1.4159	1.5208	4.076	3	0.372	3.040	0.1773	0.9895	1.0000
3.1	0.9524	0.9677	1.3762	1.2571	1.5208	9.713	3	1.508	3.040	0.1773	0.9895	1.0000
6.3	1.0000	1.0161	1.5208	1.5208	1.5208	0.000	3	0.972	3.040	0.1773	0.9895	1.0000
12.5	1.0000	1.0161	1.5208	1.5208	1.5208	0.000	3	0.972	3.040	0.1773	0.9895	1.0000
25	1.0000	1.0161	1.5208	1.5208	1.5208	0.000	3	0.972	3.040	0.1773	0.9895	1.0000
50	1.0000	1.0161	1.5208	1.5208	1.5208	0.000	3	0.972	3.040	0.1773	0.9895	1.0000
100	0.9841	1.0000	1.4641	1.3508	1.5208	6.702	3	0.000	3.040	0.1773	0.9833	0.9937

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.8683	0.884	-0.2527	1.21522
Equality of variance cannot be confirmed				

Hypothesis Test (2-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	100	>100		1	0.06714	0.06791	0.00764	0.0051	0.23734	7, 16

Linear Interpolation (200 Resamples)

Point	%	SD	95% CL(Exp)	Skew
IC05	>100			
IC10	>100			
IC15	>100			
IC20	>100			
IC25	>100			
IC40	>100			
IC50	>100			



- APPENDIX B -

Ecklonia radiata Germination and Tube Growth Bioassay

Raw Data and Statistical Analyses

Intertek GEOTECH GEOTECHNICAL SERVICES PTY LTD
41-45 Furnace Road, Welshpool, Western Australia, 6106
Locked Bag 27, Welshpool DC, Western Australia, 6986
Email: geotech@geotechnical-services.com.au
(24 hours) Telephone: (08) 9458 8877
Facsimile: (08) 9458 8857
ABN 58 050 543 194
ACN 050 543 194

Ecotoxicology Laboratory Test Report

Report Date: 3rd June 2011

Sample Details

Client	GHD Services Pty Ltd	**Lab ID No**	ECX11-0704
Attention	Oliver Glade-Wright GHD House 239 Adelaide Terrace Perth WA 6004	**Sampled By**	GHD Services Pty Ltd
		Date Sampled	06/04/11
		Date Received	07/04/11
Phone No	(08) 6222 8649	**Project Start**	07/04/11
Order No	51629	**Project Finish**	01/06/11

Bioassay Details

Test Performed	48-h Macroalgal Germination	72-h Macroalgal Tube Growth
Test Protocol	WIENV-67	WIENV-67
Reference	Burridge et al., 1999	Burridge et al., 1999
Test Species	*Ecklonia radiata*	*Ecklonia radiata*
Test Temperature	21°C	21°C

Concentration-Response Data

Brine Simulate (%)	Macroalgal Germination (% Control)	Macroalgal Tube Growth (% Control)
Control	100 ± 0	100 ± 8
1.6	100 ± 0	102 ± 5
3.1	100 ± 0	93 ± 5
6.3	100 ± 0	77 ± 6
12.5	100 ± 0	60 ± 5
25.0	91 ± 5	47 ± 5
50.0	52 ± 7	25 ± 7
100.0	21 ± 8	4 ± 2

Statistical Effects Data

Effect Concentration	Macroalgal Germination Inhibition (% Brine)	Macroalgal Tube Growth Inhibition (% Brine)
EC50	56.6	19.6
EC10	25.6	4.4
LOEC	25.0	6.3
NOEC	12.5	3.1

Quality Assurance Limits

Quality Assurance Criteria	Macroalgal Germination Inhibition	Macroalgal Tube Growth Inhibition
Reference Toxicant	Copper	Copper
Cusum Chart Limits	63 – 87 µg/L	16 – 37 µg/L
Observed EC50	74 µg/L	22 µg/L
Coefficient of Variance	18.9%	29.4%
Control Germination	>80% Germinated	>80% Germinated
Test Acceptability	YES	YES

Report Approval

David Strom
Principal Ecotoxicologist & Divisional Manager

NATA ACCREDITED FOR TECHNICAL COMPETENCE

Macroalgae - Copper Reference (acute)

Start Date:	7/04/2011	Test ID:	ECX11-0704	Sample ID:	GHD Simulated Brine
End Date:	1/06/2011	Lab ID:	FREO-Geotech Fremantle La	Sample Type:	Copper
Sample Date:	7/04/2011	Protocol:	WIENV-67	Test Species:	E. radiata
Comments:					

Conc-ug/L	1	2	3
0	1.0000	1.0000	1.0000
10	1.0000	1.0000	1.0000
25	0.8333	0.9000	0.9333
50	0.6667	0.5667	0.7333
100	0.4000	0.3333	0.4333

			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
0	1.0000	1.0000	1.5208	1.5208	1.5208	0.000	3				0	300
10	1.0000	1.0000	1.5208	1.5208	1.5208	0.000	3	0.000	2.470	0.1177	0	300
*25	0.8889	0.8889	1.2363	1.1503	1.3096	6.507	3	5.967	2.470	0.1177	34	300
*50	0.6556	0.6556	0.9452	0.8523	1.0282	9.350	3	12.074	2.470	0.1177	103	300
*100	0.3889	0.3889	0.6729	0.6155	0.7185	7.807	3	17.787	2.470	0.1177	184	300

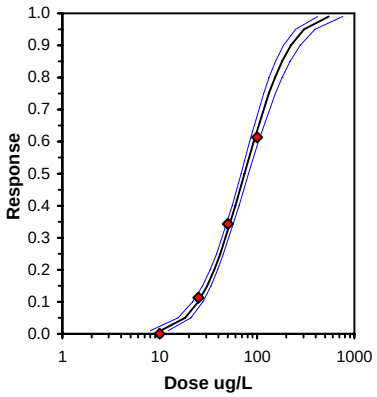
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.88686	0.835	-0.3888	0.32835
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	10	25	15.8114		0.02538	0.02544	0.41076	0.00341	2.0E-08	4, 10

Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	2.69326	0.16712	2.36572	3.02081	0	4.6744	5.99146	0.1	1.87172	0.3713	4
Intercept	-0.041	0.29081	-0.611	0.52896							
TSCR											

Point	Probits	ug/L	95% Fiducial Limits	
EC01	2.674	10.1848	8.00592	12.3353
EC05	3.355	18.2385	15.4347	20.8802
EC10	3.718	24.8818	21.8304	27.7336
EC15	3.964	30.6827	27.5081	33.6794
EC20	4.158	36.2432	32.9683	39.4064
EC25	4.326	41.8102	38.4025	45.2145
EC40	4.747	59.9309	55.5133	64.9641
EC50	5.000	74.4249	68.4992	81.7228
EC60	5.253	92.424	84.004	103.44
EC75	5.674	132.481	117.024	154.222
EC80	5.842	152.83	133.278	180.982
EC85	6.036	180.527	154.996	218.228
EC90	6.282	222.615	187.288	276.362
EC95	6.645	303.702	247.692	392.565
EC99	7.326	543.856	417.718	759.649



Macroalgae - Brine (acute)

Start Date:	7/04/2011	Test ID:	ECX11-0704	Sample ID:	GHD Simulated Brine
End Date:	1/06/2011	Lab ID:	FREO-Geotech Fremantle La	Sample Type:	Brine
Sample Date:	7/04/2011	Protocol:	WIENV-67	Test Species:	E. radiata
Comments:					

Conc-%	1	2	3
0	1.0000	1.0000	1.0000
1.6	1.0000	1.0000	1.0000
3.1	1.0000	1.0000	1.0000
6.3	1.0000	1.0000	1.0000
12.5	1.0000	1.0000	1.0000
25	0.9000	0.9667	0.8667
50	0.4667	0.6000	0.5000
100	0.1333	0.2000	0.3000

			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
0	1.0000	1.0000	1.5208	1.5208	1.5208	0.000	3				0	300
1.6	1.0000	1.0000	1.5208	1.5208	1.5208	0.000	3	0.000	2.560	0.1173	0	300
3.1	1.0000	1.0000	1.5208	1.5208	1.5208	0.000	3	0.000	2.560	0.1173	0	300
6.3	1.0000	1.0000	1.5208	1.5208	1.5208	0.000	3	0.000	2.560	0.1173	0	300
12.5	1.0000	1.0000	1.5208	1.5208	1.5208	0.000	3	0.000	2.560	0.1173	0	300
*25	0.9111	0.9111	1.2777	1.1970	1.3872	7.692	3	5.305	2.560	0.1173	26	300
*50	0.5222	0.5222	0.8078	0.7520	0.8861	8.638	3	15.564	2.560	0.1173	143	300
*100	0.2111	0.2111	0.4724	0.3738	0.5796	21.848	3	22.887	2.560	0.1173	237	300

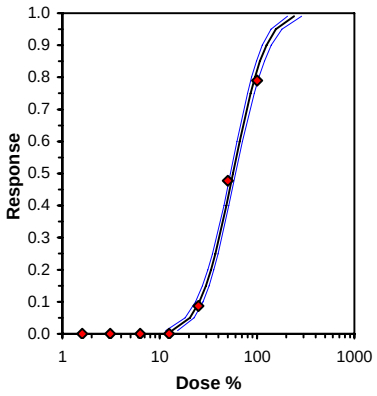
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.77432	0.884	0.61625	1.99366
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	12.5	25	17.6777	8	0.02523	0.02529	0.499	0.00315	1.4E-13	7, 16

Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	3.72367	0.18767	3.35584	4.09149	0	8.29484	11.0705	0.14	1.75275	0.26855	3
Intercept	-1.5267	0.32291	-2.1596	-0.8938							
TSCR											

Point	Probits	%	95% Fiducial Limits	
EC01	2.674	13.4278	11.4771	15.3061
EC05	3.355	20.4654	18.2301	22.5708
EC10	3.718	25.6204	23.2883	27.8133
EC15	3.964	29.8135	27.4364	32.0638
EC20	4.158	33.6303	31.2187	35.9412
EC25	4.326	37.2919	34.8385	39.6826
EC40	4.747	48.3853	45.6439	51.2512
EC50	5.000	56.5914	53.4125	60.0978
EC60	5.253	66.1892	62.2585	70.7485
EC75	5.674	85.8788	79.8059	93.3916
EC80	5.842	95.2291	87.9389	104.427
EC85	6.036	107.42	98.4017	119.03
EC90	6.282	125.001	113.264	140.449
EC95	6.645	156.487	139.368	179.681
EC99	7.326	238.504	205.231	285.8



Macroalgae - Copper Reference (chronic)

Start Date:	7/04/2011	Test ID:	ECX11-0704	Sample ID:	GHD Simulated Brine
End Date:	1/06/2011	Lab ID:	FREO-Geotech Fremantle La	Sample Type:	Copper
Sample Date:	7/04/2011	Protocol:	WIENV-67	Test Species:	E. radiata
Comments:					

Conc-ug/L	1	2	3
0	1.0000	0.9098	1.0000
10	0.7459	0.7869	0.8852
25	0.3852	0.4754	0.4426
50	0.1230	0.1721	0.0738
100	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
0	0.9699	1.0000	1.4358	1.2658	1.5208	10.252	3				9	300
*10	0.8060	0.8310	1.1195	1.0425	1.2252	8.455	3	3.942	2.420	0.1941	57	300
*25	0.4344	0.4479	0.7194	0.6696	0.7608	6.419	3	8.930	2.420	0.1941	169	300
*50	0.1230	0.1268	0.3537	0.2751	0.4278	21.622	3	13.489	2.420	0.1941	264	300
100	0.0000	0.0000	0.0500	0.0500	0.0500	0.000	3				300	300

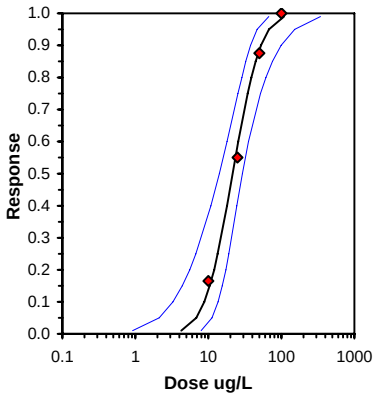
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.94109	0.805	-0.578	-0.2695
Bartlett's Test indicates equal variances (p = 0.55)	2.1131	11.3449		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	<10	10			0.08636	0.08795	0.6661	0.00965	4.6E-06	3, 8

Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	3.29615	0.33673	1.84734	4.74497	0.03	7.33247	5.99146	0.03	1.33317	0.30338	4
Intercept	0.60568	0.48891	-1.4979	2.70929							
TSCR	0.03323	0.01964	-0.0513	0.11774							

Point	Probits	ug/L	95% Fiducial Limits	
EC01	2.674	4.24022	0.91852	7.94482
EC05	3.355	6.82563	2.1124	11.2441
EC10	3.718	8.79758	3.2772	13.5966
EC15	3.964	10.4407	4.39322	15.5058
EC20	4.158	11.9627	5.53042	17.2596
EC25	4.326	13.4442	6.72022	18.9715
EC40	4.747	18.0428	10.7962	24.4874
EC50	5.000	21.536	14.0928	29.0905
EC60	5.253	25.7055	18.0011	35.317
EC75	5.674	34.4981	25.4897	51.7185
EC80	5.842	38.7704	28.711	61.3276
EC85	6.036	44.4225	32.645	75.5788
EC90	6.282	52.7191	37.9284	99.4484
EC95	6.645	67.9497	46.6543	151.67
EC99	7.326	109.381	67.075	343.37



Macroalgae - Brine (chronic)

Start Date:	7/04/2011	Test ID:	ECX11-0704	Sample ID:	GHD Simulated Brine
End Date:	1/06/2011	Lab ID:	FREO-Geotech Fremantle La	Sample Type:	Brine
Sample Date:	7/04/2011	Protocol:	WIENV-67	Test Species:	E. radiata
Comments:					

Conc-%	1	2	3
0	1.0000	0.9098	1.0000
1.6	1.0000	0.9672	1.0000
3.1	0.9344	0.9836	0.8852
6.3	0.7869	0.8115	0.7049
12.5	0.5820	0.6557	0.5656
25	0.4836	0.5082	0.4098
50	0.3115	0.1721	0.2705
100	0.0410	0.0164	0.0656

			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
0	0.9699	1.0000	1.4358	1.2658	1.5208	10.252	3				9	300
1.6	0.9891	1.0197	1.4768	1.3887	1.5208	5.163	3	-0.578	2.560	0.1814	3	300
3.1	0.9344	0.9634	1.3265	1.2252	1.4424	8.243	3	1.542	2.560	0.1814	20	300
*6.3	0.7678	0.7915	1.0697	0.9965	1.1217	6.096	3	5.165	2.560	0.1814	70	300
*12.5	0.6011	0.6197	0.8876	0.8512	0.9438	5.564	3	7.735	2.560	0.1814	119	300
*25	0.4672	0.4817	0.7524	0.6947	0.7936	6.840	3	9.642	2.560	0.1814	160	300
*50	0.2514	0.2592	0.5223	0.4278	0.5921	16.250	3	12.889	2.560	0.1814	225	300
*100	0.0410	0.0423	0.1971	0.1284	0.2590	33.262	3	17.478	2.560	0.1814	287	300

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.95509	0.884	-0.5615	-0.3618
Bartlett's Test indicates equal variances (p = 0.83)	3.52584	18.4753		

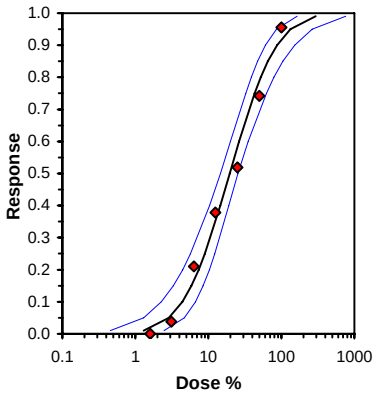
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	3.1	6.3	4.41928	32.2581	0.07872	0.08017	0.62645	0.00753	2.2E-11	7, 16

Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	1.97695	0.20078	1.46084	2.49306	0.03	25.7324	11.0705	1.0E-04	1.2913	0.50583	8
Intercept	2.44717	0.27222	1.74742	3.14692							
TSCR	0.01924	0.01555	-0.0207	0.05922							

Point	Probits	%	95% Fiducial Limits	
EC01	2.674	1.30185	0.45136	2.46748
EC05	3.355	2.87926	1.30064	4.70422
EC10	3.718	4.39592	2.27475	6.67008
EC15	3.964	5.84842	3.30476	8.47301
EC20	4.158	7.33804	4.43259	10.2806
EC25	4.326	8.91498	5.6841	12.1748
EC40	4.747	14.5595	10.4139	19.0425
EC50	5.000	19.5568	14.6504	25.4996
EC60	5.253	26.2693	20.1304	34.9605
EC75	5.674	42.9017	32.4583	62.1325
EC80	5.842	52.1212	38.7249	79.0864
EC85	6.036	65.3967	47.2821	105.413
EC90	6.282	87.005	60.3876	152.319
EC95	6.645	132.835	86.0451	265.092
EC99	7.326	293.787	164.786	760.447

Significant heterogeneity detected (p = 1.01E-04)



- APPENDIX C -

Heliocidaris erythrogramma Fertilisation and Larval Development Bioassay

Raw Data and Statistical Analyses

Intertek GEOTECH GEOTECHNICAL SERVICES PTY LTD
41-45 Furnace Road, Welshpool, Western Australia, 6106
Locked Bag 27, Welshpool DC, Western Australia, 6986
Email: geotech@geotechnical-services.com.au
(24 hours) Telephone: (08) 9458 8877
Facsimile: (08) 9458 8857
ABN 58 050 543 194
ACN 050 543 194

Ecotoxicology Laboratory Test Report

Report Date: 3rd June 2011

Sample Details

Client	GHD Services Pty Ltd	**Lab ID No**	ECX11-0704
Attention	Oliver Glade-Wright GHD House 239 Adelaide Terrace Perth WA 6004	**Sampled By**	GHD Services Pty Ltd
		Date Sampled	06/04/11
		Date Received	07/04/11
Phone No	(08) 6222 8649	**Project Start**	07/04/11
Order No	51629	**Project Finish**	01/06/11

Bioassay Details

Test Performed	1-h Echinoderm Fertilisation	72-h Echinoderm Larval Development
Test Protocol	WIENV-N/A	WIENV-N/A
Reference	Simon et al., 1997	Simon et al., 1997
Test Species	*Heliocidaris erythrogramma*	*Heliocidaris erythrogramma*
Test Temperature	21°C	21°C

Concentration-Response Data

Brine Simulate (%)	Echinoderm Fertilisation (% Control)	Echinoderm Larval Development (% Control)
Control	100 ± 0	101 ± 9
1.6	100 ± 0	94 ± 6
3.1	100 ± 0	86 ± 9
6.3	100 ± 0	75 ± 13
12.5	99 ± 2	47 ± 6
25.0	86 ± 8	28 ± 5
50.0	52 ± 5	13 ± 8
100.0	8 ± 2	1 ± 2

Statistical Effects Data

Effect Concentration	Echinoderm Fertilisation Inhibition (% Brine)	Echinoderm Larval Development Inhibition (% Brine)
EC50	51.2	12.9
EC10	19.6	5.9
LOEC	25.0	6.3
NOEC	12.5	3.1

Quality Assurance Limits

Quality Assurance Criteria	Echinoderm Fertilisation Inhibition	Echinoderm Larval Development Inhibition
Reference Toxicant	Copper	Copper
Cusum Chart Limits	37 – 61 µg/L	18 – 33 µg/L
Observed EC50	41 µg/L	22 µg/L
Coefficient of Variance	21.1%	27.9%
Control Response	>80% Fertilised	>80% Developed
Test Acceptability	YES	YES

Report Approval

David Strom
Principal Ecotoxicologist & Divisional Manager

Echinoderm - Copper Reference (acute)

Start Date:	7/04/2011	Test ID:	ECX11-0704	Sample ID:	GHD Simulated Brine
End Date:	1/06/2011	Lab ID:	FREO-Geotech Fremantle La	Sample Type:	Copper
Sample Date:	07/04/2011	Protocol:	WIENV-N/A	Test Species:	H. erythrogramma
Comments:					

Conc-ug/L	1	2	3
0	1.0000	1.0000	1.0000
10	0.9667	1.0000	0.9000
25	0.7000	0.6333	0.7333
50	0.4667	0.3667	0.5333
100	0.1667	0.1000	0.1333

			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
0	1.0000	1.0000	1.5208	1.5208	1.5208	0.000	3				0	300
10	0.9556	0.9556	1.3857	1.2490	1.5208	9.805	3	2.098	2.470	0.1590	13	300
*25	0.6889	0.6889	0.9799	0.9204	1.0282	5.590	3	8.401	2.470	0.1590	94	300
*50	0.4556	0.4556	0.7404	0.6504	0.8188	11.448	3	12.121	2.470	0.1590	163	300
*100	0.1333	0.1333	0.3720	0.3218	0.4205	13.283	3	17.843	2.470	0.1590	260	300

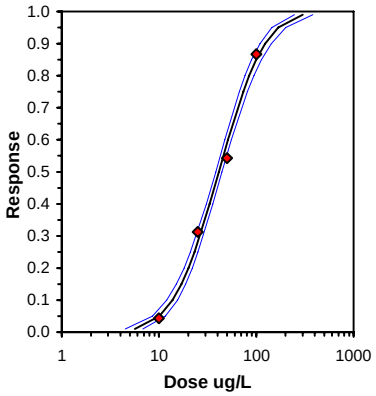
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.95513	0.835	-0.1226	0.77042
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	10	25	15.8114		0.04057	0.04067	0.66157	0.00622	3.8E-08	4, 10

Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	2.69621	0.1375	2.42671	2.96572	0	5.19453	5.99146	0.07	1.6148	0.37089	3
Intercept	0.64615	0.22311	0.20886	1.08344							
TSCR											

Point	Probits	ug/L	95% Fiducial Limits	
EC01	2.674	5.64912	4.49861	6.82206
EC05	3.355	10.1098	8.54606	11.6372
EC10	3.718	13.7875	12.0103	15.4978
EC15	3.964	16.998	15.0901	18.8275
EC20	4.158	20.0748	18.0703	22.0032
EC25	4.326	23.1547	21.0666	25.1815
EC40	4.747	33.177	30.7693	35.6539
EC50	5.000	41.1909	38.3515	44.2861
EC60	5.253	51.1406	47.4944	55.3646
EC75	5.674	73.2763	67.0122	81.1464
EC80	5.842	84.5183	76.6277	94.6811
EC85	6.036	99.8171	89.4893	113.46
EC90	6.282	123.06	108.65	142.642
EC95	6.645	167.827	144.614	200.574
EC99	7.326	300.346	246.56	381.226



Echinoderm - Brine (acute)

Start Date:	7/04/2011	Test ID:	ECX11-0704	Sample ID:	GHD Simulated Brine
End Date:	1/06/2011	Lab ID:	FREO-Geotech Fremantle La	Sample Type:	Brine
Sample Date:	7/04/2011	Protocol:	WIENV-N/A	Test Species:	H. erythrogramma
Comments:					

Conc-%	1	2	3
0	1.0000	1.0000	1.0000
1.6	1.0000	1.0000	1.0000
3.1	1.0000	1.0000	1.0000
6.3	1.0000	1.0000	1.0000
12.5	1.0000	1.0000	0.9667
25	0.9333	0.8667	0.7667
50	0.4667	0.5333	0.5667
100	0.0667	0.0667	0.1000

			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
0	1.0000	1.0000	1.5208	1.5208	1.5208	0.000	3				0	300
1.6	1.0000	1.0000	1.5208	1.5208	1.5208	0.000	3	0.000	2.560	0.1158	0	300
3.1	1.0000	1.0000	1.5208	1.5208	1.5208	0.000	3	0.000	2.560	0.1158	0	300
6.3	1.0000	1.0000	1.5208	1.5208	1.5208	0.000	3	0.000	2.560	0.1158	0	300
12.5	0.9889	0.9889	1.4762	1.3872	1.5208	5.224	3	0.984	2.560	0.1158	3	300
*25	0.8556	0.8556	1.1911	1.0667	1.3096	10.208	3	7.287	2.560	0.1158	43	300
*50	0.5222	0.5222	0.8077	0.7520	0.8523	6.317	3	15.762	2.560	0.1158	143	300
*100	0.0778	0.0778	0.2814	0.2612	0.3218	12.434	3	27.396	2.560	0.1158	276	300

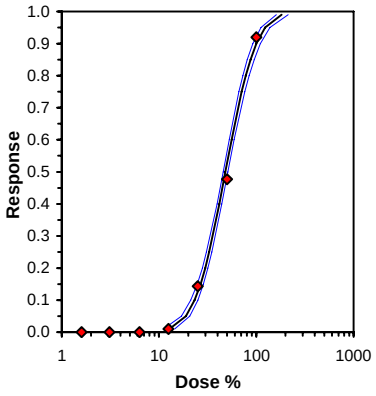
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.84219	0.884	-0.3959	3.05821
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	12.5	25	17.6777	8	0.02475	0.02481	0.6337	0.00307	1.8E-14	7, 16

Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	4.04107	0.19255	3.66366	4.41848	0	5.28901	11.0705	0.38	1.68331	0.24746	3
Intercept	-1.8024	0.32337	-2.4362	-1.1686							
TSCR											

Point	Probits	%	95% Fiducial Limits	
EC01	2.674	12.8125	11.1076	14.4516
EC05	3.355	18.8919	16.972	20.704
EC10	3.718	19.6369	21.2441	25.1152
EC15	3.964	26.7199	24.6932	28.6403
EC20	4.158	29.8567	27.8053	31.8194
EC25	4.326	32.8398	30.7606	34.8553
EC40	4.747	41.7461	39.4886	44.0613
EC50	5.000	51.2291	45.7003	50.9448
EC60	5.253	59.7189	52.7133	59.1
EC75	5.674	70.8301	66.4311	76.103
EC80	5.842	77.9069	72.7083	84.2623
EC85	6.036	87.0531	80.7183	94.9532
EC90	6.282	100.102	91.985	110.445
EC95	6.645	123.124	111.51	138.337
EC99	7.326	181.545	159.652	211.509



Echinoderm - Copper Reference (chronic)

Start Date:	7/04/2011	Test ID:	ECX11-0704	Sample ID:	GHD Simulated Brine
End Date:	1/06/2011	Lab ID:	FREO-Geotech Fremantle La	Sample Type:	Copper
Sample Date:	7/04/2011	Protocol:	WIENV-N/A	Test Species:	H. erythrogramma
Comments:					

Conc-ug/L	1	2	3
0	1.0000	1.0000	0.9167
10	0.7917	0.8750	0.9167
25	0.5000	0.4583	0.3750
50	0.1250	0.0417	0.0833
100	0.0000	0.0000	0.0417

			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
0	0.9722	1.0000	1.4398	1.2780	1.5208	9.737	3				8	300
*10	0.8611	0.8857	1.1947	1.0968	1.2780	7.655	3	3.119	2.470	0.1941	42	300
*25	0.4444	0.4571	0.7294	0.6591	0.7854	8.826	3	9.040	2.470	0.1941	166	300
*50	0.0833	0.0857	0.2866	0.2056	0.3614	27.247	3	14.674	2.470	0.1941	276	300
*100	0.0139	0.0143	0.1019	0.0500	0.2056	88.157	3	17.024	2.470	0.1941	296	300

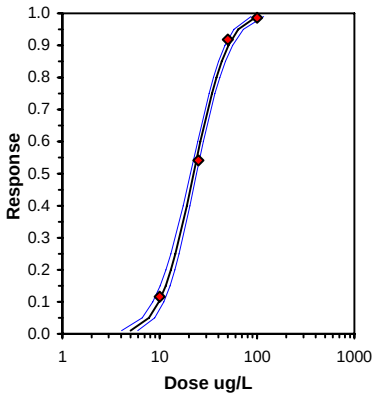
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.92321	0.835	-0.4573	-0.8805
Bartlett's Test indicates equal variances (p = 0.88)	1.18646	13.2767		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	<10	10			0.08496	0.08643	0.98168	0.00927	3.8E-08	4, 10

Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	3.58986	0.19102	3.21546	3.96425	0.02667	3.28215	5.99146	0.19	1.34854	0.27856	3
Intercept	0.15893	0.27715	-0.3843	0.70216							
TSCR	0.02789	0.00939	0.00947	0.0463							

Point	Probits	ug/L	95% Fiducial Limits	
EC01	2.674	5.01772	4.05762	5.97476
EC05	3.355	7.76865	6.58536	8.90973
EC10	3.718	9.80727	8.51602	11.0367
EC15	3.964	11.477	10.1221	12.7605
EC20	4.158	13.0046	11.6054	14.3287
EC25	4.326	14.4761	13.043	15.8353
EC40	4.747	18.9656	17.4484	20.4389
EC50	5.000	22.3121	20.7164	23.9109
EC60	5.253	26.249	24.5077	28.0741
EC75	5.674	34.3897	32.106	37.0026
EC80	5.842	38.2811	35.6303	41.4129
EC85	6.036	43.3762	40.1607	47.3023
EC90	6.282	50.7613	46.594	56.0295
EC95	6.645	64.0818	57.9088	72.2159
EC99	7.326	99.2142	86.6318	116.83



Echinoderm - Brine (chronic)

Start Date:	7/04/2011	Test ID:	ECX11-0704	Sample ID:	GHD Simulated Brine
End Date:	1/06/2011	Lab ID:	FREO-Geotech Fremantle La	Sample Type:	Brine
Sample Date:	7/04/2011	Protocol:	WIENV-N/A	Test Species:	H. erythrogramma
Comments:					

Conc-%	1	2	3
0	1.0000	1.0000	0.9167
1.6	0.9583	1.0000	0.8750
3.1	0.9583	0.8333	0.7917
6.3	0.6250	0.7500	0.8750
12.5	0.5417	0.4583	0.4167
25	0.3333	0.2500	0.2500
50	0.0417	0.2083	0.1250
100	0.0000	0.0000	0.0417

			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
0	0.9722	1.0000	1.4398	1.2780	1.5208	9.737	3				8	300
1.6	0.9444	0.9714	1.3651	1.2094	1.5208	11.403	3	0.749	2.560	0.2554	16	300
3.1	0.8611	0.8857	1.2041	1.0968	1.3652	11.799	3	2.363	2.560	0.2554	42	300
*6.3	0.7500	0.7714	1.0561	0.9117	1.2094	14.113	3	3.847	2.560	0.2554	76	300
*12.5	0.4722	0.4857	0.7575	0.7017	0.8271	8.429	3	6.840	2.560	0.2554	158	300
*25	0.2778	0.2857	0.5542	0.5236	0.6155	9.571	3	8.878	2.560	0.2554	217	300
*50	0.1250	0.1286	0.3470	0.2056	0.4740	38.846	3	10.956	2.560	0.2554	262	300
*100	0.0139	0.0143	0.1019	0.0500	0.2056	88.157	3	13.413	2.560	0.2554	296	300

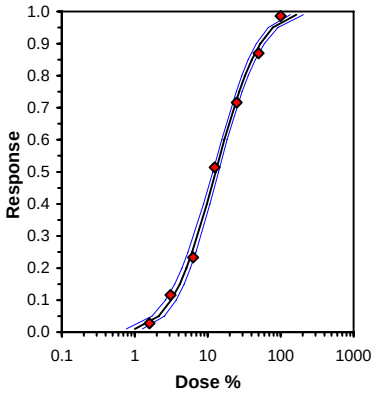
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.94469	0.884	0.0265	-1.0322
Bartlett's Test indicates equal variances (p = 0.86)	3.24418	18.4753		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	3.1	6.3	4.41928	32.2581	0.12492	0.12709	0.72422	0.01493	1.3E-09	7, 16

Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	2.10234	0.09075	1.92446	2.28022	0.02667	7.05454	11.0705	0.22	1.10984	0.47566	3
Intercept	2.66675	0.11247	2.4463	2.88719							
TSCR	0.02788	0.00854	0.01113	0.04462							

Point	Probits	%	95% Fiducial Limits	
EC01	2.674	1.00759	0.763	1.2771
EC05	3.355	2.12542	1.71602	2.554
EC10	3.718	5.86411	2.63962	3.70093
EC15	3.964	6.63852	3.52613	4.75798
EC20	4.158	7.52282	4.43492	5.81432
EC25	4.326	8.15187	5.39483	6.91113
EC40	4.747	9.75726	8.79475	10.7358
EC50	5.000	12.8776	11.7385	14.0669
EC60	5.253	16.9958	15.5794	18.5358
EC75	5.674	26.9565	24.6	29.7274
EC80	5.842	32.3714	29.3679	36.0048
EC85	6.036	40.0706	36.0258	45.1114
EC90	6.282	52.4105	46.4735	60.0567
EC95	6.645	78.0237	67.5591	92.0855
EC99	7.326	164.584	135.515	206.481



- APPENDIX D -

Mytilis edulis Survival and Larval Development Bioassay

Raw Data and Statistical Analyses

Intertek GEOTECH GEOTECHNICAL SERVICES PTY LTD
41-45 Furnace Road, Welshpool, Western Australia, 6106
Locked Bag 27, Welshpool DC, Western Australia, 6986
Email: geotech@geotechnical-services.com.au
(24 hours) Telephone: (08) 9458 8877
Facsimile: (08) 9458 8857
ABN 58 050 543 194
ACN 050 543 194

Ecotoxicology Laboratory Test Report

Report Date: 3rd June 2011

Sample Details

Client	GHD Services Pty Ltd	**Lab ID No**	ECX11-0704
Attention	Oliver Glade-Wright GHD House 239 Adelaide Terrace Perth WA 6004	**Sampled By**	GHD Services Pty Ltd
		Date Sampled	06/04/11
		Date Received	07/04/11
Phone No	(08) 6222 8649	**Project Start**	07/04/11
Order No	51629	**Project Finish**	01/06/11

Bioassay Details

Test Performed	96-h Mollusc Survival	48-h Mollusc Larval Development
Test Protocol	WIENV-66	WIENV-66
Reference	ASTM E724-98	ASTM E724-98
Test Species	*Mytilis edulis*	*Mytilis edulis*
Test Temperature	21°C	21°C

Concentration-Response Data

Brine Simulate (%)	Mollusc Survival (% Control)	Mollusc Larval Development (% Control)
Control	100 ± 0	100 ± 0
1.6	100 ± 0	100 ± 0
3.1	100 ± 0	100 ± 0
6.3	100 ± 0	66 ± 5
12.5	93 ± 6	46 ± 8
25.0	77 ± 12	28 ± 5
50.0	43 ± 15	14 ± 4
100.0	13 ± 6	7 ± 7

Statistical Effects Data

Effect Concentration	Mollusc Survival Inhibition (% Brine)	Mollusc Larval Development Inhibition (% Brine)
EC50	43.2	13.9
EC10	16.3	3.5
LOEC	25.0	6.3
NOEC	12.5	3.1

Quality Assurance Limits

Quality Assurance Criteria	Mollusc Survival Inhibition	Mollusc Larval Development Inhibition
Reference Toxicant	Copper	Copper
Cusum Chart Limits	54 – 81 µg/L	33 – 49 µg/L
Observed EC50	72 µg/L	48 µg/L
Coefficient of Variance	23.6%	17.8%
Control Response	>80% Survival	>80% Development
Test Acceptability	YES	YES

Report Approval

David Strom
Principal Ecotoxicologist & Divisional Manager

NATA
ACCREDITED FOR TECHNICAL COMPETENCE

Mollusc - Copper Reference (acute)

Start Date:	7/04/2011	Test ID:	ECX11-0704	Sample ID:	GHD Simulated Brine
End Date:	1/06/2011	Lab ID:	FREO-Geotech Fremantle La	Sample Type:	Copper
Sample Date:	7/04/2011	Protocol:	WIENV-66	Test Species:	M. edulis
Comments:					

Conc-ug/L	1	2	3
0	1.0000	1.0000	1.0000
10	0.9000	1.0000	1.0000
20	0.9000	0.8000	1.0000
40	0.9000	0.8000	0.9000
60	0.6000	0.5000	0.7000
80	0.4000	0.3000	0.3000
160	0.3000	0.1000	0.2000

			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
0	1.0000	1.0000	1.5208	1.5208	1.5208	0.000	3				0	300
10	0.9667	0.9667	1.4302	1.2490	1.5208	10.969	3	0.900	2.530	0.2547	10	300
20	0.9000	0.9000	1.2923	1.1071	1.5208	16.264	3	2.269	2.530	0.2547	30	300
*40	0.8667	0.8667	1.2017	1.1071	1.2490	6.817	3	3.168	2.530	0.2547	40	300
*60	0.6000	0.6000	0.8875	0.7854	0.9912	11.592	3	6.289	2.530	0.2547	120	300
*80	0.3333	0.3333	0.6147	0.5796	0.6847	9.870	3	8.999	2.530	0.2547	200	300
*160	0.2000	0.2000	0.4550	0.3218	0.5796	28.386	3	10.585	2.530	0.2547	240	300

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.9745	0.873	0.01374	0.07517
Equality of variance cannot be confirmed				

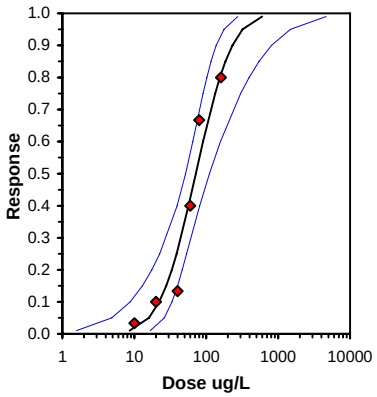
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	20	40	28.2843		0.08754	0.08776	0.50873	0.01521	1.6E-07	6, 14

Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	2.52306	0.41942	1.35857	3.68755	0	51.1265	9.48773	2.1E-10	1.85492	0.39634	4
Intercept	0.31993	0.75081	-1.7647	2.40453							
TSCR											

Point	Probits	ug/L	95% Fiducial Limits	
EC01	2.674	8.56805	1.56832	16.5946
EC05	3.355	15.9583	4.85639	26.033
EC10	3.718	22.2321	8.77675	33.4533
EC15	3.964	27.8055	12.969	39.9725
EC20	4.158	33.2157	17.5337	46.4537
EC25	4.326	38.6888	22.4935	53.3555
EC40	4.747	56.8202	39.3971	80.9034
EC50	5.000	71.6004	51.8169	110.699
EC60	5.253	90.2252	65.2981	158.088
EC75	5.674	132.509	90.7008	302.262
EC80	5.842	154.343	102.26	395.03
EC85	6.036	184.374	117.113	541.948
EC90	6.282	230.595	138.296	810.305
EC95	6.645	321.249	175.935	1479.25
EC99	7.326	598.341	273.61	4620.58

Significant heterogeneity detected (p = 2.10E-10)



Mollusc - Brine (acute)

Start Date:	7/04/2011	Test ID:	ECX11-0704	Sample ID:	GHD Simulated Brine
End Date:	1/06/2011	Lab ID:	FREO-Geotech Fremantle La	Sample Type:	Brine
Sample Date:	7/04/2011	Protocol:	WIENV-66	Test Species:	M. edulis
Comments:					

Conc-%	1	2	3
0	1.0000	1.0000	1.0000
1.6	1.0000	1.0000	1.0000
3.1	1.0000	1.0000	1.0000
6.3	1.0000	1.0000	1.0000
12.5	0.9000	1.0000	0.9000
25	0.9000	0.7000	0.7000
50	0.6000	0.3000	0.4000
100	0.1000	0.2000	0.1000

			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
0	1.0000	1.0000	1.5208	1.5208	1.5208	0.000	3				0	300
1.6	1.0000	1.0000	1.5208	1.5208	1.5208	0.000	3	0.000	2.560	0.2061	0	300
3.1	1.0000	1.0000	1.5208	1.5208	1.5208	0.000	3	0.000	2.560	0.2061	0	300
6.3	1.0000	1.0000	1.5208	1.5208	1.5208	0.000	3	0.000	2.560	0.2061	0	300
12.5	0.9333	0.9333	1.3396	1.2490	1.5208	11.711	3	2.251	2.560	0.2061	20	300
*25	0.7667	0.7667	1.0771	0.9912	1.2490	13.823	3	5.512	2.560	0.2061	70	300
*50	0.4333	0.4333	0.7168	0.5796	0.8861	21.724	3	9.989	2.560	0.2061	170	300
*100	0.1333	0.1333	0.3690	0.3218	0.4636	22.199	3	14.309	2.560	0.2061	260	300

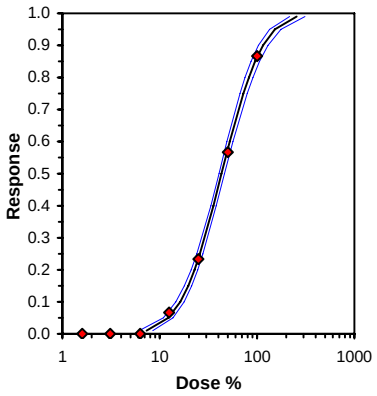
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.83917	0.884	0.95067	0.84071
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	12.5	25	17.6777	8	0.06165	0.06181	0.58719	0.00972	2.5E-10	7, 16

Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	3.01779	0.13427	2.75463	3.28096	0	3.28733	11.0705	0.66	1.63594	0.33137	3
Intercept	0.06307	0.21616	-0.3606	0.48675							
TSCR											

Point	Probits	%	95% Fiducial Limits	
EC01	2.674	7.32931	6.18082	8.47515
EC05	3.355	12.3279	10.8671	13.7465
EC10	3.718	16.2658	14.6524	17.8244
EC15	3.964	19.611	17.901	21.2686
EC20	4.158	22.7538	20.9639	24.5043
EC25	4.326	25.8486	23.9783	27.7019
EC40	4.747	35.6443	33.4152	37.9865
EC50	5.000	43.2455	40.5676	46.1938
EC60	5.253	52.4677	49.0425	56.413
EC75	5.674	72.3511	66.7506	79.2031
EC80	5.842	82.1916	75.3118	90.7711
EC85	6.036	95.3634	86.6179	106.488
EC90	6.282	114.976	103.194	130.301
EC95	6.645	151.703	133.608	175.95
EC99	7.326	255.164	216.403	309.791



Mollusc - Copper Reference (chronic)

Start Date:	7/04/2011	Test ID:	ECX11-0704	Sample ID:	GHD Simulated Brine
End Date:	1/06/2011	Lab ID:	FREO-Geotech Fremantle La	Sample Type:	Copper
Sample Date:	7/04/2011	Protocol:	WIENV-66	Test Species:	M. edulis
Comments:					

Conc-ug/L	1	2	3
0	1.0000	1.0000	1.0000
10	1.0000	1.0000	0.9667
20	0.9333	0.8667	0.7667
40	0.6667	0.6000	0.7333
60	0.5333	0.4000	0.4667
80	0.1333	0.1333	0.1000

			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
0	1.0000	1.0000	1.5208	1.5208	1.5208	0.000	3				0	300
10	0.9889	0.9889	1.4762	1.3872	1.5208	5.224	3	0.757	2.500	0.1471	3	300
*20	0.8556	0.8556	1.1911	1.0667	1.3096	10.208	3	5.601	2.500	0.1471	43	300
*40	0.6667	0.6667	0.9565	0.8861	1.0282	7.428	3	9.587	2.500	0.1471	100	300
*60	0.4667	0.4667	0.7518	0.6847	0.8188	8.914	3	13.065	2.500	0.1471	160	300
*80	0.1222	0.1222	0.3564	0.3218	0.3738	8.429	3	19.783	2.500	0.1471	264	300

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.96451	0.858	-0.2403	0.13189
Equality of variance cannot be confirmed				

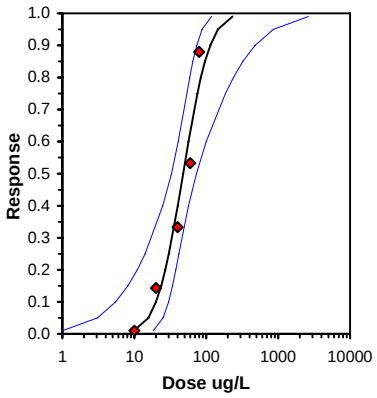
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	10	20	14.1421		0.03587	0.03596	0.60091	0.0052	1.0E-09	5, 12

Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	3.40196	0.63485	1.38159	5.42234	0	42.4889	7.81473	3.2E-09	1.679	0.29395	3
Intercept	-0.7119	1.06121	-4.0891	2.66533							
TSCR											

Point	Probits	ug/L	95% Fiducial Limits	
EC01	2.674	9.88956	1.01378	18.41
EC05	3.355	15.6856	3.08642	25.1473
EC10	3.718	20.0582	5.53737	29.9643
EC15	3.964	23.678	8.15556	33.9685
EC20	4.158	27.0153	11.0164	37.794
EC25	4.326	30.2508	14.1483	41.7402
EC40	4.747	40.2283	25.0494	56.8791
EC50	5.000	47.7532	33.0119	73.3121
EC60	5.253	56.6856	41.0124	100.237
EC75	5.674	75.3819	53.9164	183.956
EC80	5.842	84.4102	59.125	237.935
EC85	6.036	96.3074	65.4223	323.175
EC90	6.282	113.687	73.8403	478.071
EC95	6.645	145.38	87.657	860.915
EC99	7.326	230.583	119.351	2629.49

Significant heterogeneity detected (p = 3.16E-09)



Mollusc - Brine (chronic)

Start Date:	7/04/2011	Test ID:	ECX11-0704	Sample ID:	GHD Simulated Brine
End Date:	1/06/2011	Lab ID:	FREO-Geotech Fremantle La	Sample Type:	Brine
Sample Date:	7/04/2011	Protocol:	WIENV-66	Test Species:	M. edulis
Comments:					

Conc-%	1	2	3
0	1.0000	1.0000	1.0000
1.6	1.0000	1.0000	1.0000
3.1	1.0000	1.0000	1.0000
6.3	0.7000	0.6667	0.6000
12.5	0.5333	0.3667	0.4667
25	0.2667	0.2333	0.3333
50	0.1667	0.1667	0.1000
100	0.1333	0.0667	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
0	1.0000	1.0000	1.5208	1.5208	1.5208	0.000	3				0	300
1.6	1.0000	1.0000	1.5208	1.5208	1.5208	0.000	3	0.000	2.560	0.1541	0	300
3.1	1.0000	1.0000	1.5208	1.5208	1.5208	0.000	3	0.000	2.560	0.1541	0	300
*6.3	0.6556	0.6556	0.9442	0.8861	0.9912	5.657	3	9.576	2.560	0.1541	103	300
*12.5	0.4556	0.4556	0.7404	0.6504	0.8188	11.448	3	12.961	2.560	0.1541	163	300
*25	0.2778	0.2778	0.5541	0.5041	0.6155	10.206	3	16.055	2.560	0.1541	217	300
*50	0.1444	0.1444	0.3876	0.3218	0.4205	14.714	3	18.820	2.560	0.1541	256	300
*100	0.0667	0.0667	0.2283	0.0500	0.3738	71.987	3	21.465	2.560	0.1541	280	300

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.9039	0.884	-0.6117	2.96002
Equality of variance cannot be confirmed				

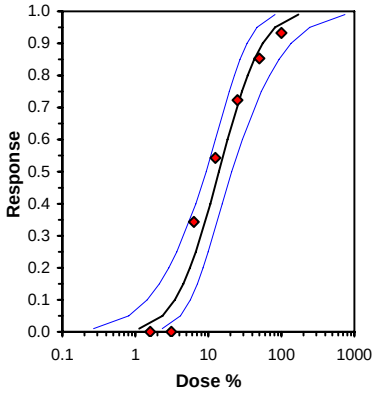
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	3.1	6.3	4.41928	32.2581	0.03861	0.0387	0.86185	0.00544	1.4E-13	7, 16

Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	2.13203	0.29299	1.37886	2.8852	0	75.6945	11.0705	6.7E-15	1.14305	0.46904	5
Intercept	2.56298	0.35879	1.64069	3.48528							
TSCR											

Point	Probits	%	95% Fiducial Limits	
EC01	2.674	1.12696	0.26761	2.33405
EC05	3.355	2.35263	0.81115	4.13967
EC10	3.718	3.48301	1.45051	5.67471
EC15	3.964	4.53863	2.13193	7.06994
EC20	4.158	5.60143	2.87775	8.47114
EC25	4.326	6.70949	3.70036	9.95148
EC40	4.747	10.5735	6.72383	15.4849
EC50	5.000	13.9011	9.30472	20.9107
EC60	5.253	18.2759	12.5011	29.085
EC75	5.674	28.8012	19.3303	53.1827
EC80	5.842	34.4985	22.6715	68.4963
EC85	6.036	42.577	27.1262	92.5898
EC90	6.282	55.4809	33.7532	136.258
EC95	6.645	82.1383	46.2147	243.948
EC99	7.326	171.471	81.8743	740.246

Significant heterogeneity detected (p = 6.66E-15)



- APPENDIX E -

Gladioferens imparipes Survival and Reproduction Bioassay

Raw Data and Statistical Analyses

Intertek GEOTECH GEOTECHNICAL SERVICES PTY LTD
41-45 Furnace Road, Welshpool, Western Australia, 6106
Locked Bag 27, Welshpool DC, Western Australia, 6986
Email: geotech@geotechnical-services.com.au
(24 hours) Telephone: (08) 9458 8877
Facsimile: (08) 9458 8857
ABN 58 050 543 194
ACN 050 543 194

Ecotoxicology Laboratory Test Report

Report Date: 3rd June 2011

Sample Details

Client	GHD Services Pty Ltd	**Lab ID No**	ECX11-0704
Attention	Oliver Glade-Wright GHD House 239 Adelaide Terrace Perth WA 6004	**Sampled By**	GHD Services Pty Ltd
		Date Sampled	06/04/11
		Date Received	07/04/11
Phone No	(08) 6222 8649	**Project Start**	07/04/11
Order No	51629	**Project Finish**	01/06/11

Bioassay Details

Test Performed	96-h Crustacean Survival	21-d Crustacean Reproduction
Test Protocol	WIENV-62	WIENV-62
Reference	USEPA 1002.0	USEPA 1002.0
Test Species	*G. imparipes*	*G. imparipes*
Test Temperature	21°C	21°C

Concentration-Response Data

Brine Simulate (%)	Crustacean Survival (% Control)	Crustacean Reproduction (% Control)
Control	94 ± 7	96 ± 9
1.6	98 ± 3	93 ± 5
3.1	97 ± 7	88 ± 2
6.3	91 ± 14	69 ± 10
12.5	84 ± 7	50 ± 8
25.0	63 ± 13	34 ± 6
50.0	22 ± 7	17 ± 1
100.0	3 ± 7	1 ± 1

Statistical Effects Data

Effect Concentration	Crustacean Survival Inhibition (% Brine)	Crustacean Reproduction Inhibition (% Brine)
EC50	30.5	14.4
EC10	12.7	3.4
LOEC	25.0	6.3
NOEC	12.5	3.1

Quality Assurance Limits

Quality Assurance Criteria	Crustacean Survival Inhibition	Crustacean Reproduction Inhibition
Reference Toxicant	Copper	Copper
Cusum Chart Limits	52 – 68 µg/L	21 – 36 µg/L
Observed EC50	58 µg/L	25 µg/L
Coefficient of Variance	17.9%	23.1%
Control Response	>80% Survival	>80% Survival
Test Acceptability	YES	YES

Report Approval

David Strom
Principal Ecotoxicologist & Divisional Manager

NATA
ACCREDITED FOR TECHNICAL COMPETENCE

Crustacean - Copper Reference (acute)

Start Date:	7/04/2011	Test ID:	ECX11-0704	Sample ID:	GHD Simulated Brine
End Date:	1/06/2011	Lab ID:	FREO-Geotech Fremantle La	Sample Type:	Copper
Sample Date:	7/04/2011	Protocol:	WIENV-62	Test Species:	G. imparipes
Comments:					

Conc-ug/L	1	2	3	4
0	0.8750	1.0000	0.8750	1.0000
15	0.8750	0.8750	1.0000	1.0000
30	0.7500	0.7500	1.0000	0.8750
60	0.5000	0.3750	0.3750	0.6250
120	0.0000	0.1250	0.1250	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
0	0.9375	1.0000	1.3651	1.2094	1.5208	13.168	4			24	400
15	0.9375	1.0000	1.3651	1.2094	1.5208	13.168	4	18.00	10.00	24	400
30	0.8438	0.9000	1.2061	1.0472	1.5208	18.510	4	14.00	10.00	62	400
*60	0.4688	0.5000	0.7538	0.6591	0.9117	16.047	4	10.00	10.00	212	400
*120	0.0625	0.0667	0.2057	0.0500	0.3614	87.390	4	10.00	10.00	376	400

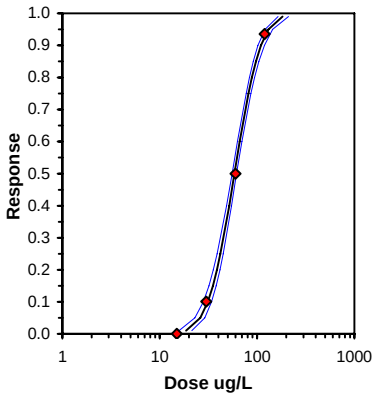
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.807	0.868	0.3648	-1.4364
Bartlett's Test indicates equal variances (p = 0.92)	0.93758	13.2767		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	30	60	42.4264	

Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	4.6924	0.26621	4.17064	5.21417	0.06	1.57869	5.99146	0.45	1.76709	0.21311	3
Intercept	-3.2919	0.48257	-4.2377	-2.3461							
TSCR	0.06064	0.00866	0.04366	0.07762							

Point	Probits	ug/L	95% Fiducial Limits	
EC01	2.674	18.6775	15.7841	21.4067
EC05	3.355	26.0947	22.9293	29.0057
EC10	3.718	31.1872	27.956	34.1337
EC15	3.964	35.1733	31.9381	38.1182
EC20	4.158	38.7017	35.4865	41.6342
EC25	4.326	42.0095	38.8252	44.9289
EC40	4.747	51.6532	48.5554	54.5946
EC50	5.000	58.4909	55.3741	61.5763
EC60	5.253	66.2337	62.9387	69.6845
EC75	5.674	81.4384	77.2302	86.3006
EC80	5.842	88.3989	83.5578	94.1762
EC85	6.036	97.2665	91.4726	104.402
EC90	6.282	109.698	102.356	119.033
EC95	6.645	131.106	120.679	144.856
EC99	7.326	183.171	163.808	210.057



Crustacean - Brine (acute)

Start Date:	7/04/2011	Test ID:	ECX11-0704	Sample ID:	GHD Simulated Brine
End Date:	1/06/2011	Lab ID:	FREO-Geotech Fremantle La	Sample Type:	Brine
Sample Date:	7/04/2011	Protocol:	WIENV-62	Test Species:	G. imparipes
Comments:					

Conc-%	1	2	3	4
0	0.8750	1.0000	0.8750	1.0000
1.6	1.0000	1.0000	0.8750	1.0000
3.1	1.0000	1.0000	0.8750	1.0000
6.3	0.8750	1.0000	1.0000	0.7500
12.5	0.8750	0.8750	0.7500	0.8750
25	0.6250	0.7500	0.5000	0.6250
50	0.3750	0.1250	0.1250	0.2500
100	0.0000	0.0000	0.0000	0.1250

			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
0	0.9375	1.0000	1.3651	1.2094	1.5208	13.168	4				24	400
1.6	0.9688	1.0333	1.4429	1.2094	1.5208	10.789	4	-0.697	2.480	0.2769	12	400
3.1	0.9688	1.0333	1.4429	1.2094	1.5208	10.789	4	-0.697	2.480	0.2769	12	400
6.3	0.9063	0.9667	1.3245	1.0472	1.5208	17.823	4	0.363	2.480	0.2769	37	400
12.5	0.8438	0.9000	1.1689	1.0472	1.2094	6.940	4	1.758	2.480	0.2769	62	400
*25	0.6250	0.6667	0.9140	0.7854	1.0472	11.697	4	4.040	2.480	0.2769	150	400
*50	0.2188	0.2333	0.4763	0.3614	0.6591	30.193	4	7.960	2.480	0.2769	313	400
*100	0.0313	0.0333	0.1279	0.0500	0.3614	121.755	4	11.082	2.480	0.2769	388	400

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.95153	0.904	-0.2216	-0.9024
Bartlett's Test indicates equal variances (p = 0.83)	3.51896	18.4753		

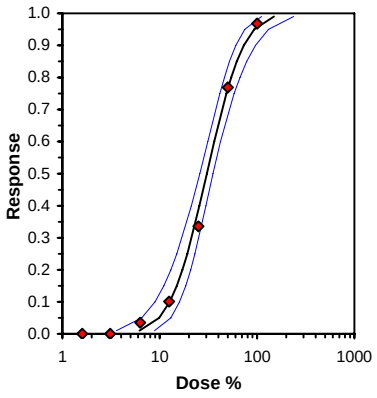
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	12.5	25	17.6777	8	0.17364	0.1812	0.96752	0.02493	1.4E-11	7, 24

Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	3.36864	0.3025	2.59104	4.14624	0.06	18.771	11.0705	2.1E-03	1.4836	0.29686	6
Intercept	0.00229	0.46935	-1.2042	1.20879							
TSCR	0.04934	0.01111	0.02077	0.0779							

Point	Probits	%	95% Fiducial Limits	
EC01	2.674	6.20883	3.58572	8.83575
EC05	3.355	9.8927	6.51394	13.0125
EC10	3.718	12.6813	8.93117	16.0374
EC15	3.964	14.9944	11.0306	18.4996
EC20	4.158	17.1301	13.0257	20.7555
EC25	4.326	19.2032	15	22.9433
EC40	4.747	25.609	21.202	29.8182
EC50	5.000	30.4509	25.8452	35.2662
EC60	5.253	36.2082	31.1708	42.1572
EC75	5.674	48.2865	41.5098	58.154
EC80	5.842	54.1302	46.1762	66.5465
EC85	6.036	61.8402	52.0928	78.1515
EC90	6.282	73.1199	60.3829	96.0554
EC95	6.645	93.7312	74.7561	131.107
EC99	7.326	149.345	110.56	237.169

Significant heterogeneity detected (p = 2.12E-03)



Crustacean - Copper Reference (chronic)

Start Date:	7/04/2011	Test ID:	ECX11-0704	Sample ID:	GHD Simulated Brine
End Date:	1/06/2011	Lab ID:	FREO-Geotech Fremantle La	Sample Type:	Copper
Sample Date:	7/04/2011	Protocol:	WIENV-62	Test Species:	G. imparipes
Comments:					

Conc-ug/L	1	2	3	4
0	0.9070	0.9767	1.0000	0.8837
5	0.7674	0.8605	0.8140	0.9767
10	0.7209	0.6744	0.6279	0.7907
25	0.4419	0.5116	0.4186	0.5349
50	0.0930	0.0233	0.0698	0.0465

			Transform: Arcsin Square Root					1-Tailed			Isotonic	
Conc-ug/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
0	0.9419	1.0000	1.3555	1.2228	1.5208	10.235	4				0.9425	1.0000
5	0.8547	0.9074	1.1995	1.0676	1.4177	12.800	4	2.077	2.360	0.1773	0.8550	0.9072
*10	0.7035	0.7469	0.9970	0.9147	1.0956	7.749	4	4.772	2.360	0.1773	0.7025	0.7454
*25	0.4767	0.5062	0.7620	0.7036	0.8203	7.287	4	7.901	2.360	0.1773	0.4750	0.5040
*50	0.0581	0.0617	0.2369	0.1531	0.3099	28.484	4	14.891	2.360	0.1773	0.0575	0.0610

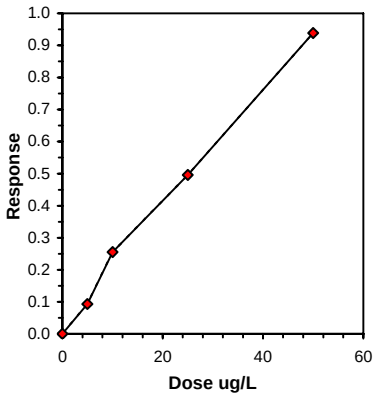
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.95583	0.868	0.6553	0.09868
Bartlett's Test indicates equal variances (p = 0.38)	4.16001	13.2767		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	5	10	7.07107		0.10071	0.10552	0.76483	0.01129	2.0E-09	4, 15

Linear Interpolation (200 Resamples)

Point	ug/L	SD	95% CL(Exp)		Skew
IC05*	2.693	1.398	0.513	7.905	0.8323
IC10	5.221	1.310	1.125	8.175	0.0031
IC15	6.766	1.265	2.327	10.030	-0.1171
IC20	8.311	1.286	3.973	12.668	0.2759
IC25	9.857	1.521	6.791	16.396	0.7317
IC40	19.033	1.694	14.503	24.195	0.0199
IC50	25.225	1.614	20.158	29.865	-0.1015

* indicates IC estimate less than the lowest concentration



Crustacean - Brine (chronic)

Start Date:	7/04/2011	Test ID:	ECX11-0704	Sample ID:	GHD Simulated Brine
End Date:	1/06/2011	Lab ID:	FREO-Geotech Fremantle La	Sample Type:	Brine
Sample Date:	7/04/2011	Protocol:	WIENV-62	Test Species:	G. imparipes
Comments:					

Conc-%	1	2	3	4
0	0.9070	0.9767	1.0000	0.8837
1.6	0.9302	1.0000	0.9070	0.9302
3.1	0.9302	0.8372	0.8837	0.8605
6.3	0.7209	0.6512	0.6047	0.7907
12.5	0.4186	0.5349	0.6047	0.4419
25	0.3488	0.3256	0.3953	0.2791
50	0.2326	0.1395	0.1628	0.1395
100	0.0000	0.0233	0.0000	0.0233

			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
0	0.9419	1.0000	1.3555	1.2228	1.5208	10.235	4				23	400
1.6	0.9419	1.0000	1.3472	1.2609	1.5208	8.721	4	0.135	2.480	0.1544	23	400
3.1	0.8779	0.9321	1.2174	1.1555	1.3035	5.225	4	2.218	2.480	0.1544	49	400
*6.3	0.6919	0.7346	0.9849	0.8908	1.0956	9.096	4	5.953	2.480	0.1544	124	400
*12.5	0.5000	0.5309	0.7855	0.7036	0.8908	11.005	4	9.157	2.480	0.1544	201	400
*25	0.3372	0.3580	0.6189	0.5566	0.6800	8.301	4	11.833	2.480	0.1544	264	400
*50	0.1686	0.1790	0.4210	0.3828	0.5032	13.509	4	15.011	2.480	0.1544	333	400
*100	0.0116	0.0123	0.1016	0.0500	0.1531	58.597	4	20.143	2.480	0.1544	396	400

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.94963	0.904	0.57066	-0.3622
Bartlett's Test indicates equal variances (p = 0.65)	5.06598	18.4753		

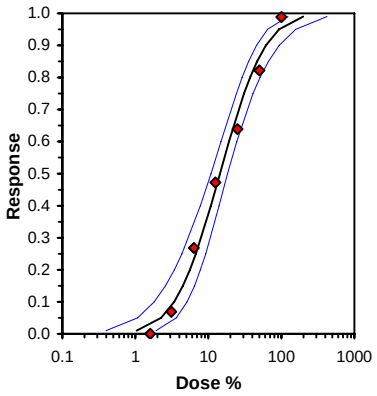
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	3.1	6.3	4.41928	32.2581	0.0849	0.08896	0.83289	0.00775	1.5E-16	7, 24

Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	2.03987	0.18714	1.5588	2.52094	0.0575	24.965	11.0705	1.4E-04	1.15881	0.49023	4
Intercept	2.63617	0.24249	2.01284	3.2595							
TSCR	0.04945	0.02073	-0.0038	0.10272							

Point	Probits	%	95% Fiducial Limits	
EC01	2.674	1.04321	0.39726	1.92341
EC05	3.355	2.25144	1.07374	3.6289
EC10	3.718	3.39283	1.81768	5.109
EC15	3.964	4.47431	2.58638	6.45122
EC20	4.158	5.57478	3.41602	7.78158
EC25	4.326	6.73225	4.32805	9.15822
EC40	4.747	10.8297	7.7539	13.9898
EC50	5.000	14.415	10.8515	18.3175
EC60	5.253	19.1872	14.9328	24.3916
EC75	5.674	30.8651	24.2832	41.0492
EC80	5.842	37.2735	29.0465	51.1721
EC85	6.036	46.4411	35.5306	66.6464
EC90	6.282	61.2444	45.4158	93.6815
EC95	6.645	92.2926	64.6574	156.827
EC99	7.326	199.184	123.246	419.559

Significant heterogeneity detected (p = 1.42E-04)



- APPENDIX F -

Pagrus auratus Survival and Growth Bioassay

Raw Data and Statistical Analyses

Intertek GEOTECH GEOTECHNICAL SERVICES PTY LTD
41-45 Furnace Road, Welshpool, Western Australia, 6106
Locked Bag 27, Welshpool DC, Western Australia, 6986
Email: geotech@geotechnical-services.com.au
(24 hours) Telephone: (08) 9458 8877
Facsimile: (08) 9458 8857
ABN 58 050 543 194
ACN 050 543 194

Ecotoxicology Laboratory Test Report

Report Date: 3rd June 2011

Sample Details

Client	GHD Services Pty Ltd	**Lab ID No**	ECX11-0704
Attention	Oliver Glade-Wright GHD House 239 Adelaide Terrace Perth WA 6004	**Sampled By**	GHD Services Pty Ltd
		Date Sampled	06/04/11
		Date Received	07/04/11
Phone No	(08) 6222 8649	**Project Start**	07/04/11
Order No	51629	**Project Finish**	01/06/11

Bioassay Details

Test Performed	96-h Fish Survival	7-d Fish Larval Development
Test Protocol	WIENV-64	WIENV-64
Reference	USEPA 1004.0	USEPA 1004.0
Test Species	*P. auratus*	*P. auratus*
Test Temperature	21°C	21°C

Concentration-Response Data

Brine Simulate (%)	Fish Survival (% Control)	Fish Larval Development (% Control)
Control	93 ± 6	100 ± 11
1.6	93 ± 6	100 ± 11
3.1	93 ± 12	100 ± 11
6.3	93 ± 6	78 ± 11
12.5	87 ± 6	70 ± 6
25.0	67 ± 6	44 ± 11
50.0	47 ± 6	19 ± 6
100.0	3 ± 6	0 ± 0

Statistical Effects Data

Effect Concentration	Fish Survival Inhibition (% Brine)	Fish Larval Development Inhibition (% Brine)
EC50	39.8	19.4
EC10	16.3	5.8
LOEC	25.0	6.3
NOEC	12.5	3.1

Quality Assurance Limits

Quality Assurance Criteria	Fish Survival Inhibition	Fish Larval Development Inhibition
Reference Toxicant	Copper	Copper
Cusum Chart Limits	82 – 114 µg/L	43 – 69 µg/L
Observed EC50	97 µg/L	48 µg/L
Coefficient of Variance	15.1%	18.6%
Control Response	>80% Survival	>80% Survival
Test Acceptability	YES	YES

Report Approval

David Strom
Principal Ecotoxicologist & Divisional Manager

NATA
ACCREDITED FOR TECHNICAL COMPETENCE

Fish - Copper Reference (acute)

Start Date:	7/04/2011	Test ID:	ECX11-0704	Sample ID:	GHD Simulated Brine
End Date:	1/06/2011	Lab ID:	FREO-Geotech Fremantle La	Sample Type:	Copper
Sample Date:	7/04/2011	Protocol:	WIENV-64	Test Species:	P. auratus
Comments:					

Conc-ug/L	1	2	3
0	0.9000	1.0000	0.9000
10	1.0000	1.0000	0.9000
25	0.9000	0.9000	0.8000
50	0.7000	0.8000	0.7000
75	0.7000	0.6000	0.7000
100	0.3000	0.4000	0.5000

			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
0	0.9333	1.0000	1.3396	1.2490	1.5208	11.711	3				20	300
10	0.9667	1.0357	1.4302	1.2490	1.5208	10.969	3	-0.994	2.500	0.2277	10	300
25	0.8667	0.9286	1.2017	1.1071	1.2490	6.817	3	1.514	2.500	0.2277	40	300
*50	0.7333	0.7857	1.0298	0.9912	1.1071	6.503	3	3.401	2.500	0.2277	80	300
*75	0.6667	0.7143	0.9561	0.8861	0.9912	6.345	3	4.210	2.500	0.2277	100	300
*100	0.4000	0.4286	0.6833	0.5796	0.7854	15.058	3	7.205	2.500	0.2277	180	300

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.96691	0.858	-0.0476	-0.5675
Bartlett's Test indicates equal variances (p = 0.74)	2.77237	15.0863		

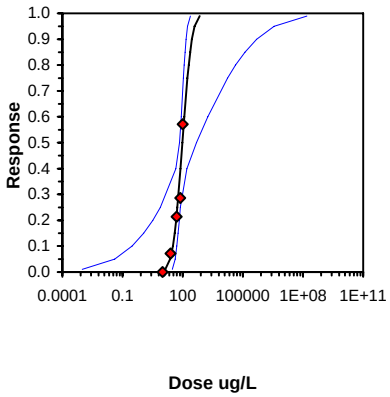
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	25	50	35.3553		0.14373	0.1517	0.2255	0.01245	3.2E-05	5, 12

Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	2.69711	0.71663	0.41648	4.97774	0.06667	17.97	7.81473	4.5E-04	1.98609	0.37077	5
Intercept	-0.3567	1.32377	-4.5695	3.85614							
TSCR	0.0537	0.02407	-0.0229	0.1303							

Point	Probits	ug/L	95% Fiducial Limits	
EC01	2.674	13.2909	0.00097	30.5235
EC05	3.355	23.7809	0.04126	42.6301
EC10	3.718	32.4287	0.30131	51.4671
EC15	3.964	39.9771	1.14024	59.0663
EC20	4.158	47.2107	3.2379	66.8292
EC25	4.326	54.4512	7.75143	75.983
EC40	4.747	78.0106	45.47	161.498
EC50	5.000	96.847	68.8514	486.606
EC60	5.253	120.232	84.849	1801.53
EC75	5.674	172.252	108.967	17490
EC80	5.842	198.67	119.045	43575.4
EC85	6.036	234.618	131.523	126719
EC90	6.282	289.23	148.61	487061
EC95	6.645	394.407	177.389	3597480
EC99	7.326	705.7	245.617	1.5E+08

Significant heterogeneity detected (p = 4.46E-04)



Fish - Brine (acute)

Start Date:	7/04/2011	Test ID:	ECX11-0704	Sample ID:	GHD Simulated Brine
End Date:	1/06/2011	Lab ID:	FREO-Geotech Fremantle La	Sample Type:	Brine
Sample Date:	7/04/2011	Protocol:	WIENV-64	Test Species:	P. auratus
Comments:					

Conc-%	1	2	3
0	1.0000	0.9000	0.9000
1.6	0.9000	1.0000	1.0000
3.1	1.0000	1.0000	0.8000
6.3	0.9000	0.9000	1.0000
12.5	0.8000	0.9000	0.9000
25	0.7000	0.7000	0.6000
50	0.5000	0.5000	0.4000
100	0.0000	0.0000	0.1000

			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
0	0.9333	1.0000	1.3396	1.2490	1.5208	11.711	3				20	300
1.6	0.9667	1.0357	1.4302	1.2490	1.5208	10.969	3	-0.763	2.560	0.3040	10	300
3.1	0.9333	1.0000	1.3829	1.1071	1.5208	17.269	3	-0.364	2.560	0.3040	20	300
6.3	0.9333	1.0000	1.3396	1.2490	1.5208	11.711	3	0.000	2.560	0.3040	20	300
12.5	0.8667	0.9286	1.2017	1.1071	1.2490	6.817	3	1.161	2.560	0.3040	40	300
*25	0.6667	0.7143	0.9561	0.8861	0.9912	6.345	3	3.229	2.560	0.3040	100	300
*50	0.4667	0.5000	0.7518	0.6847	0.7854	7.731	3	4.949	2.560	0.3040	160	300
*100	0.0333	0.0357	0.1406	0.0500	0.3218	111.583	3	10.096	2.560	0.3040	290	300

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.93111	0.884	-0.2106	-0.4395
Bartlett's Test indicates equal variances (p = 0.63)	5.27914	18.4753		

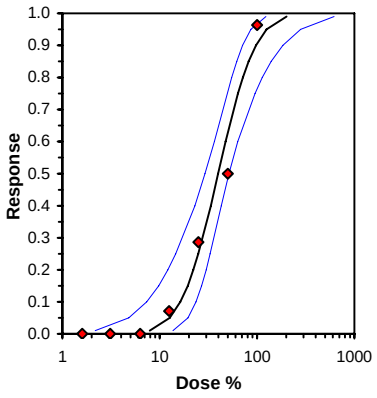
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	12.5	25	17.6777	8	0.20762	0.21912	0.58643	0.02116	8.5E-08	7, 16

Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	3.30822	0.53754	1.92644	4.69001	0.06667	37.5859	11.0705	4.6E-07	1.60006	0.30228	4
Intercept	-0.2934	0.88774	-2.5754	1.98863							
TSCR	0.0612	0.01916	0.01196	0.11044							

Point	Probits	%	95% Fiducial Limits	
EC01	2.674	7.8861	2.16645	13.7446
EC05	3.355	12.6725	4.82112	19.4894
EC10	3.718	16.3185	7.34959	23.5903
EC15	3.964	19.3541	9.7348	26.9261
EC20	4.158	22.1647	12.1346	30.0012
EC25	4.326	24.899	14.6149	33.0187
EC40	4.747	33.3797	22.8697	42.9243
EC50	5.000	39.8166	29.2346	51.4748
EC60	5.253	47.4947	36.4018	63.3721
EC75	5.674	63.6717	49.4073	94.9811
EC80	5.842	71.5265	54.9288	113.245
EC85	6.036	81.9132	61.7169	139.985
EC90	6.282	97.1511	70.9428	184.11
EC95	6.645	125.102	86.4127	278.907
EC99	7.326	201.032	123.227	617.159

Significant heterogeneity detected (p = 4.57E-07)



Fish - Copper Reference (chronic)

Start Date:	7/04/2011	Test ID:	ECX11-0704	Sample ID:	GHD Simulated Brine
End Date:	1/06/2011	Lab ID:	FREO-Geotech Fremantle La	Sample Type:	Copper
Sample Date:	7/04/2011	Protocol:	WIENV-64	Test Species:	P. auratus
Comments:					

Conc-ug/L	1	2	3
0	0.8889	1.0000	1.0000
10	1.0000	1.0000	1.0000
25	0.7778	0.7778	0.8889
50	0.6667	0.5556	0.4444
75	0.2222	0.3333	0.1111
100	0.0000	0.0000	0.1111

			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
0	0.9630	1.0000	1.4242	1.2310	1.5208	11.749	3				11	300
10	1.0000	1.0385	1.5208	1.5208	1.5208	0.000	3	-0.938	2.500	0.2574	0	300
*25	0.8148	0.8462	1.1303	1.0799	1.2310	7.716	3	2.855	2.500	0.2574	55	300
*50	0.5556	0.5769	0.8420	0.7297	0.9553	13.396	3	5.655	2.500	0.2574	133	300
*75	0.2222	0.2308	0.4821	0.3398	0.6155	28.634	3	9.152	2.500	0.2574	234	300
*100	0.0370	0.0385	0.1466	0.0500	0.3398	114.117	3	12.410	2.500	0.2574	289	300

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.96621	0.858	0.01675	-0.7491
Equality of variance cannot be confirmed				

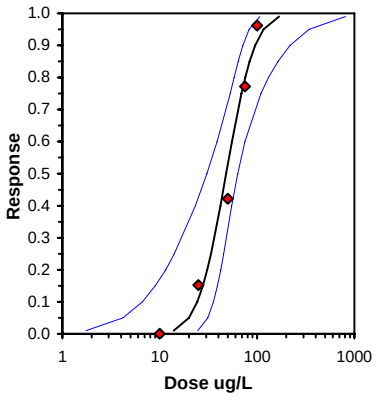
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	10	25	15.8114		0.13317	0.13608	0.87311	0.0159	7.5E-08	5, 12

Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	4.29643	0.79251	1.7743	6.81855	0.03667	35.1628	7.81473	1.1E-07	1.68336	0.23275	4
Intercept	-2.2324	1.39518	-6.6725	2.20765							
TSCR	0.02127	0.02088	-0.0452	0.08773							

Point	Probits	ug/L	95% Fiducial Limits	
EC01	2.674	13.8643	1.75335	24.4829
EC05	3.355	19.9764	4.18864	31.2389
EC10	3.718	24.2703	6.63526	35.7232
EC15	3.964	27.6775	9.02317	39.2235
EC20	4.158	30.7235	11.4899	42.3599
EC25	4.326	33.6024	14.0994	45.3712
EC40	4.747	42.1107	23.1687	54.9792
EC50	5.000	48.2346	30.4912	63.2222
EC60	5.253	55.2492	38.8384	75.1146
EC75	5.674	69.2385	52.8506	109.915
EC80	5.842	75.7264	58.0864	131.444
EC85	6.036	84.0602	64.0312	163.979
EC90	6.282	95.861	71.4765	219.339
EC95	6.645	116.467	82.8936	342.608
EC99	7.326	167.811	107.05	808.668

Significant heterogeneity detected (p = 1.13E-07)



Fish - Brine (chronic)

Start Date:	7/04/2011	Test ID:	ECX11-0704	Sample ID:	GHD Simulated Brine
End Date:	1/06/2011	Lab ID:	FREO-Geotech Fremantle La	Sample Type:	Brine
Sample Date:	7/04/2011	Protocol:	WIENV-64	Test Species:	P. auratus
Comments:					

Conc-%	1	2	3
0	0.8889	1.0000	1.0000
1.6	1.0000	1.0000	1.0000
3.1	1.0000	1.0000	0.8889
6.3	0.6667	0.8889	0.7778
12.5	0.7778	0.6667	0.6667
25	0.3333	0.5556	0.4444
50	0.2222	0.2222	0.1111
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
0	0.9630	1.0000	1.4242	1.2310	1.5208	11.749	3				11	300
1.6	1.0000	1.0385	1.5208	1.5208	1.5208	0.000	3	-0.987	2.530	0.2476	0	300
3.1	0.9630	1.0000	1.4242	1.2310	1.5208	11.749	3	0.000	2.530	0.2476	11	300
*6.3	0.7778	0.8077	1.0887	0.9553	1.2310	12.678	3	3.428	2.530	0.2476	66	300
*12.5	0.7037	0.7308	0.9968	0.9553	1.0799	7.216	3	4.367	2.530	0.2476	88	300
*25	0.4444	0.4615	0.7288	0.6155	0.8411	15.478	3	7.106	2.530	0.2476	167	300
*50	0.1852	0.1923	0.4405	0.3398	0.4909	19.796	3	10.052	2.530	0.2476	245	300
100	0.0000	0.0000	0.0500	0.0500	0.0500	0.000	3				300	300

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.92568	0.873	-0.5859	-0.5997
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	3.1	6.3	4.41928	32.2581	0.12617	0.12892	0.48494	0.01436	1.5E-07	6, 14

Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	2.43984	0.33855	1.56958	3.3101	0.03667	47.7257	11.0705	4.0E-09	1.28839	0.40986	4
Intercept	1.85652	0.46235	0.66801	3.04503							
TSCR	0.02065	0.0186	-0.0272	0.06847							

Point	Probits	%	95% Fiducial Limits	
EC01	2.674	2.16228	0.54962	4.2624
EC05	3.355	4.11368	1.46565	6.97848
EC10	3.718	5.79608	2.45756	9.13076
EC15	3.964	7.30465	3.46849	10.9923
EC20	4.158	8.77901	4.54458	12.7852
EC25	4.326	10.2789	5.70978	14.6068
EC40	4.747	15.2953	9.9151	20.9133
EC50	5.000	19.4265	13.4766	26.6127
EC60	5.253	24.6736	17.8399	34.7715
EC75	5.674	36.7149	26.7905	57.5679
EC80	5.842	42.9877	30.975	71.4698
EC85	6.036	51.6643	36.397	92.6916
EC90	6.282	65.1111	44.2077	129.666
EC95	6.645	91.74	58.3142	215.66
EC99	7.326	174.533	96.1933	570.784

Significant heterogeneity detected (p = 4.04E-09)