APPENDIX O10

Ecotoxicology studies

O10 ECOTOXICOLOGY STUDIES

O10.1 INTRODUCTION

The toxicity of simulated brine was tested using 15 species of marine flora and fauna. The tests were managed by Hydrobiology and Geotechnical Services and undertaken in laboratories in Sydney, Adelaide and Perth during 2006 and 2007.

A suite of species from Upper Spencer Gulf, or surrogates for which recognised tests were available, were tested using both chronic and acute tests by Hydrobiology in 2006. These tests are presented in Appendix O10.2.

Toxicity tests were developed for the Australian Giant Cuttlefish *Sepia apama* by Geotechnical Services in 2006. These tests are presented in Appendix O10.3.

Following a review of the 2006 reports, additional tests were undertaken in 2007 to include more local species, chronic rather than acute tests, and a consistent diluent salinity (41 g/L), and to repeat the Giant Cuttlefish tests. The 2007 tests are presented in Appendix O10.4.

Several interim species protection trigger values (SPTV) were calculated using the three suites of toxicity tests. The CSIRO subsequently reviewed all the tests and calculated an overall SPTV using the most appropriate species and tests. The CSIRO's assessment is presented in Appendix O10.5. Dr Michael Warne of the CSIRO has also provided a peer review letter of testimony for the ecotoxicological studies undertaken for the Draft EIS (see overleaf).

A number of terms are used throughout the reports, and are explained in Table O10.1.

Table O10.1 Glossary

EC50	Concentration that causes an effect on 50% of the population For example: Growth: Concentration that results in 50% less growth when compared to controls Reproduction: Concentration that results in 50% less fecundity when compared to controls Germination: Concentration that results in 50% germination of zoospores Larval development: Concentration that results in 50% of larva deformed Calculated statistically
IC50	Concentration that causes an inhibition of growth of 50% when compared with controls (Unicellular alga bioassay) Calculated statistically
EC/IC10	Concentration that causes an effect of 10% when compared with controls Calculated statistically
LOEC	Lowest observed effect concentration Function of concentration tested
NOEC	No observed effect concentration Function of concentration tested
g/L	Grams per litre (effectively the same as parts per thousand or practical salinity units)
BurrliOZ	Software designed to estimate the protecting concentrations of chemicals such that a given percentage of species will survive, by fitting a certain distribution, called the Burr III distribution, to the input data (other distributions fitted to the data are the normal and the log-logistic distributions, however, these latter distributions are provided only as a reference guide and are not used to estimate the protecting concentrations).

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Our Ref: Statement re Point Lowly Desalination Plant EIS

Mr David Wiltshire
ARUP ENSR
GPO Box 11052 Adelaide
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14 November 2008

Dear David,

As part of my work for your organisation I was asked to review the whole effluent toxicity (WET) testing reported in a report by Hydrobiology Pty Ltd "Ecotoxicity of effluent from the proposed Olympic Dam desalination plant", a report by Geotechnical Services 2006 entitled "The provision of water quality monitoring services for Cockburn Sound (WET testing only). Simulated and RO brine. Report ENV05-214 and ENV05-389" and a report by Geotechnical Services 2007/2008 entitled "The provision of reverse osmosis brine toxicity testing – Report ECX07-1805". I have done this and I am satisfied that the test procedures used for the whole effluent toxicity testing and the statistical techniques used to estimate the toxicity are appropriate. However, it was noted that the statistical distributions used did not fit the data particularly well for a number of the test species in the Geotechnical Services 2007/08 report. The toxicity of the saline brine to these species was calculated using a different method, which improved the fit to the data.

The calculation of a species protection value was correctly executed within the Geotechnical Services and Hydrobiology reports, however, it was necessary to conduct a holistic review of all tests performed to date, and select the most appropriate set of results for calculating the species protection value. This was done in the CSIRO report "Selection of species and other factors that affect dilution factors for saline brine discharge from the proposed plant at Point Lowly, South Australia." I confirm that the work presented in the reports by Hydrobiology Pty Ltd and Geotechnical Services is acceptable for the intended purpose.

In addition, I read the marine chapter of the Draft EIS (as presented to me on 11 July 2008) and I confirm that it appropriately summarises the findings of the three reports I read and my own report

Yours faithfully,

Michael Warne
Principal Research Scientist
Centre for Environmental Contaminants Research

APPENDIX O10.2

Ecotoxicity of effluent from the proposed Olympic Dam desalination plant (report by Hydrobiology, 2006)

See overleaf for report.

ARUP/HLA

Ecotoxicity of Effluent from the Proposed Olympic Dam Desalination Plant

Final

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Job Number	ARUP-HLA/0601
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Authors	Dustin Hobbs, Jenny Stauber, Anu Kumar, Ross Smith,
File Name	ARUP-HLA_0601_RO_desal_plant_ecotox_report
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EXECUTIVE SUMMARY

The assessment of a prototype desalination plant effluent on the marine biota has been undertaken as part of an assessment of the potential impacts of the proposed desalination plant to be situated in Point Lowly. This desalination plant is part of the proposed expansion of BHP Billiton's Olympic Dam copper, gold, silver and uranium mine and these investigations are to be part of the overall EIS for the proposed expansion of the mine and processing plant and associated infrastructure.

Toxicity testing was undertaken on a prototype desalination plant effluent using a suite of locally relevant organisms or organisms that could be used as surrogates for local species. This suite included:

- The microalga, *Nitzschia closterium*, used in a 72-h growth rate inhibition test;
- The sea urchin, *Heliocidaris tuberculata*, used in a 72-h larval development test;
- The yellowtail kingfish, *Seriola lalandi*, used in a 96-h survival test;
- The macroalga, *Hormisira banksii*, used in a 72-h germination test;
- The oyster, *Saccostrea commercialis,* used in a 48-h larval development test, and;
- The prawn, *Penaeus monodon*, used in a 96-h survival test.

Standard testing methods were used to evaluate the toxicity of the prototype desalination plant effluent. The tests and organisms chosen were as locally relevant as possible, including the yellowtail kingfish, *Nitzschia closterium* and *Hormisira banksii,* all of which can be found in the Spencer Gulf. In some cases, the chosen biota were surrogate species, included due to their availability for testing year round from pristine areas or from hatcheries. Tests with these species are known to be highly reproducible, with appropriate quality assurance protocols.

The effluent was toxic to the test species, with EC/LC50 values ranging from 12 to >33% effluent. The sea urchin was the most sensitive species, with a no observed effect concentration (NOEC) of 4.1% effluent concentration. The least sensitive species were the prawn and macroalgae, with NOECs of 17%. All QA/QC criteria were met for all direct toxicity assessment's undertaken.

Table 0.1 Ranking of species from most to least sensitive with their corresponding NOEC's

Ranking	Test	NOEC (%)
1	72 h sea urchin larval development test (sub-chronic)	4
2	48 h oyster larval development test (sub-chronic)	8
3	72 h microalgal growth test (chronic)	11
4	96 h fish imbalance test (acute)	12
5	72 h macroalgal germination test (chronic)	16
5	96 h prawn mortality test (acute)	17

Through the use of salinity controls the observed toxicity was attributed to salinity for all organisms except the microalgae. The microalgae results indicated that 70% of the toxicity observed was caused by the high salinity but the remaining toxicity may have been caused by the added antiscalant.

"Safe" dilutions of the discharge were determined by combining acute data (after application of an acute to chronic ratio) and chronic data in a species sensitivity distribution. To protect 95% of species (appropriate for slightly to moderately disturbed ecosystems), the effluent would need to be diluted 1:60 times. To protect 99% of species (in pristine environments) the effluent would need to be diluted at least 1:80 times. Comparisons of these estimated "safe" dilutions with the dilutions achievable by the diffuser at the edge of the mixing zone, should ensure low risk to marine biota in the vicinity of the discharge.

As the final make up of the desalination plant effluent is still unknown, and will generally not be known until the plant is set up and processing, the toxicity of the effluent may increase or decrease pending the final concentration of the brine and the additives used during the process. Further testing would be recommended once the plant is commissioned and the final make up of the effluent has been determined.

1 INTRODUCTION

This report has been prepared by Hydrobiology Pty Ltd in association with Ecotox Services Australasia Pty Ltd and CSIRO Centre for Environmental Contaminants Research for ARUP/HLA. The assessment of effluent from a prototype desalination plant has been undertaken to determine its potential toxicity to the aquatic biota of the northern Spencer Gulf, to determine a safe dilution factor for the effluent, and to offer advice on measures to mitigate any identified problems.

1.1 Background

BHP Billiton proposes to expand the existing Olympic Dam copper, uranium, gold and silver mine and mine processing plant including associated infrastructure, located approximately 570 km NNW of Adelaide, South Australia. The project is currently in the planning phase with several options for major infrastructure being investigated. One of the principal components of the proposed expansion currently under investigation is the sourcing and supply of additional water needed for the proposed mine expansion via a water pipeline from a seawater desalination plant on the northern Spencer Gulf. Currently an EIS is being prepared by an ARUP/HLA consortium for the proposed expansion of the Olympic Dam mining and processing operations. A component of these studies is the assessment of the potential impacts of the proposed desalination plant brine discharge on the marine biota, including a study of the ecotoxicology of the brine discharge on local marine biota.

The area being considered for the desalination plant is Point Lowly, 20 km north of Whyalla. The northern Spencer Gulf is a low energy, seagrass-based ecosystem, with deep off-shore channels of up to 25 m in depth into which the brine would be discharged. These off-shore channels regularly have strong tidal flows of up to 2 to 3 knots. The region supports lucrative fisheries including the Western King Prawn fishery, and aquaculture of the Yellowtail Kingfish in sea cages in Fitzgerald Bay. The Australian Giant Cuttlefish also aggregates annually for breeding in the Point Lowly area and has become a major tourist attraction in South Australia, being visited by divers from all over world.

The main concerns associated with the discharges from the desalination plant are the potential effects of the hyper-saline effluent and associated antiscalants and other possible contaminants on the marine biota in the vicinity of the outfall, with particular attention focussed on the breeding grounds of the Australian Giant Cuttlefish, the Western King Prawn and Yellowtail Kingfish, due to their economic importance to the region, and to the seagrass communities.

1.2 Study objectives

- Undertake a review of all available literature on the toxicity of discharges from desalination plants similar to that proposed for use in the Olympic Dam Development Project.

- Undertake Direct Toxicity Assessment (DTA) on a prototype discharge using a suite of temperate Australian species comprising several trophic levels and taxonomic groups.

- Use results from the DTA of prototype discharge to develop species protection values using species sensitivity distributions.

- Apply the derived species protection values to the information collected during the literature review and identify any possible ecological problems that may be associated with the discharge.

1.3 Literature review

A literature review was undertaken encompassing existing information on the toxicity of typical desalination plant discharges and the toxicants that can be associated with such outputs. As it is not yet known which chemicals will be used in the reverse osmosis RO process, the products used in the generation of the prototype effluent were researched and reported using available sources.

Hoepner (1999) identified and discussed the possible components of desalination plant discharges that may enter the sea. Those discussed which were relevant to RO processes are corrosion products (metals), antiscaling additives, antifouling additives, halogenated organic compounds formed after chlorine addition, anticorrosion additives, acid and the concentrated brine left after extraction of fresh water. The final make-up of the effluent from the proposed desalination plant will not be known until the plant is operational and running at optimum capacity at which time any of the previously mentioned components may make up the final effluent. Additives that may be used in the desalination process for this project, include, $FeCl_3$ (coagulant), chlorine gas (disinfectant), sodium metabisulphite (chlorine scavenger) and NALCO PC-1020T (antiscalant).

The $FeCl_3$ added as a coagulant to feed water for the removal of large particles before reaching the RO membranes would be settled out from the filter backwash and disposed of on land. Chlorine added to the feed water for disinfection will be removed by the use of sodium metabisulphite as a chlorine scavenger. Therefore, there is not expected to be any residual chlorine present in the effluent. Data for the toxicity of sodium metabisulphite to marine organisms is not available, although data for the response of freshwater organisms to this chemical indicate that the alga *Desmodesmus subspicatus* is the most sensitive, with an IC50 (the concentration that will inhibit growth by 50%) of 48 mg/L after a 72-h exposure.

Antiscaling chemicals are used to prevent metal hydroxides/oxides and compounds such as calcium carbonate, calcium sulfate and silicates from precipitating out of aqueous solution onto membranes and other equipment. The antiscalant used for the processing of the simulated effluent was NALCO PC 1020T, which is an organophosphonate compound. NALCO PC 1020T was used as an additive in the processing of a prototype RO desalination effluent by Geotechnical Services (2005) that was then assessed using ecotoxicity testing. These results are covered below, but it is difficult to extrapolate results from the Geotechnical Services (2005) testing to the testing undertaken for this project mainly due to the different feed waters that were used. Currently, specific ecotoxicity data are not available for this particular antiscalant.

The effect that the hypersaline brine will have on the marine ecosystem is of the greatest concern when dealing with effluent from a seawater RO desalination plant. The proposed operating water recovery rates will produce an effluent with an average salinity of 75‰, with predicted peaks of 78 ‰. Marine organisms exist in an osmotic balance with their marine environment and an increase in the concentration of salts may result in the dehydration of cells, decreasing cell turgidity and leading to death (mainly of the larvae and young individuals) (Einav *et al.*, 2002).

Few researchers have looked at the effects of hypersalinity on marine organisms. Torquemada *et al.* (2005) investigated the effect of salinity on the growth and photosynthesis of the seagrass *Halophila johnsonii*. It was found that high salinity values of 60 ‰ caused significantly higher mortality than the optimum 30 ‰ and growth was significantly reduced at 40, 50 and 60 ‰ compared with 30 ‰. Photosynthetic activity was also reduced above 50 ‰.

Blaszkoski and Moreira (1986) found that over the course of 16 days (at 30°C) larvae of the hermit crab (*Pagurus criticornus*) grow and metamorphose equally well in 25 and 35 ‰, but at 45 ‰ fewer larvae progress beyond development stage II (about 5 days).

Reynolds *et al*. (1976) determined that *Leuresthes tenuis* (California grunion) pro-larvae (larvae with a yolk sac, up to 4 days old) have an upper salinity tolerance LC50 of 41 ‰ after 24 hours exposure.

Pillard *et al*. (1999) exposed the mysid shrimp (*Mysidopsis bahia*), sheepshead minnow (*Cyprinodon variegatus*) and inland silverside minnow (*Menidia beryllina*) to balanced solutions of synthetic seawater ranging from near zero to 80 ‰ salinity. The mysid shrimp and inland silverside had 48-h LC50s of 43 and 44 ‰ respectively while the sheepshead minnow had a 48 h LC50 of 70 ‰.

An investigation of the toxicity of prototype reverse osmosis desalination plant effluent was undertaken by Geotechnical Services (2006) for the Western Australian Water Corporation. The prototype effluent was evaluated using bacteria (15 min Microtox), 72-h *Nitzschia closterium* algal growth bioassay, and a 24-d *Gladioferens imparipes* copepod reproduction test. The prototype effluent was toxic, with no observed effect concentrations (NOECs) of <6.3 % sample concentration for Microtox, 42 % for the algae and <2.6 % for the copepods.

Le Page (2005) successfully maintained sexually mature purple sea urchins (*Stronglyocentrotus purpuratus*) for 3 months in 36.2 ‰ water (the salinity that was expected to be experienced in the zone of initial dilution) blended from demonstration plant RO water (~66 ‰) and seawater (33.5 ‰). In addition eggs and sperm were harvested from these urchins and the eggs were successfully fertilised in 36 ‰ seawater (60 min sperm activation tests).

Bay and Greenstein (1992) investigated the toxicity of mixes of brine from various desalination plants and seawater. Bioassays used were 48-h spore germination and germ tube length using the giant kelp (*Macrocystis pyrifera*), 10-d survival test using amphipods (*Rhepoxynius abronius*) and 48-h fertilisation test using the sea urchin (*Strongylocentrotus purpuratus*). No effect was observed for any of these tests over a range of salinities up to 43 ‰.

From the available literature, toxicity of desalination plant effluent seems to be mainly attributable to the high salinity of the brine. Other constituents that may be used in the treatment process are not discharged at levels during normal operation that are likely to cause toxicity on marine biota.

2 METHODS

2.1 Prototype effluent

The prototype effluent was prepared and provided to the ecotoxicity testing labs, where it was refrigerated until testing was undertaken within the next 1 to 2 weeks depending on organism availability. The processing methodology for developing the effluent was provided by CITOR Pty Ltd and is attached in The samples were handled with minimal agitation to limit the loss of volatiles and were allowed to warm to the experiment temperature (20ºC) prior to testing.

2.2 Direct Toxicity Assessment (DTA) of prototype effluent

Direct toxicity assessments of the prototype RO desalination plant effluent were carried out by Ecotox Services Australasia (ESA) and CSIRO Centre for Environmental Contaminants Research (CECR). The assessments included:

- Microalgal (*Nitzschia closterium*) 72-h growth rate inhibition test (chronic). This is a chronic test with a locally relevant species that is widely distributed in Australian waters. It is particularly sensitive to metals and ammonia and has been widely used in DTA in Australia and SE Asia. This test was carried out by CECR, Sydney.

- Macroalgal germination test (chronic) using the kelp *Ecklonia radiata* was originally to be used, but due to rough seas hindering collection and the lack of gametes in those specimens that were collected, the brown alga *Hormosira banksii* (Neptune's necklace) was used in its place. This bioassay measures germination success over 72 h from fertilisation as the end-point. Both species are widely distributed throughout southern Australian waters. *Hormosira*, an intertidal species, has been widely used for DTA in Australia, and viable gametes are available all year-round. This test was carried out by ESA.

- Prawn 96-h acute toxicity test (acute). This test uses 15-day post-larvae of the tiger prawn, *Penaeus monodon*, and has become one of the most widely used tests for the assessment of effluents in Australia. Although a tropical species, post-larvae of *P. monodon* are readily available from commercial hatcheries, so it is commonly used as a surrogate for other prawn species. The native prawn species of northern Spencer Gulf were not readily available from hatcheries and have not undergone standardisation for toxicity testing. This test was carried out by ESA.

- Oyster 48-h larval development test (sub-chronic). This test can utilise either the rock oyster, *Saccostrea commercialis*, or the Pacific oyster, *Crassostrea gigas*. The Pacific oyster was proposed as the species which may be most relevant to South Australian waters, given that there is a significant oyster culture industry in the region that uses this species. However, due to the Pacific oyster not spawning at the time of testing, the rock oyster was used as a surrogate. Both species are euryhaline and so are relatively tolerant of hypo and hypersaline conditions. The test using the rock oyster has been widely used for DTA in Australia. This test was carried out by ESA.

- Sea urchin 72-h larval development test using *Heliocidaris tuberculata* (sub-chronic). This species is widely used in Australian toxicity assessment programs and has been shown to be sensitive to a range of heavy metals, ammonia and surfactants. The test has routinely been used to assess the toxicity of sewage effluents, mine tailings, pulp/paper mill effluents, sediment pore waters, landfill leachates and petroleum hydrocarbons and dispersants. As there is currently no hatchery rearing of sea urchin species in Australia, it is necessary to collect broodstock from wild populations. *H. tuberculata* is distributed on rocky reefs from Southern Queensland to central New South Wales and produces robust gametes throughout the year. It was used as a surrogate for species native to Spencer Gulf. This test was carried out by ESA.

- Fish 96-h imbalance test (acute). This test determines mortality in 8-12 mm larvae over 96 h. Yellowtail kingfish, *Seriola lalandi*, was used for the discharge DTA testing. Other fish species have routinely been used for the assessment of effluents from pulp and paper mill and sewage treatment plants and contaminants such as aquaculture chemicals, pesticides and endocrine disrupting compounds throughout Australia. This test was carried out by CECR, Adelaide.

2.3 Assessment of possible desalination effluent impacts

The data produced from the suite of DTA testing of the prototype desalination plant discharge were used in a species sensitivity distribution to derive a "safe" dilution of effluent**.** No observable effect data (NOEC) or equivalent, from acute and chronic tests, were combined (after application of an appropriate acute to chronic ratio to the acute values) and the safe dilution extrapolated from the data according to the method of ANZECC and ARMCANZ (2000). This value was then used in conjunction with the results of the literature review and other available information to determine the possible impact of the discharge on the marine ecology of the northern Spencer Gulf.

3 RESULTS

3.1 Direct Toxicity Assessment QA/QC

Quality assurance and quality control was undertaken for all testing undertaken for this study. This involved the use of controls, salinity controls and reference toxicants. All water quality criteria were met throughout testing, all tests were valid according to test validity criteria and reference toxicity indicated that responses of all the organisms were within the accepted parameters. QA/QC information can be found for each individual test in Appendix 2.

3.2 Desalination effluent DTA results

The results of the direct toxicity assessment of the desalination effluent with 6 species can be seen in Table 3.1. The test reports are given in Appendix 2.

Table 3.1 Direct toxicity assessment results for the prototype RO desalination plant effluent.

Test	NOEC (%)	LOEC(%)	EC50 (%) (95% CI)
72 h microalgal growth test	11	33	26 (25 – 27)
72 h macroalgal germination test	17	33	> 33
96 h prawn mortality test	17	33	22 (16 – 32)
48 h oyster larval development test	8.3	17	12 (11 – 12)
72 h sea urchin larval development test	4.1	8.3	12 (11 – 12)
96 h fish imbalance test	13	25	19 (17 – 22)

Toxicity was observed in all tests with NOEC's ranging from 4.1% effluent concentration for the sea urchin larval development test to 17% effluent concentration for both the macroalgal germination test and the prawn mortality test. To determine the effect of high salinity on the microalgae, macroalgae, prawn, oyster and sea urchin, salinity controls of 39, 42 and 51 ‰, were used during testing by dissolving artificial sea salts in the diluent seawater.

Salinities for each dilution were recorded during the fish testing allowing for observation of the effects of the elevated salinity. Results from the salinity controls indicated that salinity was the main cause of toxicity observed in the macroalgae, prawn, oyster and sea urchin tests. The toxicity of the effluent to the fish was also attributed to the elevated salinity, as the salinity at the observed NOEC was 45 ‰ while the salinity at the observed LOEC was 54 ‰. Toxicity of the effluent to the microalga was predominantly due to the high salinity, but salinity alone could not explain all of the observed toxicity. This unexplained observed toxicity may be attributable to the antiscalant that was used during the processing of the prototype effluent.

3.3 Derivation of 'safe' dilutions

The data for the testing undertaken in this study included both acute and chronic test results (Table 3.2). The default acute to chronic ratio of 10, as suggested by ANZECC/ARMCANZ (2000) for converting acute LC50 values to chronic NOEC values, was used to convert the acute test (prawn and fish) LC50 values to chronic NOEC equivalents for inclusion in the species sensitivity distribution (SSD). The BurrliOZ software (provided as part of the ANZECC and ARMCANZ 2000 package) was used to calculate the SSD, which is presented in

Figure 3.1. The concentration to protect 95% of species derived from the Burr Type III distribution fitted to the data was 1.66% effluent concentration, corresponding to a 'safe' dilution of 1:60. For 99% species protection, the estimate was 1.23% effluent, corresponding to a 'safe' dilution of 1:80.

Table 3.2 The values from the results of the DTA on the prototype effluent used for the derivation of the SSD

Test	Endpoint (%)	Value Used in SSD (%)
96 h prawn mortality test (acute)	22 (LC50)	2.2
96 h fish imbalance test (acute)	19 (LC50)	1.9
72 h microalgal growth test (chronic)	11 (NOEC)	11
72 h macroalgal germination test (chronic)	17 (NOEC)	17
72 h sea urchin larval development test (sub-chronic)	4.1 (NOEC)	4.1
48 h oyster larval development test (sub-chronic)	8.3 (NOEC)	8.3

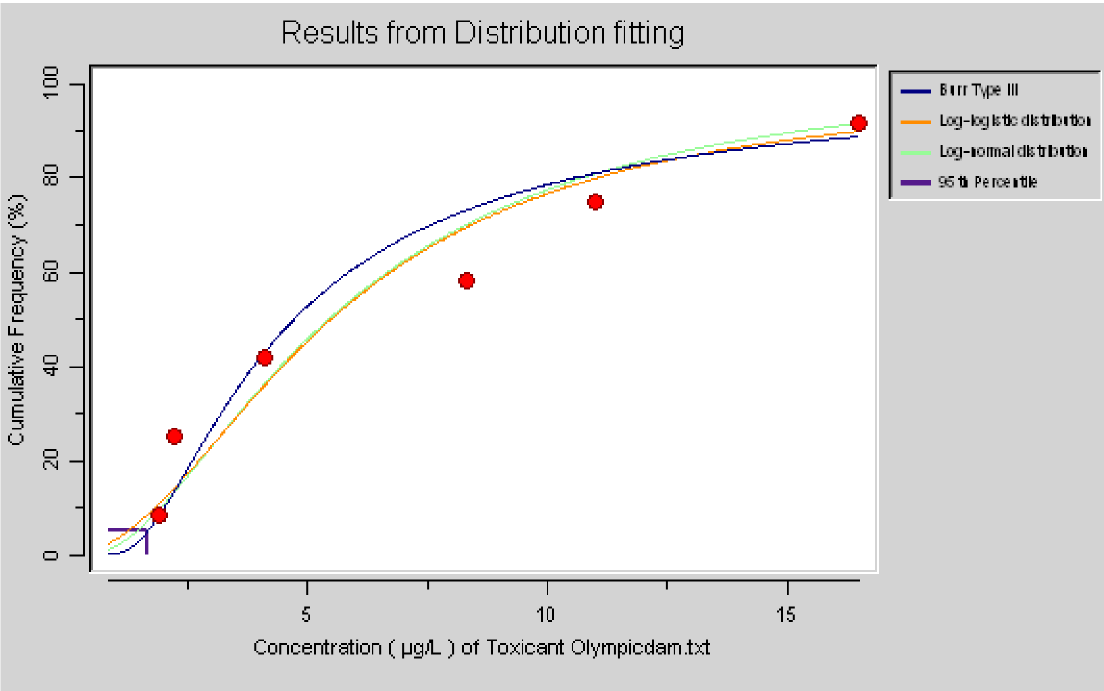

Figure 3.1 Results from distribution fitting of ecotoxicity testing results

4 DISCUSSION

The makeup of effluent from desalination plants can differ from plant to plant and can contain any number of compounds (Hoepner 1999). Those compounds in RO desalination plant effluent that may be of concern to marine biota in the receiving environment are generally removed by scavengers or are at concentrations that will not cause any toxic effect to the majority of the organisms.

Direct toxicity assessments carried out on the supplied prototype desalination plant effluent indicated that the effluent was toxic to marine biota. Sea urchin larval development was the most sensitive test, which may be due to echinoderms generally being less tolerant to salinity changes than other taxonomic groups (Graham 2005). The prawn and macro-algae tests were the least sensitive tests to the effluent.

Salinity controls were used to determine the effect high salinity was having on the test organisms. It was found that salinity was the major cause of the observed toxicity for all organisms except for the microalgae. The highest concentration tested, 33% of the desalination effluent sample concentration, caused a 70% inhibition in algal growth, while the salinity-matched control (51 ‰) caused only 54% inhibition in algal growth. This suggests that, while high salinity contributed to the reduced growth in the effluent sample, salinity did not account for all of the observed toxicity and that the added antiscalant may have been contributing to the toxicity.

Data generated from the DTA tests was then used to determine a species sensitivity distribution. A ‘safe’ dilution to protect 95% of species of 1:60 was estimated; the estimated ‘safe’ dilution to protect 99% of species was 1:80. Geotechnical Services (2006) used the results from DTAs using six species to derive “safe” dilutions for simulated desalination plant effluent that had an end salinity of 66 ‰ and treated with very similar levels of additives to that in the current study. The “safe” dilutions they derived to protect 95% and 99% of marine species were 1:71 and 1:106 respectively. These dilutions are similar to those derived in this study.

These estimated “safe” dilutions can then be compared with modeled dilution estimates achieved by the diffuser at the edge of the designated mixing zone surrounding the outfall. The higher than normal salinity of the water in the upper Spencer Gulf may also reduce the impact of the increased salinity of the effluent although Shepherd (1983) has suggested that the far northern section of the Spencer Gulf ecosystem is already under stress due to the high salinity and wide temperature fluctuations. Therefore, additional stress, such as effluent discharges, may have more serious consequences than in less stressed environments further to the south. This will all depend on the target salinity that is set for the desalination discharge.

As the final make up of the desalination plant effluent is still unknown, and will generally not be known until the plant is set up and processing, the toxicity of the effluent may increase or decrease pending the final concentration of the brine and the additives used during the process Further DTA testing would be recommended once the plant is commissioned and the final make up of the effluent has been determined.

5 CONCLUSIONS

- Prototype desalination plant effluent was found to be toxic to all tested organisms
- Toxicity was attributed to high salinity for all species except for microalgae, for which part of the observed toxicity may have been attributable to the antiscalant.
- Data from DTA tests were used to derive 95% and 99% species protective concentrations of 1.66 and 1.23% effluent concentration respectively using species sensitivity distributions.
- The corresponding 'safe' dilutions to protect 95 and 99% of species were estimated to be 1:60 and 1:80 respectively.

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APPENDIX 1

PROTOTYPE DESALINATION PLANT EFFLUENT CHEMICAL ADDITIVES

CITOR PTY. LTD.

Manufacturers of Reverse Osmosis
and associated Water Purification Equipment

A.B.N. 64 064 083 192

BHP BILLITON ECOTOXICOLOGY PROJECT

RO WATER PROCESSING REPORT 29/05/06 – 02/06/06

Conducted by Paul Fildes on 29/05/2006 to 01/06/06.
At SAAM Pty Ltd Pt Lowly Whyalla

Containers 1 – 8 had been filled with ambient Seawater on Friday 26 May by SAAM Pty Ltd.
Processing was commenced @ 1000 hrs Mon 29/05/06. TDS (Seawater): 44.2 PPT
The Containers were each dosed with NALCO Anti-scalant PC-1020T
Dosing Rate: 3.6 mg/L

Method

Five (5) x Containers (1000 L) used in the processing of Brine.
Each Container had the Brine recirculated from the RO Discharge and the Permeate discarded until the desired Salinity level was achieved.

Four (4) Containers were finished on Wed 31/05 @ 1600 hrs.
Each Containers holding approx. 500 L Brine
TDS Readings varied i.e. of the range 79.1 – 79.5 PPT
These Containers were then 'blended' to achieve a uniform TDS of 79.2 PPT in Containers 1 & 2.

Container 3 was successfully processed on Thurs 01/06.
Resulting in a final TDS reading of 79.4 PPT @ 1100 hrs.
200 L Brine was then decanted into a 200 L Drum with a final TDS result of 79.3 PPT

The 1000 L Containers were collected by Toll Logistics on Thurs 01/06 at approx. 1400 hrs.
Containers 1 & 2 held 1000 L each of Brine. Container 3 held approx. 300 L.
The remaining Containers 4 – 10 held 1000 L each of ambient Seawater.

The 200 L Drums – one holding Brine, the other ambient Seawater - were delivered to A. Kumar at the University of Adelaide, SA @ 1100 hrs Fri 02/06 by P. Fildes.

Note

All readings varied over the four (4) days of Processing and the Readings given are the final ones taken on Thurs 01/06.

Paul Fildes

08/06/2006

HEAD OFFICE AND PRODUCTION PLANT:
11 MEWS ROAD FREMANTLE. WESTERN AUSTRALIA 6160
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WEBSITE: www.citor.com.au
EMAIL: enquiries@citor.com.au

APPENDIX 2

DTA REPORTS

CSIRO

Centre for Environmental Contaminants Research
Lucas Heights Science and Technology Centre
New Illawarra Road, Lucas Heights, NSW
Private Mail Bag 7, Bangor, NSW, 2234, Australia
Telephone 61 2 9710 6808 Fax 61 2 9710 6837

Chronic Algal Growth Test Report 06178 NAG

Client: Hydrobiology Pty Ltd
Project: Olympic Dam; Desalination plant discharge
Test Performed: 72-h chronic algal growth rate toxicity test using the marine alga *Nitzschia closterium*

Samples Collected:	Prepared June 2006		
Samples Received:	27/6/2006	**Test Initiated:**	4/7/2006
CSIRO Sample No.	**Sample Name**	**Sample Description**	
WQE06178	Diluent Water	Seawater	
WQE06179	Desalination plant discharge	Brine	

Sample Physico-Chemistry and Preparation: The salinity of the pilot plant waste water was 77‰ and the pH was 7.9. The sample was diluted prior to testing with dilution water (37‰) supplied by CSIRO Land and Water. Prior to use, the dilution water was filtered (0.45 µm) to remove any microorganisms that may have been present in the seawater. To determine the effect of high salinity on the growth of *Nitzschia*, salinity controls (39, 42 and 51‰) were prepared by dissolving GP-2 artificial sea salts in natural seawater.

Sample	**Physico-chemistry**			
	pH	**‰**	**mS/cm**	**DO[a]**
WQE06178 (dilution water as received)	8.06	37	55	106
WQE06178 (dilution water filtered)	8.08	37	56	98
WQE06179 (plant discharge as received)	7.93	77	106	99
Salinity Control (39‰)	8.10	39	58	97
Salinity Control (42‰)	8.06	42	62	96
Salinity Control (51‰)	7.99	51	73	97
Natural seawater control	8.19	35	54	98

‰ = Salinity; mS/cm = Conductivity; [a] Dissolved Oxygen (%)

Test Method: This test measures the decrease (inhibition) in algal growth rate of the temperate marine alga *Nitzschia closterium* after exposure to the samples for 72 h (initial cell density 2-4 x 10^4 cells/mL). The test protocol is based on the OECD Test Guideline 201(1984) and the protocol of Stauber et. al. (1994). The 72-h IC50, NOEC and LOEC values were calculated using ToxCalc Version 5.0.23 (Tidepool Software).

Results: Algal growth in the dilution water was similar to the algal growth in the QA control (1.80 and 1.74 doublings per day respectively). Increased salinity of 39‰ and 51‰ caused a significant reduction in *Nitzschia* growth rate, however a salinity of 42‰ did not cause a reduction in algal growth.

The Olympic Dam plant discharge was toxic to *Nitzschia*, with an IC50 of 26%. There was no effect on algal growth at a concentration of 11%. The highest concentration tested, 33%, caused a 70% inhibition in algal growth, while the salinity-matched control (51‰) caused only 54% inhibition in algal growth. This suggests that while high salinity contributed to the reduced algal growth in the discharge sample (toxicity), salinity may not account for all of the observed toxicity (i.e. another toxicant(s) was contributing to toxicity).

When the highest concentrations tested (3.7, 11 and 33%) were corrected for reduced growth caused by high salinity (i.e. compared to the salinity-matched controls), the discharge was less toxic, with an IC50 of >33%. The LOEC was 33%, causing 32% inhibition in *Nitzschia* growth rate. This again indicated that salinity alone was not the sole cause of toxicity in the Olympic Dam desalination discharge.

There was no effect on algal growth rate at 11% before and after correction for effects due to high salinity.

Sample	**Growth Rate (Doublings/day)**	**% of QA/Diluent Control**	**% of Salinity Control**	**CV (%)**
QA Control (35‰)	1.74	100	–	6.0
Diluent Water (37‰)	1.80	103	–	3.3
Salinity Control 39‰	1.38	79[a]	–	1.5
Salinity Control 42‰	1.62	93	–	11
Salinity Control 51‰	0.80	46[a]	–	4.0
Olympic Dam- plant discharge				
Diluent Water	1.80	100	100	3.3
0.14%	1.84	102	102	2.6
0.4%	1.79	100	100	0.2
1.2%	1.83	102	102	1.5
3.7% (39‰)	1.73	97	125[b]	6.1
11% (42‰)	1.74	97	107	5.2
33% (51‰)	0.55	30[a]	68[a]	5.2
Sample	**IC50 (%)**	**LOEC (%)**	**NOEC (%)**	
Olympic Dam	26 (25-27)	–	11	
Olympic Dam (corrected for effects due to high salinity)	>33	33	11	

[a] Significantly less than control; [b] Significantly greater than than control;

Quality Assurance/Quality Control	**Criterion**	**This Test**	**Criterion Met?**
Control growth rate (doublings/day)	1.5 ± 0.3	1.74	Yes
Control growth rate CV	<20%	6%	Yes
Reference toxicant IC50 (measured copper, µg Cu/L)	18 ± 12	30	Yes

References:

OECD (1984) Guideline for testing of chemicals. Alga growth inhibition test. Test Guideline No. 201. Organisation for Economic Cooperation and Development, Paris, France.

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Test carried out by: Janine Wech and Monique Binet
Test supervised and reported by: Merrin Adams
Experimental Scientist (ph: 02 9710 6831)

Test report authorised by: Jenny Stauber
Senior Principal Research Scientist (ph: 02 9710 6808)
Date: 19/7/2006

APPENDIX – QA

4/07/2006 **72-h Chronic Toxicity of Olympic Dam pilot plant waste water to Nitzschia closterium: Quality Assurance**

	Sample	pH		0h	24h	48h	72h	Slope	Growth Rate (dblngs/day)		Pearson	% Control	Mean %
Flask No.		Day 0	Day 3	*All cell counts in (cells/mL) by x10^{4}*						Mean			
QA Control													
1				3.1	10.4	37.2	92.0	0.02078	1.66	1.74	99%	95%	100%
2	Control	8.12	8.5	3.1	10.0	40.6	99.5	0.02143	1.71		99%	98%	
3	(35‰)			3.1	10.1	45.6	135.4	0.02331	1.86		100%	107%	
						Mean control rate=		***0.02184***					
Measured copper concentrations (µg/L)													
4				3.1	7.0	31.3	91.0	0.02113	1.68	1.72	99%	97%	99%
5	3.5	8.13	8.52	3.1	8.4	40.9	108.2	0.02223	1.77		99%	102%	
6				3.1	8.2	32.6	99.7	0.02141	1.71		100%	98%	
7				3.1	5.9	26.3	73.5	0.01995	1.59	1.56	98%	91%	90%
8	7.2	8.12	8.46	3.1	7.5	26.0	68.7	0.01912	1.52		100%	88%	
9				3.1	7.5	28.9	74.6	0.01978	1.58		99%	91%	
10				3.1	6.1	12.2	43.5	0.01567	1.25	1.26	97%	72%	72%
11	15	8.12	8.32	3.1	5.0	15.3	42.7	0.01632	1.30		98%	75%	
12				3.1	5.9	15.3	37.6	0.01534	1.22		99%	70%	
10				3.1	4.7	9.3	14.1	0.00951	0.76	0.90	99%	44%	52%
11	29	8.09	8.26	3.1	4.8	10.4	22.7	0.01229	0.98		99%	56%	
12				3.1	2.9	9.0	20.1	0.01226	0.98		89%	56%	
Dilution Water (37‰)													
13				3.1	11.9	41.1	109.9	0.02169	1.73	1.80	100%	99%	103%
14	Dilution	8.07	8.53	3.1	13.6	45.4	137.5	0.02285	1.82		100%	105%	
15	Water			3.1	11.8	52.9	129.5	0.02305	1.84		99%	106%	
Salinity Controls													
13				3.1	10.4	28.5	52.0	0.01720	1.37	1.38	98%	79%	79%
14	39‰	8.06	8.44	3.1	11.1	31.6	51.4	0.01721	1.37		96%	79%	
15				3.1	10.9	33.3	54.4	0.01764	1.41		97%	81%	
16				3.1	9.4	32.2	53.3	0.01774	1.41	1.62	97%	81%	93%
17	42‰	8.05	8.59	3.1	9.9	41.0	97.4	0.02136	1.70		99%	98%	
18				3.1	9.9	41.4	104.5	0.02176	1.73		99%	100%	
16				3.1	5.2	12.2	16.0	0.01052	0.84	0.80	97%	48%	46%
17	51‰	8.02	8.42	3.1	6.0	11.1	15.9	0.01008	0.80		98%	46%	
18				3.1	5.4	12.4	13.9	0.00971	0.77		93%	44%	

Algal Growth Rate-Slope

Start Date:	4/07/2006	Test ID:	OD	Sample ID:	REF-Ref Toxicant
End Date:	4/07/2006	Lab ID:	CECR-Centre for Environmental	Sample Type:	Copper
Sample Date:		Protocol:	BD-Flow-FACSCalibur flow cyto	Test Species:	NC-Nitzschia closterium
Comments:	Measured concentrations of copper				

Conc-ug/L	1	2	3
Control	0.0208	0.0214	0.0233
3.5	0.0211	0.0222	0.0214
7.2	0.0199	0.0191	0.0198
15	0.0157	0.0163	0.0153
29	0.0095	0.0123	0.0123

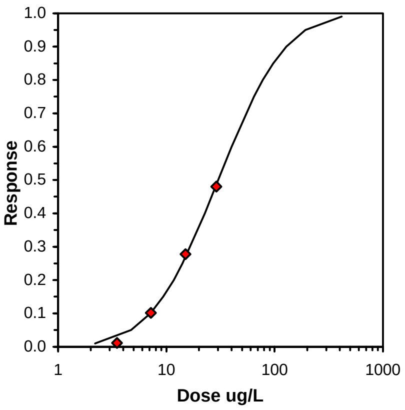
			Transform: Untransformed						1-Tailed			
Conc-ug/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
Control	0.0218	1.0000	0.0218	0.0208	0.0233	6.013	3				0.0218	0.0000
3.5	0.0216	0.9886	0.0216	0.0211	0.0222	2.631	3	0.304	2.470	0.0020	0.0216	0.0114
*7.2	0.0196	0.8982	0.0196	0.0191	0.0199	2.211	3	2.714	2.470	0.0020	0.0196	0.1018
*15	0.0158	0.7224	0.0158	0.0153	0.0163	3.143	3	7.399	2.470	0.0020	0.0158	0.2776
*29	0.0114	0.5198	0.0114	0.0095	0.0123	14.079	3	12.798	2.470	0.0020	0.0114	0.4802

Auxiliary Tests					Statistic		Critical		Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)					0.974617		0.835		-0.35583	0.35037
Bartlett's Test indicates equal variances (p = 0.33)					4.596714		13.2767			
Hypothesis Test (1-tail, 0.05)	**NOEC**	**LOEC**	**ChV**	**TU**	**MSDu**	**MSDp**	**MSB**	**MSE**	**F-Prob**	**df**
Dunnett's Test	3.5	7.2	5.01996		0.002024	0.092673	5.95E-05	1.01E-06	6.4E-07	4, 10

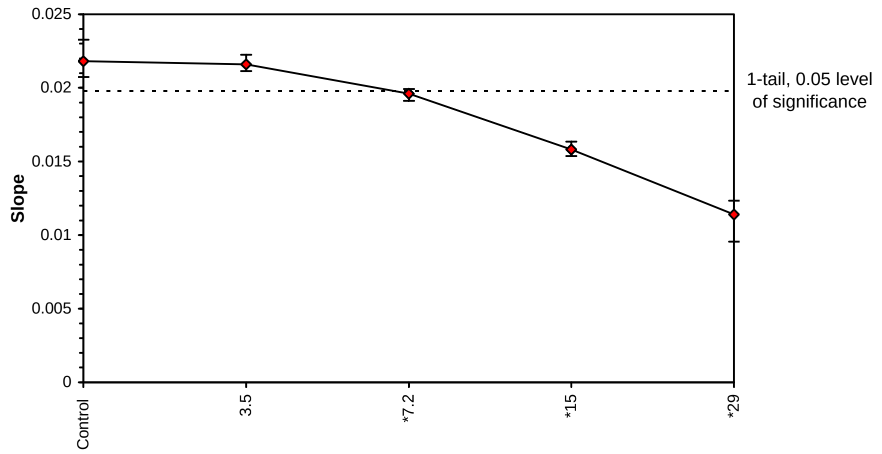
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	2.044053	1.187674	-0.28379	4.371895	0	0.020239	5.991477	0.99	1.479636	0.489224	4
Intercept	1.975545	1.547293	-1.05715	5.008239							
TSCR											

Point	Probits	ug/L	95% Fiducial Limits
EC01	2.674	2.195486	
EC05	3.355	4.730794	
EC10	3.718	7.123119	
EC15	3.964	9.388321	
EC20	4.158	11.69216	
EC25	4.326	14.1143	
EC40	4.747	22.68259	
EC50	5.000	30.17422	
EC60	5.253	40.14021	
EC75	5.674	64.50787	
EC80	5.842	77.87131	
EC85	6.036	96.98047	
EC90	6.282	127.8209	
EC95	6.645	192.4589	
EC99	7.326	414.7073	



Dose-Response Plot



Algal Growth Rate-Slope

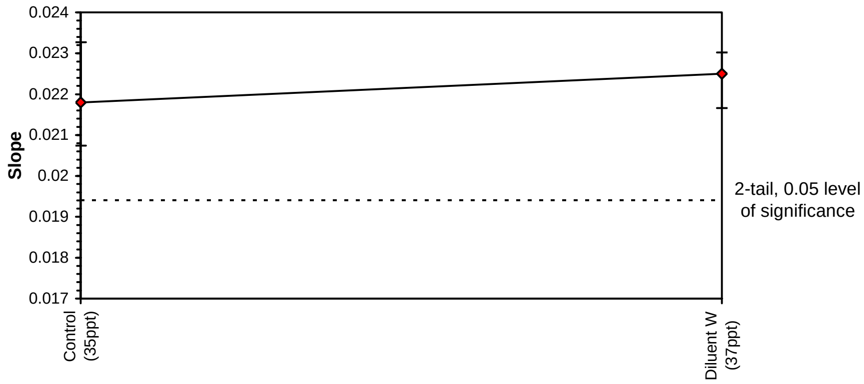
Start Date:	4/07/2006	Test ID:	OD	Sample ID:	WQE06178
End Date:	4/07/2006	Lab ID:	CECR-Centre for Environmental	Sample Type:	Diluent water (37ppt)
Sample Date:		Protocol:	BD-Flow-FACSCalibur flow cyto	Test Species:	NC-Nitzschia closterium
Comments:					

Conc-	1	2	3
Control (35ppt)	0.0208	0.0214	0.0233
luent W (37ppt)	0.0217	0.0228	0.0231

			Transform: Untransformed						2-Tailed	
Conc-	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD
Control (35ppt)	0.0218	1.0000	0.0218	0.0208	0.0233	6.013	3			
luent W (37ppt)	0.0225	1.0316	0.0225	0.0217	0.0231	3.256	3	0.794	2.776	0.0024

Auxiliary Tests	Statistic		Critical		Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.947776		0.713		0.525322	-0.67224
F-Test indicates equal variances (p = 0.48)	3.204553		199.012			
Hypothesis Test (2-tail, 0.05)	**MSDu**	**MSDp**	**MSB**	**MSE**	**F-Prob**	**df**
Homoscedastic t Test indicates no significant differences	0.002411	0.110403	7.13E-07	1.13E-06	0.471828	1, 4

Dose-Response Plot



Algal Growth Rate-Slope

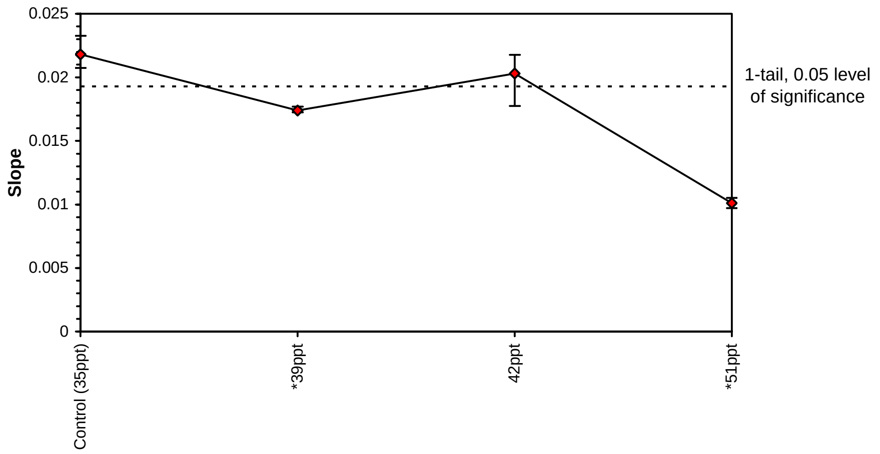
Start Date:	4/07/2006	Test ID:	OD	Sample ID:	Salinity Controls
End Date:	4/07/2006	Lab ID:	CECR-Centre for Environmental	Sample Type:	Seawater + DP2 sea salts
Sample Date:		Protocol:	BD-Flow-FACSCalibur flow cyto	Test Species:	NC-Nitzschia closterium
Comments:					

Conc-	1	2	3
Control (35ppt)	0.0208	0.0214	0.0233
39ppt	0.0172	0.0172	0.0176
42ppt	0.0177	0.0214	0.0218
51ppt	0.0105	0.0101	0.0097

			Transform: Untransformed						1-Tailed	
Conc-	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD
Control (35ppt)	0.0218	1.0000	0.0218	0.0208	0.0233	6.013	3			
*39ppt	0.0174	0.7944	0.0174	0.0172	0.0176	1.468	3	4.197	2.420	0.0026
42ppt	0.0203	0.9288	0.0203	0.0177	0.0218	10.929	3	1.454	2.420	0.0026
*51ppt	0.0101	0.4626	0.0101	0.0097	0.0105	3.982	3	10.972	2.420	0.0026

Auxiliary Tests	Statistic		Critical		Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.925348		0.805		-0.79515	1.458977
Bartlett's Test indicates equal variances (p = 0.06)	7.580256		11.34488			
Hypothesis Test (1-tail, 0.05)	**MSDu**	**MSDp**	**MSB**	**MSE**	**F-Prob**	**df**
Dunnett's Test indicates significant differences	0.002589	0.118535	8.13E-05	1.72E-06	1.9E-05	3, 8

Dose-Response Plot



APPENDIX – Olympic Dam, desalination pilot plant discharge

4/07/2006 **72-h Chronic Toxicity of Olympic Dam pilot Plant waste water to *Nitzschia closterium***

	Sample	pH		Day 0	24h	48h	72h	Slope	Growth Rate (dblngs/day)		Pearson	% Control	Mean %
Flask No.		Day 0	Day 3	*All cell counts in (cells/mL) by x10^4*						Mean			
Samples and Controls													
25	Dilution Water (WQE06178)	8.07	8.53	3.1	11.9	41.1	109.9	0.02169	1.73	1.80	100%	96%	100%
26				3.1	13.6	45.4	137.5	0.02285	1.82		100%	101%	
27				3.1	11.8	52.9	129.5	0.02305	1.84		99%	102%	
						Mean control rate=		***0.02253***					
Brisbane water- Pilot Plant waste water (WQE06179)													
28	0.14%	8.07	8.62	3.1	12.6	39.8	129.1	0.02240	1.78	1.84	100%	99%	102%
29				3.1	11.5	43.0	149.7	0.02350	1.87		100%	104%	
30				3.1	10.2	47.6	134.4	0.02332	1.86		99%	104%	
31	0.4%	8.07	8.52	3.1	10.9	42.7	121.6	0.02246	1.79	1.79	100%	100%	100%
32				3.1	10.4	41.5	121.5	0.02249	1.79		100%	100%	
33				3.1	11.3	38.4	129.9	0.02257	1.80		100%	100%	
34	1.2%	8.06	8.59	3.1	9.8	42.8	140.2	0.02342	1.87	1.83	100%	104%	102%
35				3.1	11.2	42.1	133.8	0.02291	1.83		100%	102%	
36				3.1	9.1	46.3	117.4	0.02275	1.81		99%	101%	
37	3.7%	8.06	8.52	3.1	8.4	36.6	77.9	0.02023	1.61	1.73	99%	90%	97%
38				3.1	11.3	43.3	126.0	0.02262	1.80		100%	100%	
39				3.1	12.1	38.7	129.8	0.02244	1.79		100%	100%	
40	11%	8.07	8.53	3.1	7.7	28.9	95.3	0.02106	1.68	1.74	100%	93%	97%
41				3.1	7.1	35.3	88.9	0.02119	1.69		99%	94%	
42				3.1	7.7	38.3	126.3	0.02309	1.84		99%	102%	
43	33%	8.06	8.28	3.1	4.2	6.7	9.4	0.00695	0.55	0.55	100%	31%	30%
44				3.1	4.7	7.3	8.7	0.00648	0.52		97%	29%	
45				3.1	4.6	7.3	9.9	0.00717	0.57		99%	32%	

Algal Growth Rate-Slope

Start Date:	4/07/2006	Test ID:	OD	Sample ID:	WQE06179
End Date:	4/07/2006	Lab ID:	CECR-Centre for Environmental	Sample Type:	Olympic Dam PPWW
Sample Date:		Protocol:	BD-Flow-FACSCalibur flow cyto	Test Species:	NC-Nitzschia closterium
Comments:	Pilot Plant waste water prepared June06				

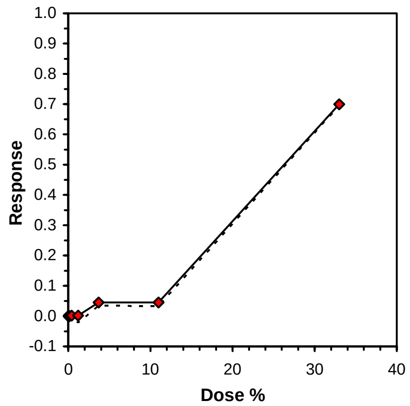
Conc-%	1	2	3
Diluent W	0.0217	0.0228	0.0231
0.14	0.0224	0.0235	0.0233
0.4	0.0225	0.0225	0.0226
1.2	0.0234	0.0229	0.0228
3.7	0.0202	0.0226	0.0224
11	0.0211	0.0212	0.0231
33	0.0070	0.0065	0.0072

			Transform: Untransformed						1-Tailed		Isotonic	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
Diluent W	0.0225	1.0000	0.0225	0.0217	0.0231	3.256	3				0.0228	1.0000
0.14	0.0231	1.0242	0.0231	0.0224	0.0235	2.559	3	-0.861	2.530	0.0016	0.0228	1.0000
0.4	0.0225	0.9991	0.0225	0.0225	0.0226	0.241	3	0.033	2.530	0.0016	0.0228	0.9985
1.2	0.0230	1.0222	0.0230	0.0228	0.0234	1.524	3	-0.789	2.530	0.0016	0.0228	0.9985
3.7	0.0218	0.9661	0.0218	0.0202	0.0226	6.115	3	1.209	2.530	0.0016	0.0218	0.9548
11	0.0218	0.9667	0.0218	0.0211	0.0231	5.212	3	1.186	2.530	0.0016	0.0218	0.9548
*33	0.0069	0.3048	0.0069	0.0065	0.0072	5.185	3	24.767	2.530	0.0016	0.0069	0.3011

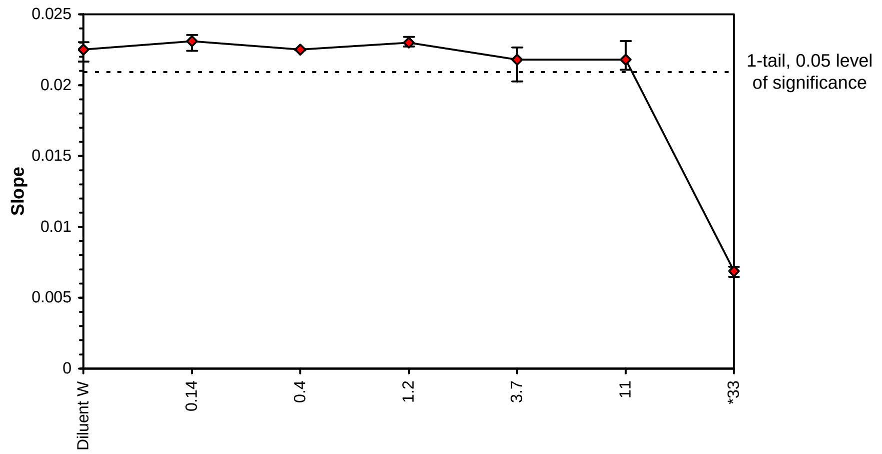
Auxiliary Tests					Statistic		Critical		Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)					0.984758		0.873		-0.33021	0.505271
Bartlett's Test indicates equal variances (p = 0.06)					12.19023		16.81187			
Hypothesis Test (1-tail, 0.05)	**NOEC**	**LOEC**	**ChV**	**TU**	**MSDu**	**MSDp**	**MSB**	**MSE**	**F-Prob**	**df**
Dunnett's Test	11	33	19.05256	9.090909	0.0016	0.071018	0.000105	6E-07	2.4E-12	6, 14

Linear Interpolation (200 Resamples)

Point	%	SD	95% CL(Exp)		Skew
IC05	11.163	3.781	0.000	13.502	-0.1910
IC10	12.846	0.649	10.082	15.064	0.2413
IC15	14.529	0.598	11.965	16.577	0.2526
IC20	16.211	0.548	13.784	18.107	0.2640
IC25	17.894	0.499	15.650	19.618	0.2744
IC40	22.942	0.365	21.277	24.321	0.2706
IC50	26.308	0.294	25.020	27.412	0.1665



Dose-Response Plot



APPENDIX – Olympic Dam, desalination pilot plant discharge; Data corrected for effects due to high salinity

4/07/2006 **72-h Chronic Toxicity of Olympic Dam pilot plant waste water to *Nitzschia closterium***

	Sample	pH		Day 0	24h	48h	72h	Slope	Growth Rate (dblngs/day)		Pearson	% Control	Mean %
Flask No.		Day 0	Day 3	*All cell counts in (cells/mL) by x10^4*						Mean			
Samples and Controls													
25				3.1	11.9	41.1	109.9	0.02169	1.73	1.80	100%	96%	100%
26	Dilution Water	8.07	8.53	3.1	13.6	45.4	137.5	0.02285	1.82		100%	101%	
27	(WQE06178)			3.1	11.8	52.9	129.5	0.02305	1.84		99%	102%	
							Mean control rate=	*0.02253*					
Pilot Plant waste water (WQE06179)													
28				3.1	12.6	39.8	129.1	0.02240	1.78	1.84	100%	99%	102%
29	0.14%	8.07	8.62	3.1	11.5	43.0	149.7	0.02350	1.87		100%	104%	
30				3.1	10.2	47.6	134.4	0.02332	1.86		99%	104%	
31				3.1	10.9	42.7	121.6	0.02246	1.79	1.79	100%	100%	100%
32	0.4%	8.07	8.52	3.1	10.4	41.5	121.5	0.02249	1.79		100%	100%	
33				3.1	11.3	38.4	129.9	0.02257	1.80		100%	100%	
34				3.1	9.8	42.8	140.2	0.02342	1.87	1.83	100%	104%	102%
35	1.2%	8.06	8.59	3.1	11.2	42.1	133.8	0.02291	1.83		100%	102%	
36				3.1	9.1	46.3	117.4	0.02275	1.81		99%	101%	
37				3.1	8.4	36.6	77.9	0.02023	1.61	1.73	99%	117%	125%
38	3.7%*	8.06	8.52	3.1	11.3	43.3	126.0	0.02262	1.80		100%	130%	
39	(39‰)			3.1	12.1	38.7	129.8	0.02244	1.79		100%	129%	
40				3.1	7.7	28.9	95.3	0.02106	1.68	1.74	100%	104%	107%
41	11%*	8.07	8.53	3.1	7.1	35.3	88.9	0.02119	1.69		99%	104%	
42	(42‰)			3.1	7.7	38.3	126.3	0.02309	1.84		99%	114%	
43				3.1	4.2	6.7	9.4	0.00695	0.55	0.55	100%	69%	68%
44	33%*	8.06	8.28	3.1	4.7	7.3	8.7	0.00648	0.52		97%	64%	
45	(51‰)			3.1	4.6	7.3	9.9	0.00717	0.57		99%	71%	
Salinity Controls													
16				3.1	10.4	28.5	52.0	0.01720	1.37	1.38	98%	76%	77%
17	39‰	8.06	8.44	3.1	11.1	31.6	51.4	0.01721	1.37		96%	76%	
18				3.1	10.9	33.3	54.4	0.01764	1.41		97%	78%	
							Mean 39‰ control rate=	*0.01735*					
19				3.1	9.4	32.2	53.3	0.01774	1.41	1.62	97%	79%	90%
20	42‰	8.05	8.59	3.1	9.9	41.0	97.4	0.02136	1.70		99%	95%	
21				3.1	9.9	41.4	104.5	0.02176	1.73		99%	97%	
							Mean 42‰ control rate=	*0.02028*					
22				3.1	5.2	12.2	16.0	0.01052	0.84	0.80	97%	47%	45%
23	51‰	8.02	8.42	3.1	6.0	11.1	15.9	0.01008	0.80		98%	45%	
24				3.1	5.4	12.4	13.9	0.00971	0.77		93%	43%	
							Mean 51‰ control rate=	*0.01010*					

* % Control data expressed as a percentage of appropriate salinity-matched control

Algal Growth Rate-% of Control

Start Date:	4/07/2006	Test ID:	OD	Sample ID:	WQE06179
End Date:	4/07/2006	Lab ID:	CECR-Centre for Environmental	Sample Type:	Olympic Dam PPWW
Sample Date:		Protocol:	BD-Flow-FACSCalibur flow cyto	Test Species:	NC-Nitzschia closterium

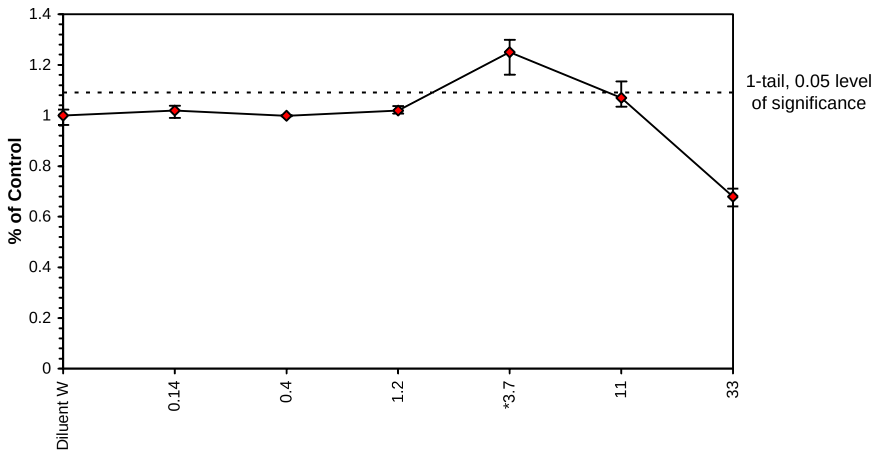
Comments: Pilot Plant waste water prepared June06, data corrected for effeects due to high salinity

Conc-%	1	2	3
Diluent W	0.9628	1.0141	1.0232
0.14	0.9943	1.0431	1.0351
0.4	0.9970	0.9984	1.0017
1.2	1.0397	1.0169	1.0099
3.7	1.1660	1.3036	1.2936
11	1.0383	1.0445	1.1382
33	0.6879	0.6410	0.7100

			Transform: Untransformed						1-Tailed	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD
Diluent W	1.0000		1.0000	0.9628	1.0232	3.256	3			
0.14	1.0242		1.0242	0.9943	1.0431	2.559	3	0.707	2.530	0.0864
0.4	0.9991		0.9991	0.9970	1.0017	0.241	3	-0.027	2.530	0.0864
1.2	1.0222		1.0222	1.0099	1.0397	1.524	3	0.649	2.530	0.0864
*3.7	1.2544		1.2544	1.1660	1.3036	6.115	3	7.448	2.530	0.0864
11	1.0737		1.0737	1.0383	1.1382	5.212	3	2.156	2.530	0.0864
33	0.6797		0.6797	0.6410	0.7100	5.185	3	-9.378	2.530	0.0864

Auxiliary Tests					Statistic		Critical		Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)					0.971166		0.873		-0.50592	0.776482
Bartlett's Test indicates equal variances (p = 0.04)					12.88323		16.81187			
Hypothesis Test (1-tail, 0.05)	**NOEC**	**LOEC**	**ChV**	**TU**	**MSDu**	**MSDp**	**MSB**	**MSE**	**F-Prob**	**df**
Dunnett's Test	33	>33		3.030303	0.086425	0	0.08672	0.00175	1.2E-08	6, 14

Dose-Response Plot



Algal Growth Rate-% of Control

Start Date:	4/07/2006	Test ID:	OD	Sample ID:	WQE06179
End Date:	4/07/2006	Lab ID:	CECR-Centre for Environmental	Sample Type:	Olympic Dam PPWW
Sample Date:		Protocol:	BD-Flow-FACSCalibur flow cyto	Test Species:	NC-Nitzschia closterium
Comments:	Pilot Plant waste water prepared June06, data corrected for effects due to high salinity				

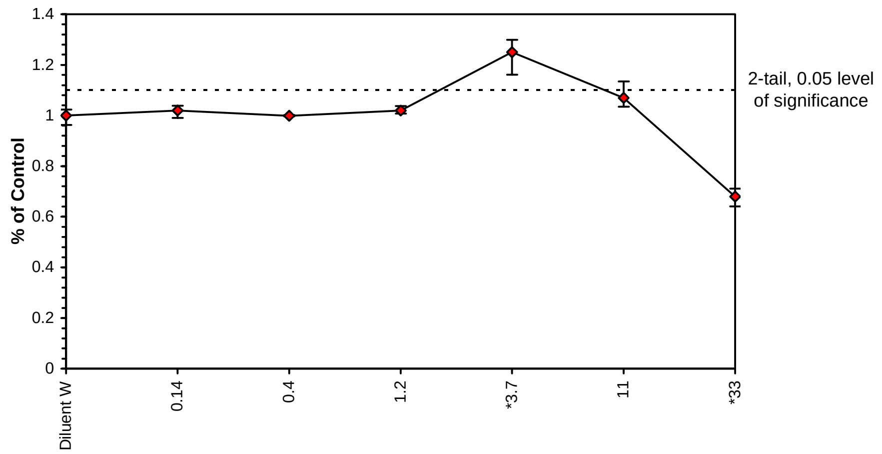
Conc-%	1	2	3
Diluent W	0.9628	1.0141	1.0232
0.14	0.9943	1.0431	1.0351
0.4	0.9970	0.9984	1.0017
1.2	1.0397	1.0169	1.0099
3.7	1.1660	1.3036	1.2936
11	1.0383	1.0445	1.1382
33	0.6879	0.6410	0.7100

			Transform: Untransformed						2-Tailed	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD
Diluent W	1.0000		1.0000	0.9628	1.0232	3.256	3			
0.14	1.0242		1.0242	0.9943	1.0431	2.559	3	0.707	3.020	0.1032
0.4	0.9991		0.9991	0.9970	1.0017	0.241	3	0.027	3.020	0.1032
1.2	1.0222		1.0222	1.0099	1.0397	1.524	3	0.649	3.020	0.1032
*3.7	1.2544		1.2544	1.1660	1.3036	6.115	3	7.448	3.020	0.1032
11	1.0737		1.0737	1.0383	1.1382	5.212	3	2.156	3.020	0.1032
*33	0.6797		0.6797	0.6410	0.7100	5.185	3	9.378	3.020	0.1032

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.971166	0.873	-0.50592	0.776482
Bartlett's Test indicates equal variances (p = 0.04)	12.88323	16.81187		

Hypothesis Test (2-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	11	33	19.05256	9.090909	0.103163	0	0.08672	0.00175	1.2E-08	6, 14

Dose-Response Plot



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Toxicity Assessment of Desalination Plant Waste Water

Hydrobiology for ARUP / HLA

Test Report

August 2006

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Toxicity Assessment of Desalination Plant Waste Water

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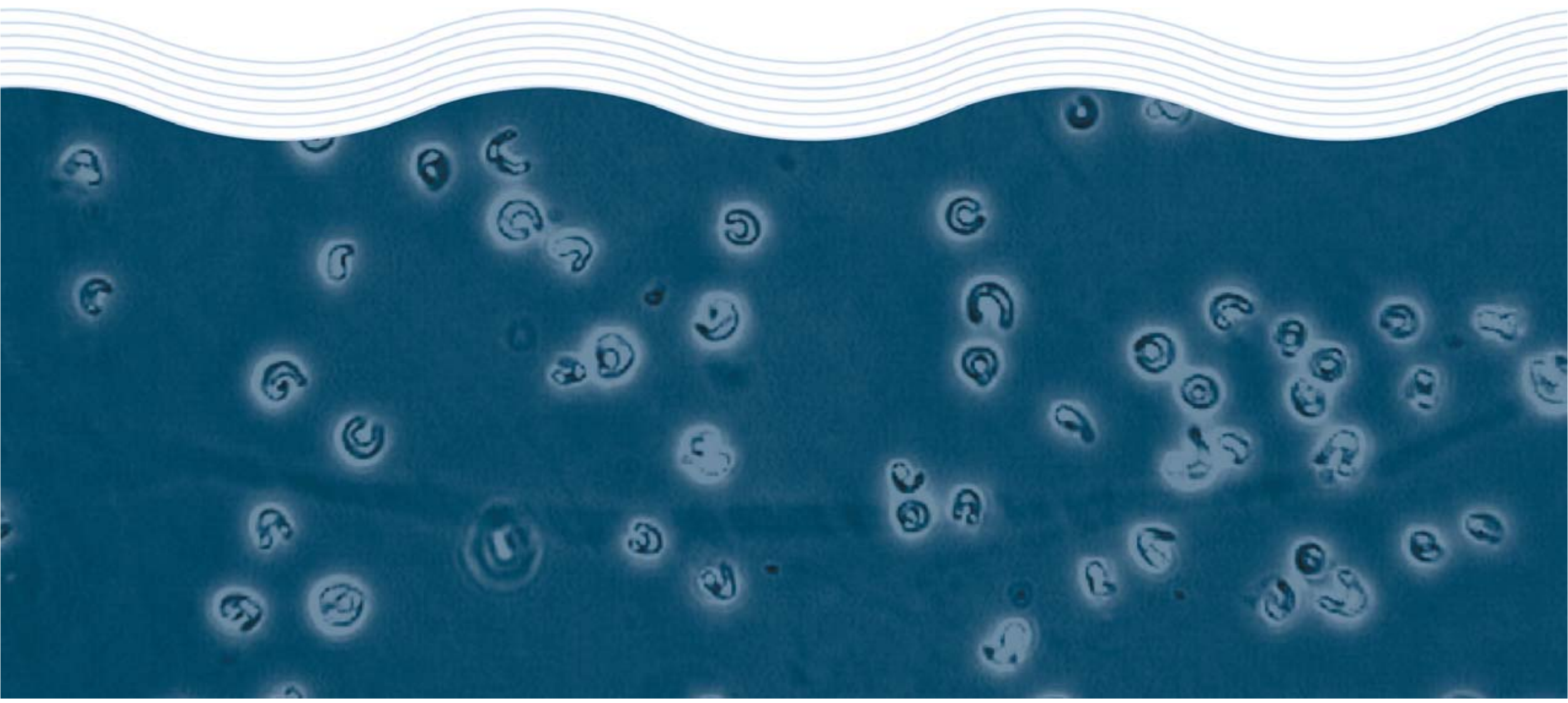

Toxicity Test Report: TR0226/1

Client:	Hydrobiology PO Box 2050 Milton QLD 4064	**ESA Job #:** **Date Sampled:** **Date Received:**	PR0226 Not supplied 30 June 2006
Attention:	Dustin Hobbs	**Sampled By:**	Not supplied
Contract #:		**Quote #:**	PL0226_q01

Lab ID No.:	**Sample Name:**	**Sample Description:**
1715	Olympic Dam	Desalination plant effluent, conductivity 109.8 mS/cm, pH 8.0

Test Performed:	72-hr Sea urchin larval development test using *Heliocidaris tuberculata*
Test Protocol:	ESA SOP 105, based on APHA (1998) and Simon and Laginestra (1996)
Deviations from Protocol:	Nil
Source of Test Organisms:	Field collected from South Maroubra NSW on 13 July 2006
Test Initiated:	13 July 2006 at 1500 h

Salinity Controls		Sample 1715: *Olympic Dam*		*Vacant*
Treatment	**% Normal Larvae (Mean ± SD)**	**Concentration (%) (salinity in brackets)**	**% Normal Larvae (Mean ± SD)**	
FSW Control	95.3 ± 1.9	FSW Control (36.3‰)	95.3 ± 1.9	
40.5‰	92.8 ± 3.1	2.1 (37.5‰)	92.5 ± 2.4	
44.4‰	0.0 ± 0.0	4.1 (38.5‰)	93.3 ± 1.3	
51.8‰	0.0 ± 0.0	8.3 (40.5‰)	91.5 ± 2.7	
		16.5 (44.4‰)	0.0 ± 0.0	
		33 (51.8‰)	0.0 ± 0.0	
		72 hr EC50 = 11.5 (11.3-11.7)% (TSK Trim value = 2.5%) NOEC = 4.1% LOEC = 8.3%		

* Significantly lower % normally developed larvae compared with the FSW Control treatment (Dunnett's Test, 1 tailed, P=0.05, df=3,12).

QA/QC Parameter	**Criterion**	**This Test**	**Criterion met?**
Control minimum % normal larvae	>70%	95.3%	Yes
Test Temperature limits	20.0 ± 1°C	20.0°C	Yes
Reference Toxicant within cusum chart limits	6.8-10.8 µg/L	8.8 µg/L	Yes

Test Report Authorised by: Dr Rick Krassoi, Director on 18 August 2006

Results are based on the samples in the condition as received by ESA

NATA Accredited Laboratory Number: 14709

The tests, calibrations or methods covered by this document have been performed in accordance with NATA requirements which include the requirements of ISO/IEC 17025 and are traceable to Australian national standards of measurement. This document shall not be reproduced except in full.

Toxicity Test Report: TR0226/2

Client:	Hydrobiology PO Box 2050 Milton QLD 4064	**ESA Job #:** **Date Sampled:** **Date Received:**	PR0226 Not supplied 30 June 2006
Attention:	Dustin Hobbs	**Sampled By:**	Not supplied
Contract #:		**Quote #:**	PL0226_q01

Lab ID No.:	**Sample Name:**	**Sample Description:**
1715	Olympic Dam	Desalination plant effluent, conductivity 109.8 mS/cm, pH 8.0

Test Performed:	72-hr Macroalgal germination test using *Hormosira banksii*
Test Protocol:	ESA SOP 116, based on Gunthorpe *et al*. (1997) and Kevekordes and Clayton (1996)
Deviations from Protocol:	Nil
Source of Test Organisms:	Field collected from Bilgola Beach NSW on 27 July 2006
Test Initiated:	28 July 2006 at 1200 h

Salinity Controls		Sample 1715: *Olympic Dam*		*Vacant*
Treatment	**% Germinated (Mean ± SD)**	**Concentration (%) (salinity in brackets)**	**% Germinated (Mean ± SD)**	
FSW Control	92.8 ± 2.5	FSW Control (36.8‰)	92.8 ± 2.5	
40‰	93.5 ± 2.7	2.1 (37.7‰)	92.5 ± 3.1	
44‰	91.8 ± 2.9	4.4 (38.5‰)	93.0 ± 2.2	
51‰	85.0 ± 3.6*	8.3 (40.2‰)	94.0 ± 4.1	
		16.5 (43.7 ‰)	94.8 ± 1.7	
		33 (50.8 ‰)	77.8 ± 6.9**	
		72 hr EC50 = >33% **NOEC = 16.5%** **LOEC = 33%**		

* Significantly lower % germination compared with the FSW Control treatment (Dunnett's Test, 1 tailed, $P<0.05$, df=3,12).
** Significantly lower % germination compared with the FSW Control treatment (Dunnett's Test, 1 tailed, $P<0.05$, df=5,18).

QA/QC Parameter	**Criterion**	**This Test**	**Criterion met?**
Control minimum % germinated	>70 %	92.8%	Yes
Test Temperature limits	18.0 ± 1 ºC	18.5ºC	Yes
Reference Toxicant within cusum chart limits	48.6-173.6µg/L	165.1 µg/L	Yes

Test Report Authorised by: Dr Rick Krassoi, Director on 18 August 2006

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Toxicity Test Report: TR0226/3

Client:	Hydrobiology PO Box 2050 Milton QLD 4064	**ESA Job #:** **Date Sampled:** **Date Received:**	PR0226 Not supplied 30 June 2006
Attention:	Dustin Hobbs	**Sampled By:**	Not supplied
Order #:		**Quote #:**	PL0226_q01

Lab ID No.:	**Sample Name:**	**Sample Description:**
1715	Olympic Dam	Desalination plant effluent, conductivity 109.8 mS/cm, pH 8.0

Test Performed:	48-hour larval development test using the rock oyster *Saccostrea commercialis*
Test Protocol:	ESA SOP 106, based on APHA (1998)
Deviations from Protocol:	Nil
Source of Test Organisms:	Farm reared, Merrimbula NSW
Test Initiated:	13 July 2006 at 1830 h

Salinity Controls		Sample 1715: *Olympic Dam*		*Vacant*
Treatment	**% Alive Normal (Mean ± SD)**	**Concentration (%) (salinity in brackets)**	**% Alive Normal (Mean ± SD)**	
FSW Control	73.9 ± 3.0	FSW Control (36.3‰)	73.9 ± 3.0	
40.5‰	76.3 ± 5.6	2.1 (37.5‰)	72.8 ± 6.1	
44.4‰	0.0 ± 0.0	4.1 (38.5‰)	76.3 ± 4.9	
51.8‰	0.0 ± 0.0	8.3 (40.5‰)	72.8 ± 6.9	
		16.5 (44.4‰)	0.0 ± 0.0	
		33 (51.8‰)	0.0 ± 0.0	
		72 hr EC50 = 11.5 (11.4-11.7)% (TSK Trim value = 0.0%) NOEC = 8.3% LOEC = 16.5%		

QA/QC Parameter	**Criterion**	**This Test**	**Criterion met?**
Control minimum % normal surviving larvae	>70 %	73.9%	Yes
Test Temperature limits	23.0 ± 1 ºC	23.0ºC	Yes
Reference Toxicant within cusum chart limits	16.7 – 22.9 µg/L	21.0 µg/L	Yes

Test Report Authorised by: Dr Rick Krassoi, Director on 18 August 2006

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Toxicity Test Report: TR0226/4 (page 1 of 1)

Client:	Hydrobiology PO Box 2050 Milton QLD 4064	**ESA Job #:** **Date Sampled:** **Date Received:**	PR0226 Not supplied 30 June 2006
Attention:	Dustin Hobbs	**Sampled By:**	Not supplied
Contract #:		**Quote #:**	PL0226_q01

Lab ID No.:	**Sample Name:**	**Sample Description:**
1715	Olympic Dam	Desalination plant effluent, conductivity 109.8 mS/cm, pH 8.0

Test Performed:	96-hr acute (survival) toxicity test using the tiger prawn *Penaeus monodon*
Test Protocol:	ESA SOP 107, based on USEPA (1994, 1996)
Deviations from Protocol:	Nil
Source of Test Organisms:	Hatchery-reared, Cairns Qld
Test Initiated:	14 July 2006 at 0900 h

Salinity Controls		Sample 1715: *Olympic Dam*		*Vacant*
Treatment	**% Survival (Mean ± SD)**	**Concentration (%) (salinity in brackets)**	**% Survival (Mean ± SD)**	
FSW Control	90.0 ± 20.0	FSW Control (36.3‰)	90.0 ± 20.0	
40.5‰	100 ± 0.0	2.1 (37.5‰)	85.0 ± 19.2	
44.4‰	55.0 ± 10.0	4.1 (38.2‰)	85.0 ± 10.0	
51.8‰	0.0 ± 0.0	8.3 (40.2‰)	95.0 ± 10.0	
		16.5 (43.9‰)	65.0 ± 10.0	
		33 (51.7‰)	20.0 ± 16.3*	
		96 hr LC50 = 22.4 (15.8-31.6)% (TSK Trim value = 22.2%) NOEC = 16.5% LOEC = 33%		

* Significantly reduced survival compared with the FSW Control treatment (Dunnett's Test, 1 tailed, P<0.05, df=5,18).

QA/QC Parameter	**Criterion**	**This Test**	**Criterion met?**
Control % survival	≥90%	90%	Yes
Test Temperature limits	25.0 ± 1°C	25.0°C	Yes
Reference Toxicant within cusum chart limits	7.4-20.1mg/L	13.1 mg/L	Yes

Test Report Authorised by: Dr Rick Krassoi, Director on 18 August 2006

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Statistical Printouts for the Rock Oyster Larval Development Tests

Bivalve Larval Survival and Development Test-Proportion Alive/Normal

Start Date:	13/07/06 18:30	Test ID:	PR0226/08	Sample ID:	Olympic Dam`
End Date:	15/07/06 18:30	Lab ID:	1715	Sample Type:	Desalination Plant Effluent
Sample Date:		Protocol:	ESASOP106	Test Species:	SR-Saccostrea commercialis
Comments:					

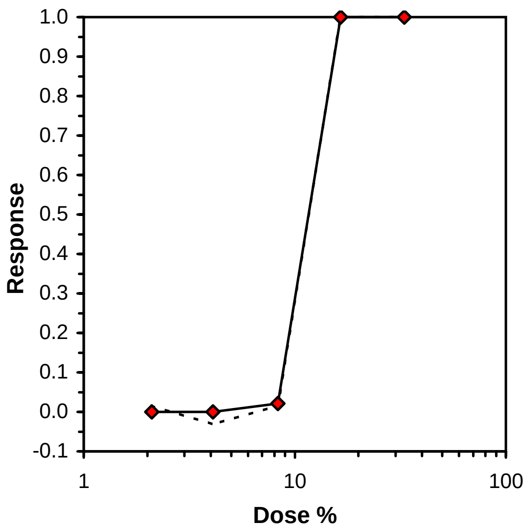
Conc-%	1	2	3	4
FSW Control	0.7746	0.7277	0.7042	0.7512
2.1	0.6573	0.7981	0.7512	0.7042
4.1	0.7512	0.7746	0.7042	0.8216
8.3	0.7042	0.7277	0.8216	0.6573
16.5	0.0000	0.0000	0.0000	0.0000
33	0.0000	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root						1-Tailed		Number
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp
FSW Control	0.7394	1.0000	1.0356	0.9958	1.0762	3.340	4				44
2.1	0.7277	0.9841	1.0236	0.9454	1.1048	6.699	4	0.270	2.290	0.1013	46
4.1	0.7629	1.0317	1.0638	0.9958	1.1347	5.439	4	-0.638	2.290	0.1013	40
8.3	0.7277	0.9841	1.0244	0.9454	1.1347	7.818	4	0.252	2.290	0.1013	46
16.5	0.0000	0.0000	0.0767	0.0767	0.0767	0.000	4				170
33	0.0000	0.0000	0.0767	0.0767	0.0767	0.000	4				170

Auxiliary Tests					Statistic		Critical		Skew
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)					0.961195		0.844		0.399127
Bartlett's Test indicates equal variances (p = 0.63)					1.75237		11.34487		
Hypothesis Test (1-tail, 0.05)	**NOEC**	**LOEC**	**ChV**	**TU**	**MSDu**	**MSDp**	**MSB**	**MSE**	**F-Prob**
Dunnett's Test	8.3	16.5	11.70256	12.04819	0.093199	0.125967	0.00141	0.003915	0.782957

Trimmed Spearman-Karber

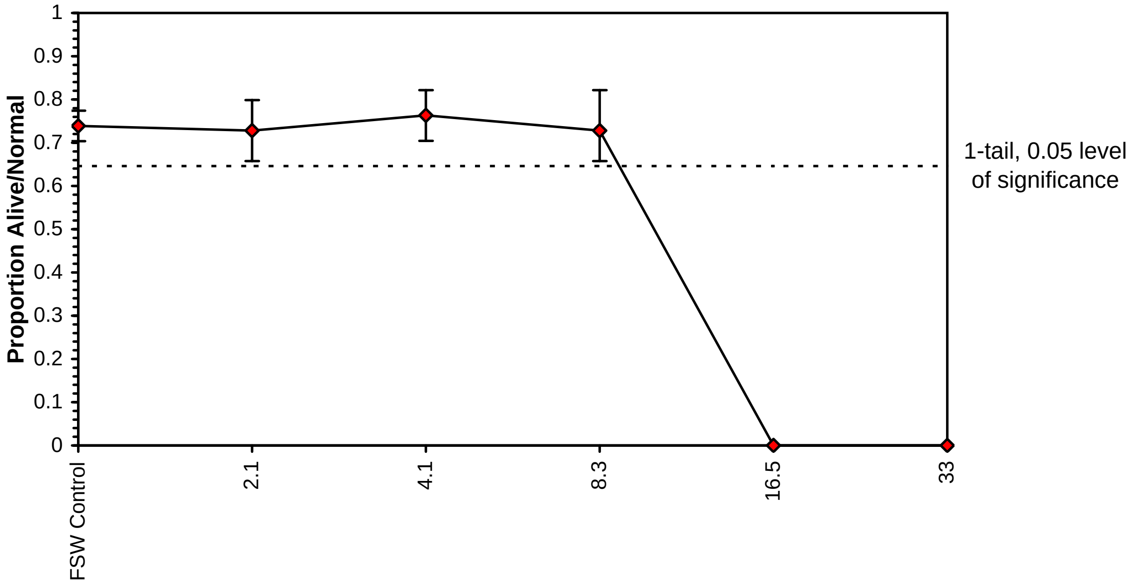
Trim Level	EC50	95% CL	
0.0%	11.532	11.357	11.710
5.0%	11.616	11.525	11.708
10.0%	11.616	11.525	11.708
20.0%	11.616	11.525	11.708
Auto-0.0%	11.532	11.357	11.710



Bivalve Larval Survival and Development Test-Proportion Alive/Normal

Start Date:	13/07/06 18:30	Test ID:	PR0226/08	Sample ID:	Olympic Dam`
End Date:	15/07/06 18:30	Lab ID:	1715	Sample Type:	Desalination Plant Effluent
Sample Date:		Protocol:	ESASOP106	Test Species:	SR-Saccostrea commercialis
Comments:					

Dose-Response Plot



Bivalve Larval Survival and Development Test-Proportion Alive/Normal

Start Date:	13/07/06 18:30	Test ID:	PR0226/08	Sample ID:	Olympic Dam`
End Date:	15/07/06 18:30	Lab ID:	1715	Sample Type:	Desalination Plant Effluent
Sample Date:		Protocol:	ESASOP106	Test Species:	SR-Saccostrea commercialis
Comments:					

		Auxiliary Data Summary					
Conc-%	**Parameter**	**Mean**	**Min**	**Max**	**SD**	**CV%**	**N**
FSW Control	%Alive Normal	73.94	70.42	77.46	3.03	2.35	4
2.1		72.77	65.73	79.81	6.06	3.38	4
4.1		76.29	70.42	82.16	4.89	2.90	4
8.3		72.77	65.73	82.16	6.91	3.61	4
16.5		0.00	0.00	0.00	0.00		4
33		0.00	0.00	0.00	0.00		4
FSW Control	Temp C	23.00	23.00	23.00	0.00	0.00	1
2.1		23.00	23.00	23.00	0.00	0.00	1
4.1		23.00	23.00	23.00	0.00	0.00	1
8.3		23.00	23.00	23.00	0.00	0.00	1
16.5		23.00	23.00	23.00	0.00	0.00	1
33		23.00	23.00	23.00	0.00	0.00	1
FSW Control	pH	8.10	8.10	8.10	0.00	0.00	1
2.1		8.10	8.10	8.10	0.00	0.00	1
4.1		8.10	8.10	8.10	0.00	0.00	1
8.3		8.10	8.10	8.10	0.00	0.00	1
16.5		8.10	8.10	8.10	0.00	0.00	1
33		8.10	8.10	8.10	0.00	0.00	1
FSW Control	Salinity ppt	36.30	36.30	36.30	0.00	0.00	1
2.1		37.50	37.50	37.50	0.00	0.00	1
4.1		38.50	38.50	38.50	0.00	0.00	1
8.3		40.50	40.50	40.50	0.00	0.00	1
16.5		44.40	44.40	44.40	0.00	0.00	1
33		51.80	51.80	51.80	0.00	0.00	1
FSW Control	DO (%sat)	107.30	107.30	107.30	0.00	0.00	1
2.1		106.20	106.20	106.20	0.00	0.00	1
4.1		104.70	104.70	104.70	0.00	0.00	1
8.3		103.20	103.20	103.20	0.00	0.00	1
16.5		102.80	102.80	102.80	0.00	0.00	1
33		102.80	102.80	102.80	0.00	0.00	1

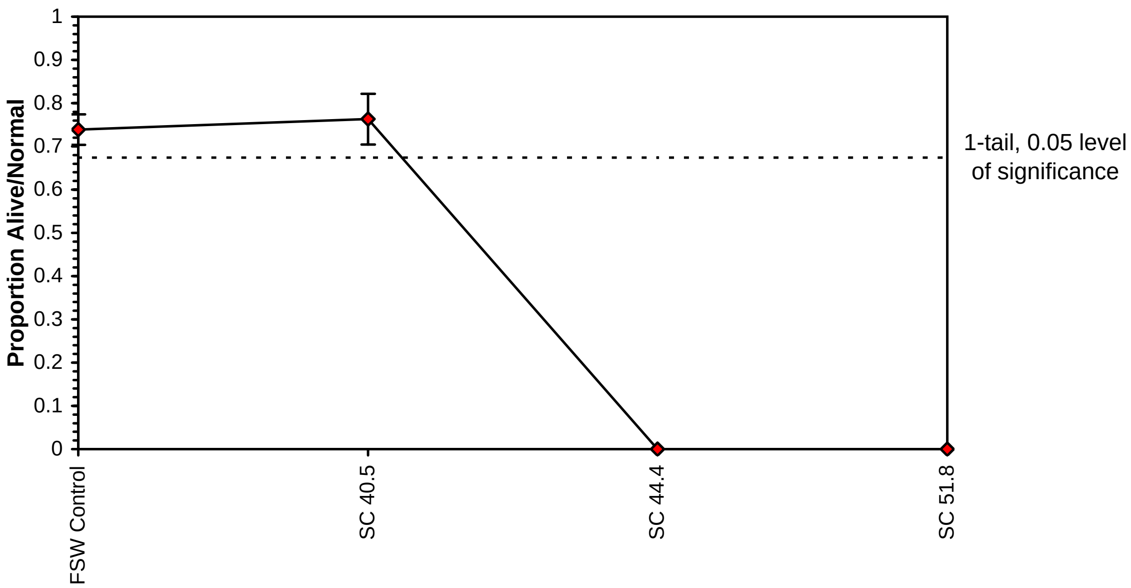
Bivalve Larval Survival and Development Test-Proportion Alive/Normal			
Start Date: 13/07/06 18:30	Test ID: PR0226/07	Sample ID:	Salinity Controls
End Date: 15/07/06 18:30	Lab ID: 1715	Sample Type:	Salinity Controls
Sample Date:	Protocol: ESASOP106	Test Species:	SR-Saccostrea commercialis
Comments:			

Conc-ppt	1	2	3	4
FSW Control	0.7746	0.7277	0.7042	0.7512
SC 40.5	0.7981	0.7277	0.7042	0.8216
SC 44.4	0.0000	0.0000	0.0000	0.0000
SC 51.8	0.0000	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root						1-Tailed	
Conc-ppt	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD
FSW Control	0.7394	1.0000	1.0356	0.9958	1.0762	3.340	4			
SC 40.5	0.7629	1.0317	1.0643	0.9958	1.1347	6.209	4	-0.770	1.943	0.0725
SC 44.4	0.0000	0.0000	0.0767	0.0767	0.0767	0.000	4			
SC 51.8	0.0000	0.0000	0.0767	0.0767	0.0767	0.000	4			

Auxiliary Tests	Statistic		Critical		Skew
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.949272		0.749		0.035188
F-Test indicates equal variances (p = 0.32)	3.650608		47.46723		
Hypothesis Test (1-tail, 0.05)	**MSDu**	**MSDp**	**MSB**	**MSE**	**F-Prob**
Homoscedastic t Test indicates no significant differences	0.065879	0.089042	0.001648	0.002782	0.470693

Dose-Response Plot



Bivalve Larval Survival and Development Test-Proportion Alive/Normal

Start Date:	13/07/06 18:30	Test ID:	PR0226/07	Sample ID:	Salinity Controls
End Date:	15/07/06 18:30	Lab ID:	1715	Sample Type:	Salinity Controls
Sample Date:		Protocol:	ESASOP106	Test Species:	SR-Saccostrea commercialis
Comments:					

		Auxiliary Data Summary					
Conc-ppt	**Parameter**	**Mean**	**Min**	**Max**	**SD**	**CV%**	**N**
FSW Control	%Alive Normal	73.94	70.42	77.46	3.03	2.35	4
SC 40.5		76.29	70.42	82.16	5.59	3.10	4
SC 44.4		0.00	0.00	0.00	0.00		4
SC 51.8		0.00	0.00	0.00	0.00		4
FSW Control	Temp C	23.00	23.00	23.00	0.00	0.00	1
SC 40.5		23.00	23.00	23.00	0.00	0.00	1
SC 44.4		23.00	23.00	23.00	0.00	0.00	1
SC 51.8		23.00	23.00	23.00	0.00	0.00	1
FSW Control	pH	8.10	8.10	8.10	0.00	0.00	1
SC 40.5		8.10	8.10	8.10	0.00	0.00	1
SC 44.4		8.10	8.10	8.10	0.00	0.00	1
SC 51.8		8.10	8.10	8.10	0.00	0.00	1
FSW Control	Salinity ppt	36.30	36.30	36.30	0.00	0.00	1
SC 40.5		40.50	40.50	40.50	0.00	0.00	1
SC 44.4		44.40	44.40	44.40	0.00	0.00	1
SC 51.8		51.80	51.80	51.80	0.00	0.00	1
FSW Control	DO (%sat)	107.30	107.30	107.30	0.00	0.00	1
SC 40.5		109.70	109.70	109.70	0.00	0.00	1
SC 44.4		107.20	107.20	107.20	0.00	0.00	1
SC 51.8		108.40	108.40	108.40	0.00	0.00	1

Statistical Printouts for the Sea Urchin Larval Development Test

Sea Urchin Larval Development Test-Proportion Normal

Start Date:	13/07/06 15:00	Test ID:	PR0226/02	Sample ID:	Olympic Dam
End Date:	16/07/06 15:00	Lab ID:	1715	Sample Type:	Desalination Plant
Sample Date:		Protocol:	ESASOP105	Test Species:	HT-Heliocidaris tuberculata
Comments:					

Conc-%	1	2	3	4
FSW Control	0.9400	0.9800	0.9500	0.9400
2.1	0.9400	0.9000	0.9500	0.9100
4.1	0.9300	0.9300	0.9200	0.9500
8.3	0.9000	0.8900	0.9200	0.9500
16.5	0.0000	0.0000	0.0000	0.0000
33	0.0000	0.0000	0.0000	0.0000

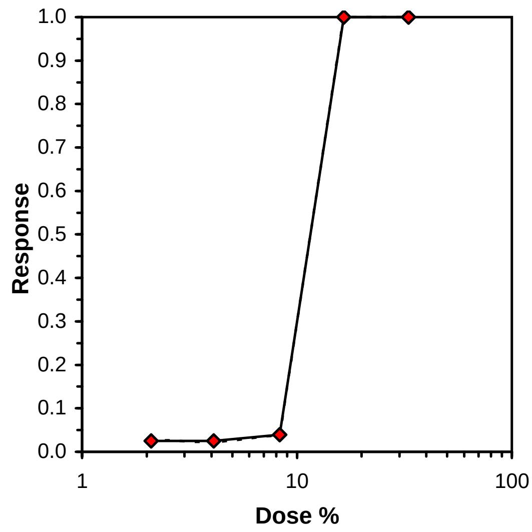
			Transform: Arcsin Square Root						1-Tailed		Number
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp
FSW Control	0.9525	1.0000	1.3552	1.3233	1.4289	3.705	4				19
2.1	0.9250	0.9711	1.2959	1.2490	1.3453	3.529	4	1.902	2.290	0.0713	30
4.1	0.9325	0.9790	1.3088	1.2840	1.3453	1.978	4	1.488	2.290	0.0713	27
*8.3	0.9150	0.9606	1.2778	1.2327	1.3453	3.900	4	2.485	2.290	0.0713	34
16.5	0.0000	0.0000	0.0500	0.0500	0.0500	0.000	4				400
33	0.0000	0.0000	0.0500	0.0500	0.0500	0.000	4				400

Auxiliary Tests	Statistic	Critical	Skew
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.899043	0.844	0.710577
Bartlett's Test indicates equal variances (p = 0.74)	1.269327	11.34487	

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob
Dunnett's Test	4.1	8.3	5.833524	24.39024	0.034334	0.035981	0.004374	0.001941	0.134603

Trimmed Spearman-Karber

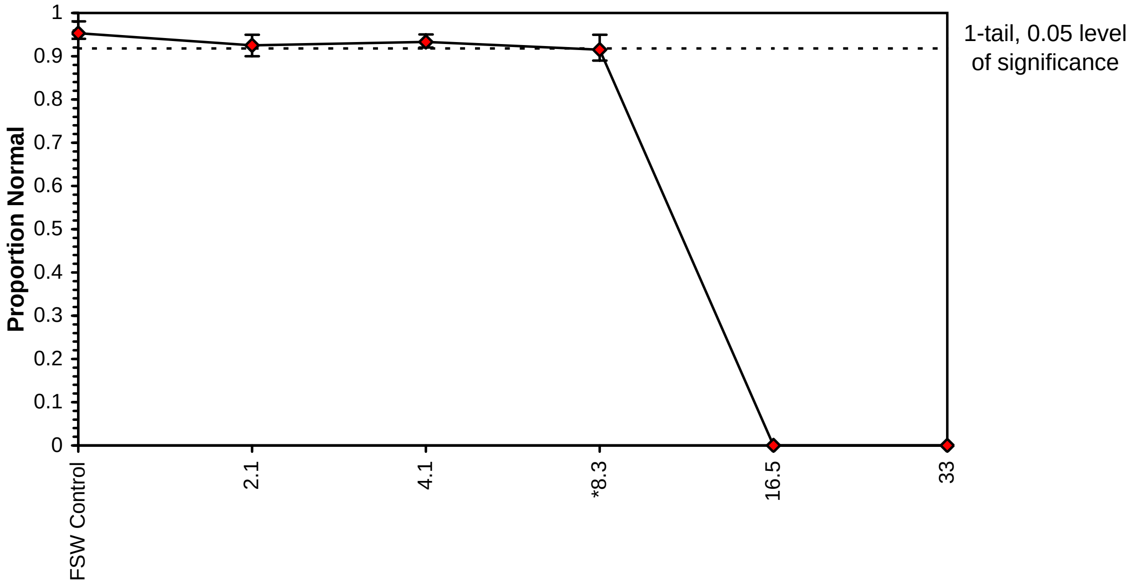
Trim Level	EC50	95% CL	
0.0%			
5.0%	11.539	11.456	11.623
10.0%	11.539	11.456	11.623
20.0%	11.539	11.456	11.623
Auto-2.5%	11.478	11.303	11.656



Sea Urchin Larval Development Test-Proportion Normal

Start Date:	13/07/06 15:00	Test ID:	PR0226/02	Sample ID:	Olympic Dam
End Date:	16/07/06 15:00	Lab ID:	1715	Sample Type:	Desalination Plant
Sample Date:		Protocol:	ESASOP105	Test Species:	HT-Heliocidaris tuberculata
Comments:					

Dose-Response Plot



Sea Urchin Larval Development Test-Proportion Normal

Start Date:	13/07/06 15:00	Test ID:	PR0226/02	Sample ID:	Olympic Dam
End Date:	16/07/06 15:00	Lab ID:	1715	Sample Type:	Desalination Plant
Sample Date:		Protocol:	ESASOP105	Test Species:	HT-Heliocidaris tuberculata
Comments:					

		Auxiliary Data Summary					
Conc-%	**Parameter**	**Mean**	**Min**	**Max**	**SD**	**CV%**	**N**
FSW Control	% Normal	95.25	94.00	98.00	1.89	1.44	4
2.1		92.50	90.00	95.00	2.38	1.67	4
4.1		93.25	92.00	95.00	1.26	1.20	4
8.3		91.50	89.00	95.00	2.65	1.78	4
16.5		0.00	0.00	0.00	0.00		4
33		0.00	0.00	0.00	0.00		4
FSW Control	Temp C	20.00	20.00	20.00	0.00	0.00	1
2.1		20.00	20.00	20.00	0.00	0.00	1
4.1		20.00	20.00	20.00	0.00	0.00	1
8.3		20.00	20.00	20.00	0.00	0.00	1
16.5		20.00	20.00	20.00	0.00	0.00	1
33		20.00	20.00	20.00	0.00	0.00	1
FSW Control	pH	8.10	8.10	8.10	0.00	0.00	1
2.1		8.10	8.10	8.10	0.00	0.00	1
4.1		8.10	8.10	8.10	0.00	0.00	1
8.3		8.10	8.10	8.10	0.00	0.00	1
16.5		8.10	8.10	8.10	0.00	0.00	1
33		8.10	8.10	8.10	0.00	0.00	1
FSW Control	Salinity ppt	36.30	36.30	36.30	0.00	0.00	1
2.1		37.50	37.50	37.50	0.00	0.00	1
4.1		38.50	38.50	38.50	0.00	0.00	1
8.3		40.50	40.50	40.50	0.00	0.00	1
16.5		44.40	44.40	44.40	0.00	0.00	1
33		51.80	51.80	51.80	0.00	0.00	1
FSW Control	DO (%sat)	107.30	107.30	107.30	0.00	0.00	1
2.1		106.20	106.20	106.20	0.00	0.00	1
4.1		104.70	104.70	104.70	0.00	0.00	1
8.3		103.20	103.20	103.20	0.00	0.00	1
16.5		102.80	102.80	102.80	0.00	0.00	1
33		102.80	102.80	102.80	0.00	0.00	1

Sea Urchin Larval Development Test-Proportion Normal

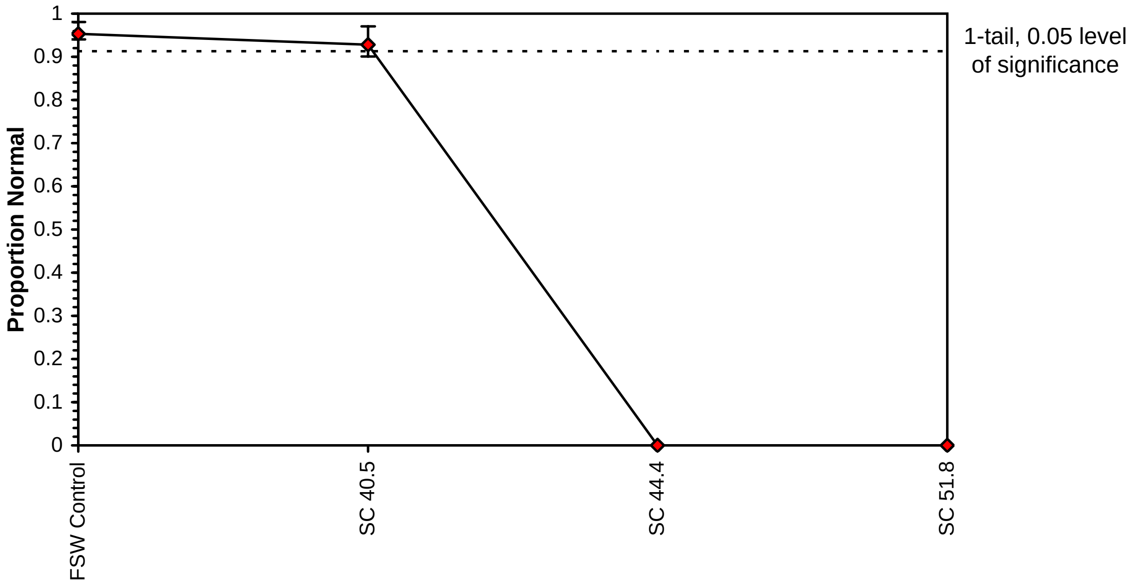
Start Date:	13/07/06 15:00	Test ID:	PR0226/01	Sample ID:	Salinity Controls
End Date:	16/07/06 15:00	Lab ID:	1715	Sample Type:	Salinity Controls
Sample Date:		Protocol:	ESASOP105	Test Species:	HT-Heliocidaris tuberculata
Comments:					

Conc-%	1	2	3	4
FSW Control	0.9400	0.9800	0.9500	0.9400
SC 40.5	0.9300	0.9700	0.9100	0.9000
SC 44.4	0.0000	0.0000	0.0000	0.0000
SC 51.8	0.0000	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root						1-Tailed	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD
FSW Control	0.9525	1.0000	1.3552	1.3233	1.4289	3.705	4			
SC 40.5	0.9275	0.9738	1.3037	1.2490	1.3967	5.059	4	1.242	1.943	0.0805
SC 44.4	0.0000	0.0000	0.0500	0.0500	0.0500	0.000	4			
SC 51.8	0.0000	0.0000	0.0500	0.0500	0.0500	0.000	4			

Auxiliary Tests	Statistic		Critical		Skew
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.835737		0.749		1.098615
F-Test indicates equal variances (p = 0.66)	1.726196		47.46723		
Hypothesis Test (1-tail, 0.05)	**MSDu**	**MSDp**	**MSB**	**MSE**	**F-Prob**
Homoscedastic t Test indicates no significant differences	0.039395	0.041284	0.005302	0.003436	0.260502

Dose-Response Plot



Sea Urchin Larval Development Test-Proportion Normal

Start Date:	13/07/06 15:00	Test ID:	PR0226/01	Sample ID:	Salinity Controls
End Date:	16/07/06 15:00	Lab ID:	1715	Sample Type:	Salinity Controls
Sample Date:		Protocol:	ESASOP105	Test Species:	HT-Heliocidaris tuberculata
Comments:					

Conc-%	Parameter	Auxiliary Data Summary Mean	Min	Max	SD	CV%	N
FSW Control	% Normal	95.25	94.00	98.00	1.89	1.44	4
SC 40.5		92.75	90.00	97.00	3.10	1.90	4
SC 44.4		0.00	0.00	0.00	0.00		4
SC 51.8		0.00	0.00	0.00	0.00		4
FSW Control	Temp C	20.00	20.00	20.00	0.00	0.00	1
SC 40.5		20.00	20.00	20.00	0.00	0.00	1
SC 44.4		20.00	20.00	20.00	0.00	0.00	1
SC 51.8		20.00	20.00	20.00	0.00	0.00	1
FSW Control	pH	8.10	8.10	8.10	0.00	0.00	1
SC 40.5		8.10	8.10	8.10	0.00	0.00	1
SC 44.4		8.10	8.10	8.10	0.00	0.00	1
SC 51.8		8.10	8.10	8.10	0.00	0.00	1
FSW Control	Salinity ppt	36.30	36.30	36.30	0.00	0.00	1
SC 40.5		40.50	40.50	40.50	0.00	0.00	1
SC 44.4		44.40	44.40	44.40	0.00	0.00	1
SC 51.8		51.80	51.80	51.80	0.00	0.00	1
FSW Control	DO (%sat)	107.30	107.30	107.30	0.00	0.00	1
SC 40.5		109.70	109.70	109.70	0.00	0.00	1
SC 44.4		107.20	107.20	107.20	0.00	0.00	1
SC 51.8		108.40	108.40	108.40	0.00	0.00	1

Statistical Printouts for the Acute *Hormosira* Cell Germination Test

Macroalgal Germination Test-Germination

Start Date:	28/07/06 12:00	Test ID:	PR0226/05	Sample ID:	Olympic Dam
End Date:	31/07/06 12:00	Lab ID:	1715	Sample Type:	Desalination Plant
Sample Date:		Protocol:	ESASOP116	Test Species:	HB-Hormosira banksii
Comments:					

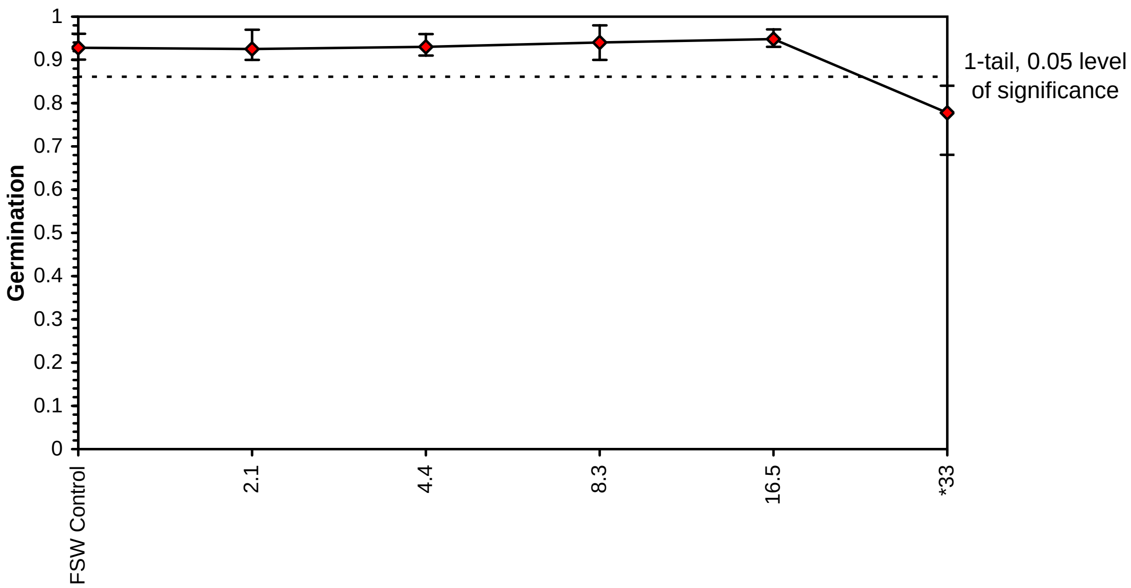
Conc-%	1	2	3	4
FSW Control	0.9600	0.9300	0.9200	0.9000
2.1	0.9200	0.9100	0.9700	0.9000
4.4	0.9300	0.9200	0.9100	0.9600
8.3	0.9800	0.9000	0.9100	0.9700
16.5	0.9400	0.9700	0.9300	0.9500
33	0.8000	0.7900	0.8400	0.6800

			Transform: Arcsin Square Root						1-Tailed	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD
FSW Control	0.9275	1.0000	1.3014	1.2490	1.3694	3.886	4			
2.1	0.9250	0.9973	1.2990	1.2490	1.3967	5.135	4	0.052	2.410	0.1109
4.4	0.9300	1.0027	1.3057	1.2661	1.3694	3.456	4	-0.093	2.410	0.1109
8.3	0.9400	1.0135	1.3352	1.2490	1.4289	6.804	4	-0.735	2.410	0.1109
16.5	0.9475	1.0216	1.3421	1.3030	1.3967	3.002	4	-0.885	2.410	0.1109
*33	0.7775	0.8383	1.0827	0.9695	1.1593	7.430	4	4.755	2.410	0.1109

Auxiliary Tests	Statistic	Critical	Skew
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.965597	0.884	0.080912
Bartlett's Test indicates equal variances (p = 0.73)	2.789603	15.08627	

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob
Dunnett's Test	16.5	33	23.33452	6.060606	0.066925	0.072028	0.037823	0.004232	2.1E-04

Dose-Response Plot



Macroalgal Germination Test-Germination

Start Date:	28/07/06 12:00	Test ID:	PR0226/05	Sample ID:	Olympic Dam
End Date:	31/07/06 12:00	Lab ID:	1715	Sample Type:	Desalination Plant
Sample Date:		Protocol:	ESASOP116	Test Species:	HB-Hormosira banksii
Comments:					

		Auxiliary Data Summary					
Conc-%	**Parameter**	**Mean**	**Min**	**Max**	**SD**	**CV%**	**N**
FSW Control	% Germinated	92.75	90.00	96.00	2.50	1.70	4
2.1		92.50	90.00	97.00	3.11	1.91	4
4.4		93.00	91.00	96.00	2.16	1.58	4
8.3		94.00	90.00	98.00	4.08	2.15	4
16.5		94.75	93.00	97.00	1.71	1.38	4
33		77.75	68.00	84.00	6.85	3.37	4
FSW Control	Temp C	18.50	18.50	18.50	0.00	0.00	1
2.1		18.50	18.50	18.50	0.00	0.00	1
4.4		18.50	18.50	18.50	0.00	0.00	1
8.3		18.50	18.50	18.50	0.00	0.00	1
16.5		18.50	18.50	18.50	0.00	0.00	1
33		18.50	18.50	18.50	0.00	0.00	1
FSW Control	pH	8.00	8.00	8.00	0.00	0.00	1
2.1		8.10	8.10	8.10	0.00	0.00	1
4.4		8.10	8.10	8.10	0.00	0.00	1
8.3		8.10	8.10	8.10	0.00	0.00	1
16.5		8.10	8.10	8.10	0.00	0.00	1
33		8.00	8.00	8.00	0.00	0.00	1
FSW Control	Salinity ppt	36.80	36.80	36.80	0.00	0.00	1
2.1		37.70	37.70	37.70	0.00	0.00	1
4.4		38.50	38.50	38.50	0.00	0.00	1
8.3		40.20	40.20	40.20	0.00	0.00	1
16.5		43.70	43.70	43.70	0.00	0.00	1
33		50.80	50.80	50.80	0.00	0.00	1
FSW Control	DO (%sat)	100.40	100.40	100.40	0.00	0.00	1
2.1		103.60	103.60	103.60	0.00	0.00	1
4.4		103.20	103.20	103.20	0.00	0.00	1
8.3		102.20	102.20	102.20	0.00	0.00	1
16.5		102.10	102.10	102.10	0.00	0.00	1
33		102.10	102.10	102.10	0.00	0.00	1

Macroalgal Germination Test-Germination

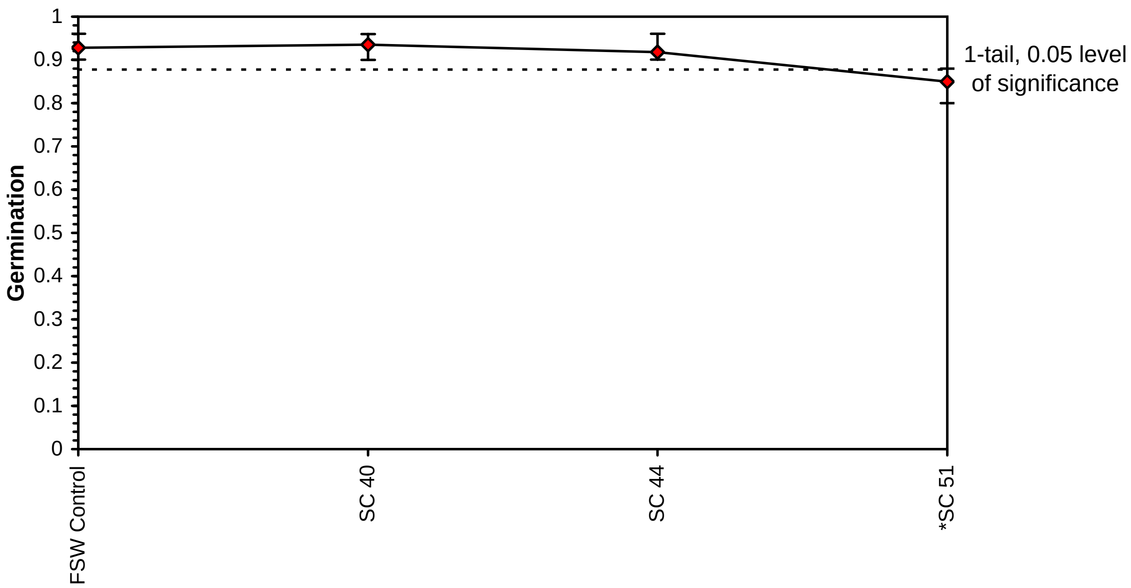
Start Date:	28/07/06 12:00	Test ID:	PR0226/04	Sample ID:	Salinity Controls
End Date:	31/07/06 12:00	Lab ID:	1715	Sample Type:	Salinity Controls
Sample Date:		Protocol:	ESASOP116	Test Species:	HB-Hormosira banksii
Comments:					

Conc-%	1	2	3	4
FSW Control	0.9600	0.9300	0.9200	0.9000
SC 40	0.9500	0.9600	0.9300	0.9000
SC 44	0.9600	0.9000	0.9000	0.9100
SC 51	0.8800	0.8500	0.8700	0.8000

			Transform: Arcsin Square Root						1-Tailed	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD
FSW Control	0.9275	1.0000	1.3014	1.2490	1.3694	3.886	4			
SC 40	0.9350	1.0081	1.3167	1.2490	1.3694	4.010	4	-0.412	2.290	0.0852
SC 44	0.9175	0.9892	1.2834	1.2490	1.3694	4.513	4	0.483	2.290	0.0852
*SC 51	0.8500	0.9164	1.1748	1.1071	1.2171	4.141	4	3.403	2.290	0.0852

Auxiliary Tests	Statistic		Critical		Skew
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.961144		0.844		0.26364
Bartlett's Test indicates equal variances (p = 0.99)	0.090511		11.34487		
Hypothesis Test (1-tail, 0.05)	**MSDu**	**MSDp**	**MSB**	**MSE**	**F-Prob**
Dunnett's Test indicates significant differences	0.049704	0.053493	0.016539	0.002767	0.009855

Dose-Response Plot



Macroalgal Germination Test-Germination

Start Date:	28/07/06 12:00	Test ID:	PR0226/04	Sample ID:	Salinity Controls
End Date:	31/07/06 12:00	Lab ID:	1715	Sample Type:	Salinity Controls
Sample Date:		Protocol:	ESASOP116	Test Species:	HB-Hormosira banksii
Comments:					

		Auxiliary Data Summary					
Conc-%	**Parameter**	**Mean**	**Min**	**Max**	**SD**	**CV%**	**N**
FSW Control	% Germinated	92.75	90.00	96.00	2.50	1.70	4
SC 40		93.50	90.00	96.00	2.65	1.74	4
SC 44		91.75	90.00	96.00	2.87	1.85	4
SC 51		85.00	80.00	88.00	3.56	2.22	4
FSW Control	Temp C	18.50	18.50	18.50	0.00	0.00	1
SC 40		18.50	18.50	18.50	0.00	0.00	1
SC 44		18.50	18.50	18.50	0.00	0.00	1
SC 51		18.50	18.50	18.50	0.00	0.00	1
FSW Control	pH	8.00	8.00	8.00	0.00	0.00	1
SC 40		8.10	8.10	8.10	0.00	0.00	1
SC 44		8.30	8.30	8.30	0.00	0.00	1
SC 51		8.40	8.40	8.40	0.00	0.00	1
FSW Control	Salinity ppt	36.80	36.80	36.80	0.00	0.00	1
SC 40		40.10	40.10	40.10	0.00	0.00	1
SC 44		43.90	43.90	43.90	0.00	0.00	1
SC 51		51.00	51.00	51.00	0.00	0.00	1
FSW Control	DO (%sat)	100.40	100.40	100.40	0.00	0.00	1
SC 40		101.20	101.20	101.20	0.00	0.00	1
SC 44		102.60	102.60	102.60	0.00	0.00	1
SC 51		103.10	103.10	103.10	0.00	0.00	1

ecotox
SERVICES AUSTRALASIA

Statistical Printouts for the Juvenile Tiger Prawn Tests

Juvenile Tiger Prawn Survival Test-96 hr survival

Start Date:	14/07/06 09:00	Test ID:	PR0226/11	Sample ID:	Olympic Dam
End Date:	18/07/06 09:00	Lab ID:	1715	Sample Type:	Desalination Plant Effluent
Sample Date:		Protocol:	ESASOP107	Test Species:	PM-Penaeus monodon
Comments:					

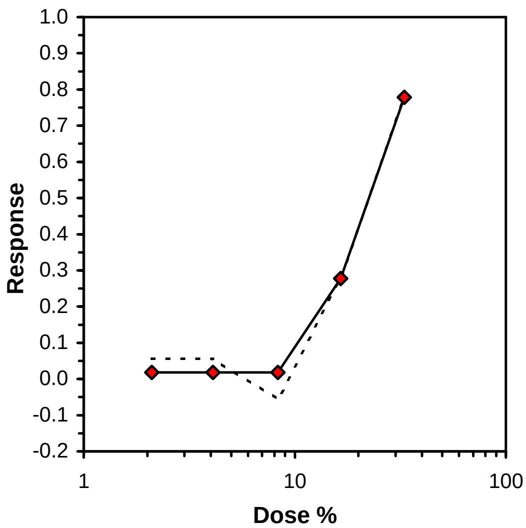
Conc-%	1	2	3	4
FSW Control	1.0000	0.6000	1.0000	1.0000
2.1	0.6000	0.8000	1.0000	1.0000
4.1	0.8000	0.8000	1.0000	0.8000
8.3	1.0000	0.8000	1.0000	1.0000
16.5	0.8000	0.6000	0.6000	0.6000
33	0.0000	0.4000	0.2000	0.2000

			Transform: Arcsin Square Root						1-Tailed		Number
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp
FSW Control	0.9000	1.0000	1.2305	0.8861	1.3453	18.660	4				2
2.1	0.8500	0.9444	1.1709	0.8861	1.3453	18.840	4	0.490	2.410	0.2928	3
4.1	0.8500	0.9444	1.1667	1.1071	1.3453	10.206	4	0.525	2.410	0.2928	3
8.3	0.9500	1.0556	1.2857	1.1071	1.3453	9.261	4	-0.455	2.410	0.2928	1
16.5	0.6500	0.7222	0.9413	0.8861	1.1071	11.742	4	2.380	2.410	0.2928	7
*33	0.2000	0.2222	0.4594	0.2255	0.6847	40.823	4	6.347	2.410	0.2928	16

Auxiliary Tests					Statistic		Critical		Skew
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)					0.935624		0.884		-0.62657
Bartlett's Test indicates equal variances (p = 0.71)					2.906309		15.08627		
Hypothesis Test (1-tail, 0.05)	**NOEC**	**LOEC**	**ChV**	**TU**	**MSDu**	**MSDp**	**MSB**	**MSE**	**F-Prob**
Dunnett's Test	16.5	33	23.33452	6.060606	0.238648	0.268569	0.381349	0.029521	2.0E-05

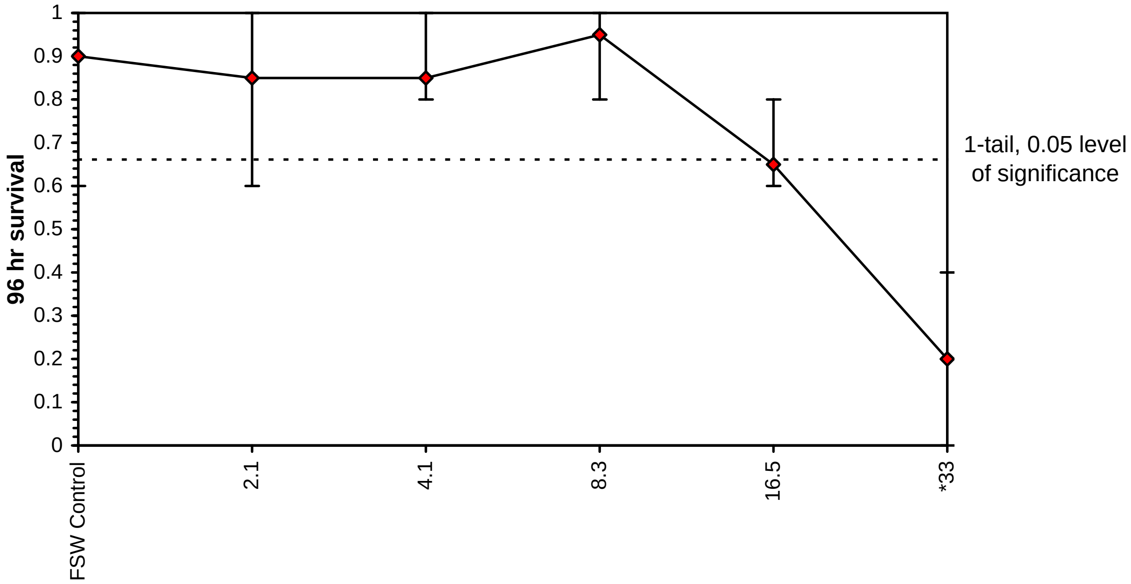
Trimmed Spearman-Karber

Trim Level	EC50	95% CL	
0.0%			
5.0%			
10.0%			
20.0%			
Auto-22.2%	22.374	15.840	31.604



Juvenile Tiger Prawn Survival Test-96 hr survival					
Start Date:	14/07/06 09:00	Test ID: PR0226/11		Sample ID:	Olympic Dam
End Date:	18/07/06 09:00	Lab ID: 1715		Sample Type:	Desalination Plant Effluent
Sample Date:		Protocol: ESASOP107		Test Species:	PM-Penaeus monodon
Comments:					

Dose-Response Plot



Juvenile Tiger Prawn Survival Test-96 hr survival					
Start Date:	14/07/06 09:00	Test ID:	PR0226/11	Sample ID:	Olympic Dam
End Date:	18/07/06 09:00	Lab ID:	1715	Sample Type:	Desalination Plant Effluent
Sample Date:		Protocol:	ESASOP107	Test Species:	PM-Penaeus monodon
Comments:					

		Auxiliary Data Summary					
Conc-%	**Parameter**	**Mean**	**Min**	**Max**	**SD**	**CV%**	**N**
FSW Control	% Survival	90.00	60.00	100.00	20.00	4.97	4
2.1		85.00	60.00	100.00	19.15	5.15	4
4.1		85.00	80.00	100.00	10.00	3.72	4
8.3		95.00	80.00	100.00	10.00	3.33	4
16.5		65.00	60.00	80.00	10.00	4.87	4
33		20.00	0.00	40.00	16.33	20.21	4
FSW Control	Temp C	25.00	25.00	25.00	0.00	0.00	1
2.1		25.00	25.00	25.00	0.00	0.00	1
4.1		25.00	25.00	25.00	0.00	0.00	1
8.3		25.00	25.00	25.00	0.00	0.00	1
16.5		25.00	25.00	25.00	0.00	0.00	1
33		25.00	25.00	25.00	0.00	0.00	1
FSW Control	pH	8.10	8.10	8.10	0.00	0.00	1
2.1		8.10	8.10	8.10	0.00	0.00	1
4.1		8.00	8.00	8.00	0.00	0.00	1
8.3		8.00	8.00	8.00	0.00	0.00	1
16.5		8.00	8.00	8.00	0.00	0.00	1
33		8.10	8.10	8.10	0.00	0.00	1
FSW Control	Salinity ppt	36.30	36.30	36.30	0.00	0.00	1
2.1		37.50	37.50	37.50	0.00	0.00	1
4.1		38.20	38.20	38.20	0.00	0.00	1
8.3		40.20	40.20	40.20	0.00	0.00	1
16.5		43.90	43.90	43.90	0.00	0.00	1
33		51.70	51.70	51.70	0.00	0.00	1
FSW Control	DO (%sat)	103.60	103.60	103.60	0.00	0.00	1
2.1		102.80	102.80	102.80	0.00	0.00	1
4.1		103.20	103.20	103.20	0.00	0.00	1
8.3		105.40	105.40	105.40	0.00	0.00	1
16.5		104.10	104.10	104.10	0.00	0.00	1
33		105.40	105.40	105.40	0.00	0.00	1

Juvenile Tiger Prawn Survival Test-96 hr survival

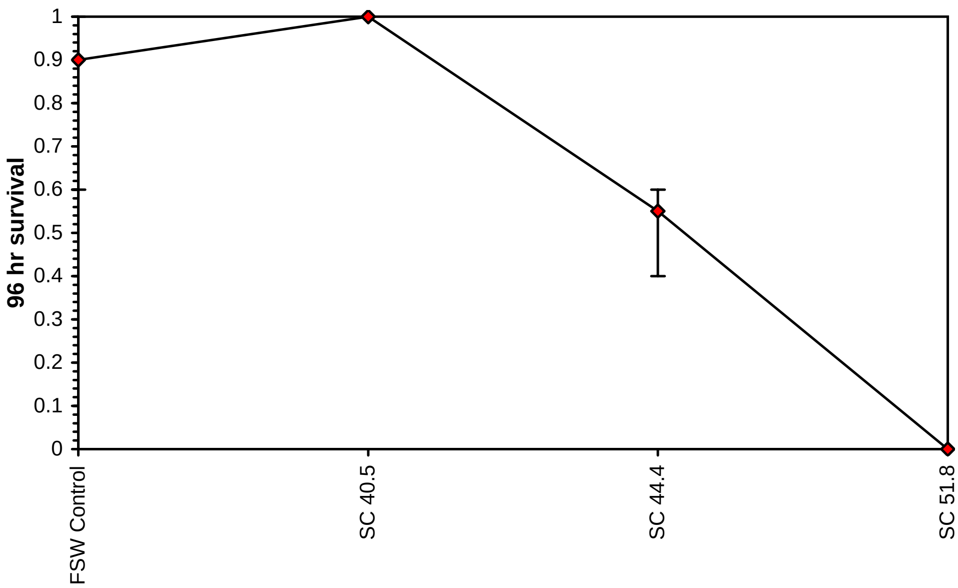
Start Date:	14/07/06 09:00	Test ID:	PR0226/10	Sample ID:	Salinity Controls
End Date:	18/07/06 09:00	Lab ID:	1715	Sample Type:	Salinity Controls
Sample Date:		Protocol:	ESASOP107	Test Species:	PM-Penaeus monodon
Comments:					

Conc-ppt	1	2	3	4
FSW Control	1.0000	0.6000	1.0000	1.0000
SC 40.5	1.0000	1.0000	1.0000	1.0000
SC 44.4	0.6000	0.4000	0.6000	0.6000
SC 51.8	0.0000	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root					Rank	1-Tailed
Conc-ppt	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical
FSW Control	0.9000	1.0000	1.2305	0.8861	1.3453	18.660	4		
SC 40.5	1.0000	1.1111	1.3453	1.3453	1.3453	0.000	4	20.00	11.00
SC 44.4	0.5500	0.6111	0.8357	0.6847	0.8861	12.047	4	11.50	11.00
SC 51.8	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	4		

Auxiliary Tests	Statistic	Critical	Skew
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.769409	0.805	-1.91384
Equality of variance cannot be confirmed			
Hypothesis Test (1-tail, 0.05)			
Steel's Many-One Rank Test indicates no significant differences			

Dose-Response Plot



Juvenile Tiger Prawn Survival Test-96 hr survival					
Start Date:	14/07/06 09:00	Test ID:	PR0226/10	Sample ID:	Salinity Controls
End Date:	18/07/06 09:00	Lab ID:	1715	Sample Type:	Salinity Controls
Sample Date:		Protocol:	ESASOP107	Test Species:	PM-Penaeus monodon
Comments:					

		Auxiliary Data Summary					
Conc-ppt	**Parameter**	**Mean**	**Min**	**Max**	**SD**	**CV%**	**N**
FSW Control	% Survival	90.00	60.00	100.00	20.00	4.97	4
SC 40.5		100.00	100.00	100.00	0.00	0.00	4
SC 44.4		55.00	40.00	60.00	10.00	5.75	4
SC 51.8		0.00	0.00	0.00	0.00		4
FSW Control	Temp C	25.00	25.00	25.00	0.00	0.00	1
SC 40.5		25.00	25.00	25.00	0.00	0.00	1
SC 44.4		25.00	25.00	25.00	0.00	0.00	1
SC 51.8		25.00	25.00	25.00	0.00	0.00	1
FSW Control	pH	8.10	8.10	8.10	0.00	0.00	1
SC 40.5		8.10	8.10	8.10	0.00	0.00	1
SC 44.4		8.10	8.10	8.10	0.00	0.00	1
SC 51.8		8.30	8.30	8.30	0.00	0.00	1
FSW Control	Salinity ppt	36.30	36.30	36.30	0.00	0.00	1
SC 40.5		40.30	40.30	40.30	0.00	0.00	1
SC 44.4		44.40	44.40	44.40	0.00	0.00	1
SC 51.8		51.60	51.60	51.60	0.00	0.00	1
FSW Control	DO (%sat)	103.60	103.60	103.60	0.00	0.00	1
SC 40.5		105.00	105.00	105.00	0.00	0.00	1
SC 44.4		107.40	107.40	107.40	0.00	0.00	1
SC 51.8		104.70	104.70	104.70	0.00	0.00	1

CSIRO

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Toxicity of brine to Juvenile yellowtail kingfish (*Seriola lalandi*)

Client: **Arup/HAL – Dr David Wiltshire and Dr Emma Cronin**

Project: **Olympic Dam Development Study: Ecotoxicological studies**

Test Performed: **96 h acute toxicity test of brine water to juvenile yellowtail kingfish, *Seriola lalandi***

TEST METHODS:

Fish fingerling (approximately 1 g in weight) were collected from the Spencer Gulf Aquaculture hatchery, South Australia and transported to Adelaide laboratory. Fish were acclimatised to the laboratory conditions in 200 L tanks for at least a week before they were use for the range-finding or the definitive bioassays. These fish tanks were constantly aerated and fingerlings were fed twice a day with brine shrimp nauplii *(Artemia salina)*. Fish were kept under constant temperature conditions (20 ± 1°C) with a 16 h light: 8 h dark photoperiod using cool white fluorescent lamps. Filtered seawater from SARDI, West Lakes was used during acclimation and as a diluent for testing (Table 1).

Fish were collected from the hatchery at three different time intervals

Table 1. Water quality characteristics of seawater from SARDI

Water quality parameters	Sea Water
pH	7.5
Salinity	40 g/L
Dissolved oxygen (DO%)	> 80% saturation
Temperature (°C)	20 ± 0.5

96 h acute toxicity test:

This test measures the survival of fish fingerlings after exposure to the toxicant or effluents for 96 h and is based on USEPA protocol (2002a)

Brine water sampling:

200 L of brine sample was provided by Mr Paul Hochman on 6th May 2006 to the CSIRO Adelaide laboratory. Samples were transferred into 20 L carboys and stored at 4ºC until testing. Samples were handled with minimal agitation to limit the loss of volatiles and were allowed to warm to testing temperature (20ºC) prior to testing. The detailed processing methodology was provided by Dr Emma Cronin and is included as an Appendix 1 in this report.

Experimental design:

Toxicity tests were conducted with brine water sample using semi-static renewal exposure regime. The brine water was tested at 100, 50, 25, 12.5, 6.25 and 1 % dilution in 10 L glass aquaria containing 5 L of the test sample. Brine was diluted with the filtered seawater from SARDI. Each test concentration consisted of four replicate aquaria. Five fish fingerling were randomly added to each aquarium resulting in total of 20 fish/ test concentration.

Initial testing confirmed that fingerlings could not withstand starving condition for more than 24 hours. Therefore, the fish fingerlings were fed brine shrimp nauplii (once/day) for the duration of the test. The test water was renewed every 24 h and fresh nauplii was added to each test aquaria until the next renewal. The test conditions were maintained at a constant temperature of (20 ± 0.5°C) with a photoperiod of 16-h light and 8-h darkness. This test was conducted twice using two batches of fish collected from the Spencer Gulf hatchery.

Copper chloride was used as a reference toxicant. Sine no background data was available on the copper toxicity to yellowtails, three independent tests were run with three batches of fingerling to develop such a database. As brine water solution, reference test solutions were also renewed every 24 h and fingerlings were fed with brine shrimp nauplii for the duration of the test.

Water quality parameters (pH, conductivity, temperature, dissolved oxygen) were measured at test commencement and before and after the renewal of test solutions.

DATA ANALYSES:

The objective of the test series for brine water was to determine:

- the No Observed Effect Concentration (NOEC), where no statistical difference ($P \leq 0.05$) was found between exposed and unexposed (or control) fish;
- the Lowest Observed Effect Concentration (LOEC), where the smallest statistical difference ($P \leq 0.05$) was found between exposed and unexposed (or control) fish; and
- the median lethal concentration (LC50), the concentration of the brine water in solution that was estimated to be effective in producing 50% mortality in the exposed fish.

The 96 h LC50 was computed by Trimmed-Spearman Karber method of Hamilton *et al.* (1977; 1978). Statistical significance was determined at $\alpha = 0.05$. Data were tested for normality and homogeneity of variance using Toxstat (1994). An analysis of variance (ANOVA) with Bonferroni (unequal replicates) or Dunnett tests (Toxstat, 1994). This information was used for the estimation of the LOEC and NOEC.

RESULTS:

The health of the fish fingerlings used for testing was determined using $CuCl_2$ as the reference toxicant. Control survival was also used for quality assurance. Both responses in the reference toxicant test and the control survival were within the criterion (Table 2).

Table 2. Quality Assurance/Quality Control for fish fingerlings exposed to reference toxicant, $CuCl_2$

Quality Assurance/Quality Control	Criterion	This Test	Criterion Met?
Control Survival	>80%	100%	Yes
Reference toxicant ($CuCl_2$, µg/L)	435 ± 112	406	Yes

Average water quality parameters are given in Table 3. Brine water exhibited toxicity yellowtail fingerling, with a 96 h LC50 of 19%. There was no observable effect on fish fingerling survival at 12.5% dilution of brine water and the lowest observable dilution of brine to cause an effect was 25% (Table 4).

Table 3. Water quality parameters for toxicity tests with Brine water

Water quality parameters	100	50	25	12.5	6.25
pH	7.69 (0.05)	7.57 (0.04)	7.55 (0.07)	7.43 (0.12)	7.55 (0.17)
Salinity (g/L)	84 (1)	60 (2)	54 (3)	45 (1)	43 (2)
DO (mg/L)	6.3 (0.4)	6.7 (0.3)	6.5 (0.5)	7.2 (0.3)	7.3 (0.4)
Temperature (°C)			20.2 (0.4)		

[a]Standard error in parenthesis, n=8.

Table 4. Summary of the 96 h toxicity of brine water to yellowtail kingfish fingerling

Endpoint	Brine water (%)
LC50	**18.95** (16.58, 21.65)[a]
LOEC	**25**
NOEC	**12.5**

[a]Standard error in parenthesis

CONCLUSIONS:

- Brine water sample was toxic to kingfish yellowtail fingerlings with an LC50 value of 19%.
- The no observable effect concentration was 12.5% and the lowest observable effect concentration was 25%.

RECOMMENDATION:
Chronic toxicity of brine should be assessed by conducting long-term exposures based on the standard US EPA protocol (USEPA 2002b). Physiological end-points such as growth, histopathological changes in fish gills can be used to assess chronic effects due to contaminants and high salinity of the brine.

References:

Hamilton MA, Russo RC and Thurston RV. 1977. Trimmed Spearman–Karber method for estimating median lethal concentrations in toxicity bioassays. *Environ Sci Technol.* **11:** 714–719.

Hamilton MA, Russo RC and Thurston RV. 1978. Correction to trimmed Spearman–Karber method for estimating median lethal concentrations in toxicity bioassays. *Environ Sci Technol.* **12:** 417.

Toxstat. 1994. WEST, Inc. and Gulley, University of Wyoming (authors), Western EcoSystems Technology Inc., Cheyenne, WY

USEPA, U.S. Environmental Protection Agency, 2002a. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms. Fifth Edition. U.S. Environmental Protection Agency Report. EPA-821-R-02-012. Washington, DC

USEPA, U.S. Environmental Protection Agency, 2002b. Short term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. U.S. Environmental Protection Agency Report EPA-821-R-02-013
EPA/600/4-85/ 014. Washington, DC

Test carried out by:	Hai Doan and Anu Kumar
Test supervised by:	Anu Kumar
Test report prepared by:	Anu Kumar Tel: (08 8303 8597)
Date:	10/07/06

APPENDIX O10.3

Effects of RO brine on the development of Giant Cuttlefish (*Sepia apama*) embryos (report by Geotechnical Services, 2006)

See overleaf for report.

EFFECTS OF RO BRINE ON THE DEVELOPMENT OF GIANT CUTTLEFISH (*Sepia apama*) EMBRYOS

Report
ENV06-128

Prepared for
BHP BILLITON

Prepared by
Dr Jill Woodworth

NOVEMBER 2006

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Contents

GLOSSARY

EC50	Concentration that causes an effect on 50% of the population Eg. Growth: Concentration that results in 50% less growth when compared to controls Reproduction: Concentration that results in 50% less fecundity when compared to controls Germination: Concentration that results in 50% germination of zoospores Larval development: Concentration that results in 50% of larva deformed Calculated statistically
IC50	Concentration that causes an inhibition of growth of 50% when compared with controls (Unicellular alga bioassay) Calculated statistically
EC/IC10	Concentration that causes an effect of 10% when compared with controls Calculated statistically
LOEC	Lowest Observed Effect Concentration Function of concentration tested
NOEC	No Observed Effect Concentration Function of concentration tested
‰	Parts per thousand

1. EXECUTIVE SUMMARY

1.1 Introduction

This report has been prepared by Geotechnical Services for Arup/HLA as part of the proposed BHP Billiton Olympic Dam mine expansion. A desalination plant is planned for Point Lowly, near Whyalla. The discharge of return water is planned to have a salinity of up to 78 ppt. The assessment of effluent from a prototype desalination plant was undertaken to determine its potential toxicity to the development and survival of the giant cuttlefish (*Sepia apama*) resident in Upper Spencer Gulf.

The Australian Giant Cuttlefish aggregates annually for breeding in the Point Lowly area and has become a major tourist attraction in South Australia, being visited by divers from all over world. This study was instigated as the potential impacts of the prototype desalination plant effluent (RO brine) on the local marine environment, in particular the spawning aggregation of the Australian giant cuttlefish were unknown. This document reports on the impacts of the RO brine on the development and growth of giant cuttlefish (*S. apama*) embryos and survival after hatch of the juveniles. A summary of the toxicity results of the exposure of the *S. apama* eggs and embryos are located in Table 1.1.

1.2 Test Endpoints

The most environmentally relevant endpoints for the 5 month exposure of the *S. apama* embryos were the:

- Embryo Development
- Number of Days to Hatch
- and Survival Post Hatch

The EC10 values generated from these endpoints and the at hatch data, post hatch data and stage data (In bold in Table 1.1) were used in the BurrliOZ (Campbell *et al.* 2000) statistics package to determine the level of RO dilution required to protect the giant cuttlefish embryos in the receiving ecosystem.

1.3 Protection Values

The Protection Values based on the EC10 data would theoretically protect X% of species from experiencing inhibitory impacts greater than 10%. Therefore, to protect 99% of the cuttlefish from experiencing inhibitory impacts greater than 10%, the RO brine would need to be diluted approximately 100 times (Table 1.2).

New Protection Values should be generated using results from the actual brine discharged from the desalination plant after commissioning of the plant.

The use of sub-lethal testing is recommended to remove the correction factors that are required when using LC50 data. Future testing should include lower test concentrations.

Table 1.1 Summary of EC50, EC10. LOEC and NOEC Data

End Point	EC50 %	EC10 %	LOEC* %	NOEC* %
Embryo Development	**5.81**	**2.37**	**1.5**	**<1.5**
Day 30 Stage	**10.34**	**4.61**	**6.25**	**3.13**
Day 30 Length	>100	>100	>100	100
Day 30 Width	>100	>100	>100	100
Day 30 Weight	>100	>100	>100	100
Day 60 Stage	**6.61**	**3.16**	**6.25**	**3.13**
Day 60 Length	>100	>100	>100	100
Day 60 Width	>100	19.39	12.5	6.25
Day 60 Weight	>100	20.87	12.5	6.25
Day 90 Stage	2.56	0.66	1.5	<1.5
Day 90 Length	6.5	2.8	3.125	1.5
Day 90 Width	7.4	4.3	1.5	<1.5
Day 90 Weight	11.17	5.15	1.5	<1.5
Days to Hatch	**5.42**	**3.16**	**1.5**	**<1.5**
Length at Hatch	**7.51**	**6.51**	**6.25**	**3.125**
Width at Hatch	**7.42**	**6.24**	**6.25**	**3.125**
Weight at Hatch	**6.40**	**5.74**	**6.25**	**3.125**
Length 30 Days Post Hatch	**7.18**	**6.30**	**3.125**	**1.5**
Width 30 Days Post Hatch	**7.10**	**6.18**	**1.5**	**<1.5**
Weight 30 Days Post Hatch	**7.38**	**6.24**	**6.25**	**3.125**
Survival Post Hatch	**4.06**	**1.86**	**12.5**	**6.25**

**Note: NOEC and LOEC are calculated by Dunnett's hypothesis test and are a function of the concentrations tested. These results do not assimilate data* *from all concentrations and, as such, should not be used for regulatory requirements*.

Table 1.2 BurrliOZ Protection Levels for Giant Cuttlefish for RO Brine

Protection Level	Protection Value %	Dilution Factor
99	0.97	103
95	1.89	53
90	2.52	40
80	3.35	30

2. INTRODUCTION

This report has been prepared by Geotechnical Services for Arup/HLA on behalf of BHP Billiton. The assessment of effluent from a prototype desalination plant was undertaken to determine its potential toxicity to the development and survival of the giant cuttlefish (*Sepia apama*) resident in Upper Spencer Gulf.

As part of the proposed BHP Billiton Olympic Dam mine expansion, a desalination plant located at Port Bonython, near Whyalla is planned in order to provide 120 ML $^{-1}$ per day of fresh water for the mine expansion and possibly also water for Yorke Peninsular communities currently reliant on Murray River and ground water. A discharge of 196 M. l $^{-1}$ per day of concentrated seawater up to 78 ppt is proposed.

The Australian Giant Cuttlefish aggregates annually for breeding in the Point Lowly area and has become a major tourist attraction in South Australia, being visited by divers from all over world. This study was instigated as the potential impacts of the prototype desalination plant effluent (RO brine) on the local marine environment, in particular the spawning aggregation of the Australian giant cuttlefish were unknown. This document reports on the impacts of the RO brine on the development and growth of giant cuttlefish (*S. apama*) embryos and survival after hatch of the juveniles

Toxicity testing was also undertaken on the RO brine by other organisations using a suite of locally relevant organisms or organisms that could be used as surrogates for local species. This suite included:

- The microalga, *Nitzschia closterium*, used in a 72-h growth rate inhibition test;
- The sea urchin, *Heliocidaris tuberculata*, used in a 72-h larval development test;
- The yellowtail kingfish, *Seriola lalandi*, used in a 96-h survival test;
- The macroalga, *Hormisira banksii*, used in a 72-h germination test;
- The oyster, *Saccostrea commercialis,* used in a 48-h larval development test, and;
- The prawn, *Penaeus monodon,* used in a 96-h survival test.

The results for this suite of bioassays are reported elsewhere and are not discussed in this report. The tests listed above are tests performed routinely with documented protocols and a large data base used for quality control. However, at present there are no recognised protocols for the use of giant cuttlefish for toxicity tests. Therefore, the data generated and presented in this report is experimental and there is no QA/QC data available. Geotech is a quality assured laboratory and operates under the ISO 9002 Quality Assurance System with many of our routine tests NATA registered. As such, the cuttlefish test was performed according to Geotech's in-house quality assurance and Geotech has confidence in the results generated from the study.

All data and information are proprietary of BHP Billiton and are regarded as highly confidential by all Geotech personnel.

Geotechnical Services has endeavoured to use techniques and equipment to achieve results and information as accurately as it possibly can. However, such equipment and techniques are not necessarily perfect. Therefore, Geotechnical Services shall not be held responsible or liable for the results of any actions taken on a basis of the information contained in this document. Moreover, this document should not be the sole reference when considering issues that may have commercial or environmental implications.

3. RO BRINE AND DILUENT SEAWATER

The prototype reverse osmosis (RO) brine was prepared by Arup/HLA for BHP Billiton on the 1st June 2006 and delivered to Geotech's Welshpool laboratory. The diluent seawater was collected from Point Lowly, South Australia. The RO brine was processed on site with 3.6 mg/l of NALCO PC-1020T antiscalant added to the seawater prior to processing which concentrated the antiscalant to 7.0 ppm. The RO Water Processing Report is located in Appendix 3. Both the RO brine and diluent seawater were transported in 1000 litre containers in a refrigerated truck at 4°C from South Australia to Perth, Western Australia. The RO brine and seawater were refrigerated at 4°C until use. Prior to use in the bioassays the seawater was filtered to 0.45 micron in 100 – 150 L batches as required and transported to Geotech's Fremantle Ecotoxicology Laboratory in 25 L HDPE containers. Brine sample were tested as received.

Table 3.1 Sample Information Sheet

Job Number	ENV06 -128
Contact Details Company	Arup/HLA and Partners for BHP Billiton
Contact Person	Dr Emma Cronin
Contact Phone Number	08 8104 8314
Contact Address	Level 2 Optus Centre 431-439 King William St Adelaide SA 5000
Number of Samples	RO brine x 3 1000L Seawater x 7 1000L
Sample Type	RO Brine Seawater
Date Collected	1st June 2006
Time Collected	NA
Location Collected	Point Lowly, South Australia
Sample Collected by	Dr Emma Cronin
pH	RO Brine 7.95 Seawater 7.99
Salinity	78 ppt 45 ppt
Transport Conditions	Refrigerated Truck at 4°C
Date of Arrival at Geotech	6th June 2006
Time of Arrival at Geotech	1:00 pm
Sample Temperature on Arrival	9 °C
Sample Received by	J. Woodworth
Tests Required	Giant cuttlefish bioassay

4. GIANT CUTTLEFISH EXPOSURE METHODOLOGY

4.1 Giant Cuttlefish Eggs

S. apama eggs were collected over two days at Point Lowly by Dr Emma Cronin in the week beginning 5th June 2006. Dr Cronin transported the eggs in seawater in insulated containers to Fremantle on Thursday 8th June 2006. Approximately 450 eggs were delivered to Geotech's Fremantle laboratory. The eggs had an average weight of 4.81g and an average length 4.17 cm (Picture 4.1). Upon receipt at the laboratory 11 eggs were randomly allocated to each replicate. The 11 eggs were sampled according to Table 4.1.

Table 4.1 Sampling procedure

Number of Eggs/ Embryos per Replicate	Time	Measurements Taken
2	Day 30	Egg length, width, weight and stage of development
2	Day 60	Egg length, width, weight and stage of development
2	Day 90	Egg length, width, weight and stage of development
2	At Hatch	Mantle length and width, weight
3	1 Month Post Hatch	Mantle length and width, weight

Picture 4.1 Giant Cuttlefish Eggs Day 0

4.2 Test Chambers

Glass 5 litre vessels were used as test chambers for the exposure assay. The eggs were suspended in the test chamber by fishing line attached to wooden skewers with a slip knot around the attaching point of the eggs (Picture 4.2). Five litres of test solution were placed in each chamber prior to the addition of the eggs. Each chamber had aeration via an air stone with adjustable flow rate. A medium flow rate was used for embryo development.

4.3 Test Concentrations

The RO brine was diluted with filtered Point Lowly seawater to selected concentrations. The concentrations selected for this assay were: 100%, 50%, 25%, 12.5%, 6.25%, 3.125%, 1.5% and 0% RO brine. Each concentration consisted of 5 replicates (Picture 4.3).

4.4 Maintenance

The *S. apama* embryos were maintained in a temperature controlled laboratory with a photoperiod of 8 hours light and 16 hours dark until hatching when the photoperiod was changed to 12 hours light and 12 hours dark. The laboratory was maintained at 14.5 ± 0.25 °C for the duration of the test. This temperature was selected in preference to the 11 – 12 °C that the eggs would have been exposed to in their natural environment, to ensure that the embryos hatched with a 3 – 4 month period.

A 50% water exchange was performed daily to ensure high water quality was maintained. Water quality parameters were tested weekly on each chamber for the duration of the test.

After the embryos hatched the juveniles were placed in the same 5 litre containers with a layer of sand and coral rubble and PVC pipe for shelter with the same concentration of brine as their development (Picture 4.4). The juveniles were fed on *Artemia*, fish flesh, prawns and copepods. A 50% water exchange was performed daily

Picture 4.2 Test Chamber

Picture 4.3 Test Set-up

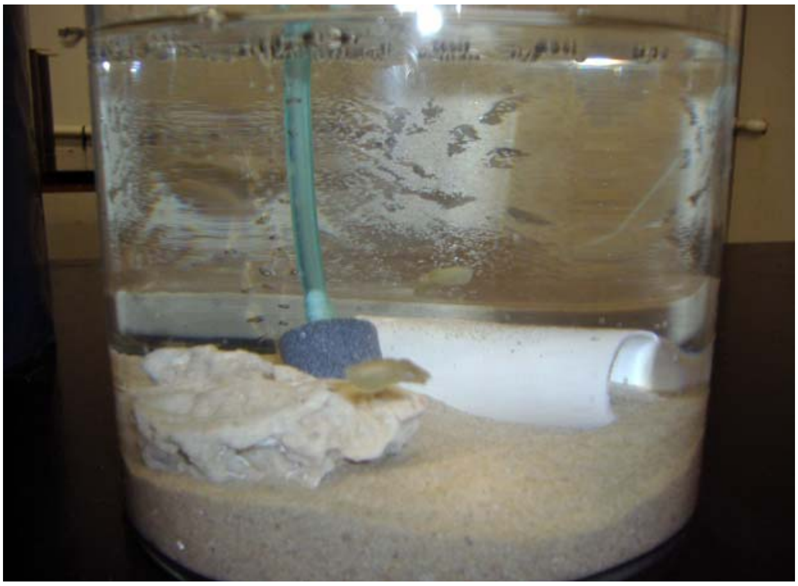
4.5 Measurements

At days 30, 60 and 90 two embryos from each replicate were sacrificed and the length, width, weight and stage of each were measured and recorded. The length and width were measured to 0.01 cm using Vernier calipers. The weight was measured to 0.01g and each embryo was staged using a staging guide (Cronin 2000). After the measurements were taken each embryo was fixed in 10% formalin in seawater.

Upon hatch the hatchling was anaesthetised in a saltwater ice bath after which, the mantle length and width was measured to 0.01 cm and the weight was measured to 0.01 g and recorded for 2 hatchlings. These hatchlings were then fixed in 10% formaldehyde in seawater. The remaining three hatchlings were maintained for one month after which the same measurements were taken.

The number of days to hatch was also recorded and survival post hatch was also recorded. All of the above data was used to calculate the EC50, EC10, LOEC and NOEC concentrations for the RO brine

Picture 4.4 Test Chamber for Juveniles



4.6 Staging of Embryos Collected from Point Lowly

On the 10th August 2006 approximately 20 embryos were collected from the same site as previous by Dr Emma Cronin. The stage of these embryos were assessed to determine how the stage of development compared with the embryos maintained in the laboratory. The embryos arrived at the laboratory on the 17th August and the temperature upon arrival was 16°C.

5. RESULTS

5.1 Physicochemistry Results

Water parameters were monitored and recorded weekly for all replicates. The temperature, salinity, pH and dissolved oxygen were monitored and the results are found in Table 5.1.

Table 5.1 Physicochem Data from Giant Cuttlefish Bioassay

Concentration %	pH	Temperature °C	DO ppm	Salinity ppt
0	8.08 ± 0.04	14.49 ± 0.25	3.95 ± 0.65	45.11 ± 0.29
1.5	8.10 ± 0.03	14.50 ± 0.20	3.64 ± 0.38	46.01 ± 0.06
3.1	8.11 ± 0.04	14.47 ± 0.19	3.47 ± 0.33	46.52 ± 0.10
6.25	8.11 ± 0.04	14.46 ± 0.19	3.32 ± 0.27	47.08 ± 0.24
12.5	8.12 ± 0.05	14.40 ± 0.25	3.26 ± 0.32	49.99 ± 0.06
25	8.13 ± 0.04	14.42 ± 0.23	3.18 ± 0.27	53.39 ± 0.88
50	8.13 ± 0.06	14.50 ± 0.24	3.12 ± 0.27	62.88 ± 1.38
100	8.13 ± 0.08	14.49 ± 0.23	3.06 ± 0.22	78.13 ± 0.33

5.2 Day 0

The weight (4.81 ± 0.51g) and length (4.18 ± 0.29 cm) of ten *S. apama* eggs were measured upon receipt at the laboratory.

5.3 Days 30, 60, 90 Samplings

The weight, length and width of two embryos from each replicate were measured and recorded on the 12th July 2006 for Day 30, the 8th August for Day 60 and the 6th September 2006 for Day 90. Each embryo was also assessed for development using the staging guide supplied by Dr Emma Cronin. After measurements were completed the embryos were placed in 10% formalin in seawater.

The results for Day 30 are found in Table 5.2. The results for Day 60 are found in Table 5.3. The results for Day 90 are found in Table 5.4. Photos 5.1 and 5.2 show embryos sampled on Day 30. Photos 5.3 and 5.4 show embryos sampled on Day 60. Photo 5.5 shows embryos at low concentrations sampled on day 90. All measurements were analyzed using a one way Anova in Excel. The measurements obtained from Day 30, 60 and 90 sampling were used to calculate the EC50, EC10, LOEC and NOEC values of the RO brine in the Tidepool Scientific Toxcalc statistics package developed for the

USEPA. These results are found in Table 5.5. Toxicity data summary sheets are found in Appendix 1.

Table 5.2 Day 30 Summary

Concentration %	Length cm n =10	Width cm n = 10	Weight g n = 10	Stage n = 10
0	3.90 ± 0.31	1.59 ± 0.10	4.00 ± 0.54	21.30 ± 2.98
1.5	3.99 ± 0.45	1.55 ± 0.13	3.81± 0.60	23.60 ± 2.50
3.1	3.72 ± 0.38	1.57 ± 0.09	3.53 ± 0.44	20.00± 1.89
6.25	3.92 ± 0.46	1.56 ± 0.12	3.93 ± 0.86	18.40 ± 0.84
12.5	3.75 ± 0.43	1.48 ± 0.09	3.45 ± 0.51	3.60 ± 7.59
25	4.24 ± 0.64	1.54 ± 0.11	3.79 ± 0.67	3.60 ± 7.59
50	3.81 ± 0.34	1.52 ± 0.08	3.69 ± 0.44	0.0 ± 0.0
100	3.86 ± 0.51	1.50 ± 0.10	3.61 ± 0.52	0.0 ± 0.0

Photo 5.1 Embryos from Low Concentrations Day 30

Photo 5.2 Embryos from High Concentrations Day 30

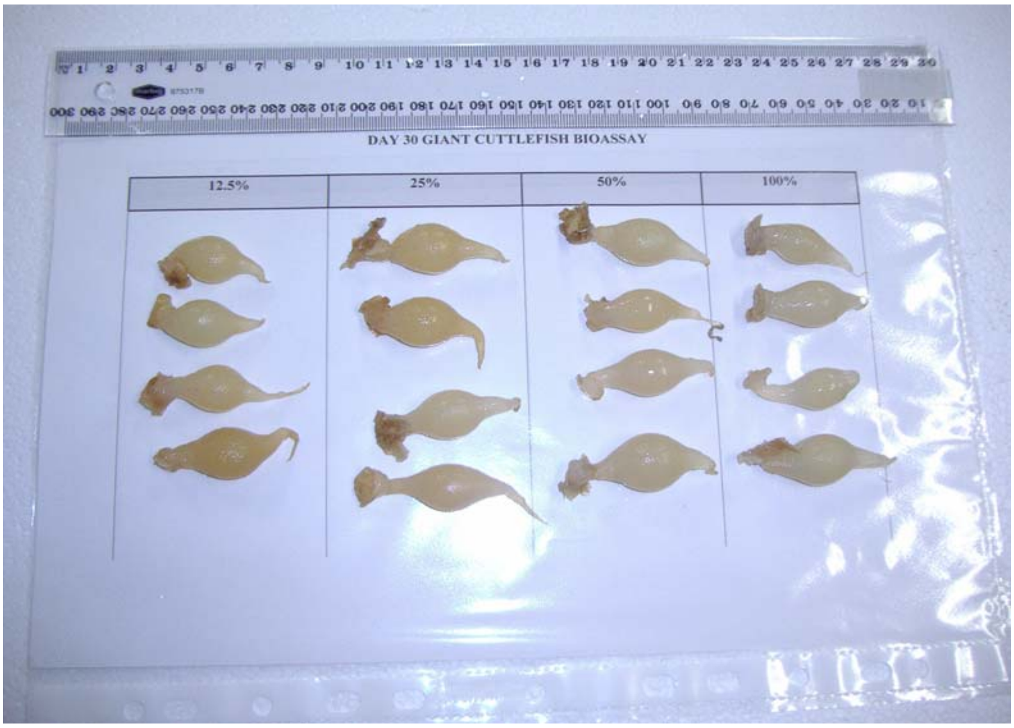


Table 5.3 Day 60 Summary

Concentration %	Length cm n =10	Width cm n = 10	Weight g n = 10	Stage n = 10
0	4.02 ± 0.25	1.78 ± 0.13	4.56 ± 1.17	27.20 ± 0.63
1.5	3.65 ± 0.54	1.67 ± 0.16	4.23 ± 0.91	26.00 ± 2.83
3.1	4.03 ± 0.33	1.72 ± 0.10	4.38 ± 0.54	24.10 ± 8.49
6.25	3.96 ± 0.25	1.73 ± 0.12	4.63 ± 0.58	22.30 ± 7.96
12.5	3.71 ± 0.33	1.50 ± 0.07	3.46 ± 0.43	0.0 ± 0.0
25	3.69 ± 0.42	1.54 ± 0.12	3.51 ± 0.52	0.0 ± 0.0
50	3.74 ± 0.58	1.50 ± 0.11	3.52 ± 0.76	0.0 ± 0.0
100	3.74 ± 0.60	1.51 ± 0.13	3.52 ± 0.59	0.0 ± 0.0

Photo 5.3 Embryos from Low Concentrations Day 60

Photo 5.4 Embryos from High Concentrations Day 60

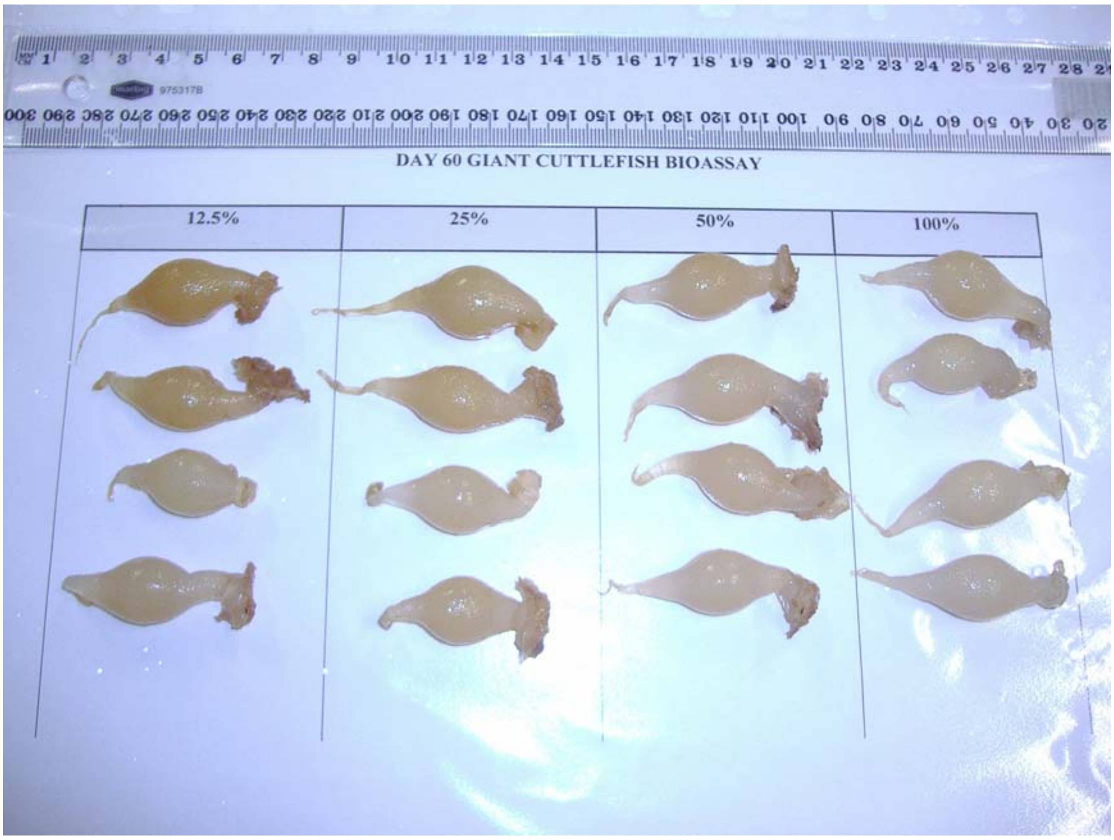


Table 5.4 Day 90 Summary

Concentration %	Length cm n =10	Width cm n = 10	Weight g n = 10	Stage n = 10
0	3.15 ± 0.51	2.12 ± 0.14	6.74 ± 1.11	29.80 ± 0.42
1.5	2.75 ± 0.69	1.78 ± 0.22	4.58 ± 1.52	14.8 ± 15.60
3.1	2.50 ± 0.66	1.96 ± 0.23	5.41 ± 1.83	20.30 ± 14.01
6.25	2.11 ± 0.25	1.52 ± 0.21	3.03 ± 1.20	7.40 ± 12.23
12.5	2.27 ± 0.16	1.68 ± 0.32	3.80 ± 0.45	0.0 ± 0.0

5.4 Staging of Embryos Collected from Point Lowly

The average stage of the embryos delivered to the laboratory on the 17th August 2006 was 21.92 ± 6.91.

Photo 5.5 Embryos from Low Concentrations Day 90

Table 5.5 Toxicity Data for Days 30,60 and 90

End Point	EC50 %	EC10 %	LOEC %	NOEC %
Day 30 Stage	10.34	4.61	6.25	3.13
Day 30 Length	>100	>100	>100	100
Day 30 Width	>100	>100	>100	100
Day 30 Weight	>100	>100	>100	100
Day 60 Stage	6.61	3.16	6.25	3.13
Day 60 Length	>100	>100	>100	100
Day 60 Width	>100	19.39	12.5	6.25
Day 60 Weight	>100	20.87	12.5	6.25
Day 90 Stage	2.56	0.66	1.5	<1.5
Day 90 Length	6.5	2.8	3.125	1.5
Day 90 Width	7.4	4.3	1.5	<1.5
Day 90 Weight	11.17	5.15	1.5	<1.5

5.5 Embryo Development

The number of developing embryos were determined in each replicate and the final number of developed embryos were used to calculate the EC50, EC10, LOEC and NOEC for Embryo Development. The results are in Table 5.6 and the toxicity results are in Table 5.7. The toxicity data summary sheet is found in Appendix 1.

Table 5.6 Embryo Development

Concentration %	Total Number	Embryos Developed
0	55	55
1.5	55	49
3.125	55	46
6.25	55	41
12.5	55	0
25	55	0
50	55	0
100	55	0

Table 5.7 Embryo Development Toxicity Data

End Point	EC50 %	EC10 %	LOEC %	NOEC %
Embryo Development	5.81	2.37	1.5	<1.5

5.6 Hatching

The number of days to hatch were determined for the five remaining cuttlefish in each replicate (Table 5.8) and these were used to calculate the EC50, EC10, LOEC and NOEC values (Table 5.9). Two of the cuttlefish were measured for length and width of mantle and weight (Table 5.10). These values were used to calculate the EC50, EC10, LOEC and NOEC (Table 5.11). Toxicity data summary sheets are found in Appendix 1.

Table 5.8 Days to Hatch

Concentration/ Replicate	Hatchling 1 Days	Hatchling 2 Days	Hatchling 3 Days	Hatchling 4 Days	Hatchling 5 Days
0%					
1	108	109	110	110	112
2	108	109	112	114	116
3	99	99	112	114	120
4	100	103	104	115	116
5	100	101	102	102	103
1.5%					
1	115	115	116	117	NH
2	112	113	113	114	115
3	113	114	116	116	118
4	102	103	114	115	116
5	105	111	112	116	118
3.125%					
1	117	117	119	NH	NH
2	108	110	112	115	NH
3	107	114	115	115	NH
4	94	94	105	106	115
5	111	112	112	117	118
6.25					
1	116	118	120	120	125
2	114	116	118	NH	NH
3	115	116	118	118	120
4	119	120	NH	NH	NH
5	111	115	117	117	119

NH = No Hatch

Table 5.9 Toxicity Data for Days to Hatch

End Point	EC50 %	EC10 %	LOEC %	NOEC %
Days to Hatch	5.42	3.16	1.5	<1.5

Table 5.10 Weight, Mantle Length and Width at Hatch

Concentration	Length cm n=10	Width cm n=10	Weight g n=10
0%	1.27 ± 0.11	1.05 ± 0.11	0.60 ± 0.12
1.5%	1.19 ± 0.11	1.04 ± 0.11	0.50 ± 0.17
3.125%	1.19 ± 0.10	1.03 ± 0.12	0.57 ± 0.18
6.25%	1.13 ± 0.06	0.94 ± 0.09	0.31 ± 0.11

Table 5.11 Toxicity Data at Hatch

End Point	EC50 %	EC10 %	LOEC %	NOEC %
Length at Hatch	7.51	6.51	6.25	3.125
Width at Hatch	7.42	6.24	6.25	3.125
Weight at Hatch	6.40	5.74	6.25	3.125

5.7 Post Hatch

The hatchlings were maintained for 30 days post hatch. Survival of the hatchlings was recorded (Table 5.12). On the 27th October the hatchlings were anaesthetized and the mantle length, mantle width and weight were measured and recorded (Table 5.13) (Photo 5.6). These data were used to calculate the EC50, EC10, LOEC and NOEC (Table 5.14). Toxicity data summary sheets are found in Appendix 1.

Table 5.12 Post Hatch Survival

Concentration %	Total Number	Number live
0	15	12
1.5	15	14
3.125	15	6
6.25	15	5
12.5	15	0

Table 5.13 Weight, Mantle Length and Width at Day 30 Post Hatch

Concentration	Length cm n=15	Width cm n=15	Weight g n=15
0%	1.38 ± 0.10	1.10 ± 0.09	0.63 ± 0.11
1.5%	1.32 ± 0.08	1.05 ± 0.08	0.61 ± 0.08
3.125%	1.29 ± 0.05	1.06 ± 0.05	0.58 ± 0.04
6.25%	1.22 ± 0.05	0.93 ± 0.02	0.52 ± 0.03

Table 5.14 Toxicity Data at Day 30 Post Hatch

End Point	EC50 %	EC10 %	LOEC %	NOEC %
Length at Day 30 Post Hatch	7.18	6.30	3.125	1.5
Width at Day 30 Post Hatch	7.11	6.18	1.5	<1.5
Weight at Day 30 Post Hatch	7.38	6.24	6.25	3.125
30 Day Post Hatch Survival	4.06	1.86	12.5	6.25

Photo 5.6 Hatchlings 30 Days Post Hatch

6. DISCUSSION

6.1 Physicochemistry

The water parameters remained stable for the 21 week duration of the bioassay due to the frequent water changes. The salinity ranged from 45.11 for the control Spencer Gulf seawater to 78.13 for the RO brine. The temperature of the test was maintained at 14 ± 0.25°C instead of the 11 – 12°C that would have been found in Spencer Gulf during winter. The reason for the increase in temperature was so that the test could finish in the required time frame. In their natural habitat the giant cuttlefish embryos take up to 5 months to hatch. This time frame was unrealistic for the test as the results were required mid November. The use of control embryos and embryos later collected from Spencer Gulf ensured that the higher temperature used in the test had no adverse impacts on the development and subsequent hatching of *S. apama*. Further, Palmegiano and D'Apote (1983) have shown that there are no interactive effects between salinity and temperature.

6.2 Day 30 Sampling

There were no significant differences between the length (P>0.05), width (P>0.05) and weight (P>0.05) of the eggs at day 30. However, there was a significant difference (P<0.05) between the stage data of the embryos at Day 30 for the different concentrations, with the 0% - 6.25% concentrations showing embryonic development and 12.5% - 100% showing minimal, if any, development. The toxicity data shows that a concentration of ~10% RO brine is sufficient to affect embryo development in 50% of exposed eggs. These results show that the stage of development is the most sensitive end point.

6.3 Day 60 Sampling

There was no significant difference between the length (P>0.05) of the eggs at Day 60, however, there was a trend towards shorter eggs at high concentrations. There were significant differences between widths (P<0.05) and weights (P<0.05) of eggs from different concentrations as the eggs became fatter and heavier as the embryos inside grew.

The stage of the embryos was significantly different (P<0.05) as embryos were developing in the eggs from 0% to 6.25% concentrations but not from the 12.5% to 100% concentrations. This is supported by the width and weight data. Again, at Day 60 the stage data is the most sensitive with an EC50 of 6.61%.

At this point the 25%, 50% and 100% treatments were terminated and all eggs fixed in a 10% formaldehyde in seawater solution.

6.4 Day 90 Sampling

The lengths, widths and weights of the eggs at day 90 were all significantly different (P<0.05) and were indicative of the growth of the embryos inside the eggs. Eggs became rounder and took up water as the embryos neared hatch. The staging data at Day 90 was again the most sensitive, with an EC50 of ~2.6%. However, the staging data is skewed as undeveloped embryos were removed from the 1.5% treatment, resulting in a sample that was not representative. This anomaly has been rectified in the embryo development data which includes all 11 embryos from each replicate.

6.5 Embryo Development

The embryo development toxicity data was calculated by using data from all 11 embryos in each replicate. The EC50 of 5.8% shows that 50% of exposed embryos will develop at this concentration. The NOEC is < 1.5% which was the lowest concentration tested. In hindsight, it would have been useful to have used a concentration of 0.75% to obtain a NOEC. The embryo development test has the most environmentally relevant endpoint generated from this study, as it encompasses RO brine exposure during all the sensitive developmental life stages of *S. apama*.

6.6 Days to hatch

Not all embryos in all concentrations hatched, with 25/25 hatching in 0%, 24/25 hatching in 1.5%, 21/25 hatching in 3.125%, 20/25 hatching in 6.25% and 0/25 hatching in 12.5% (Table 5.8). The time to hatch ranged from 94 up to 125 days (3 months – 4 months). The embryos in the higher concentrations took significantly (P<0.05) longer to hatch than the control embryos. A concentration of ~5.4% resulted in a 50% delay in hatching. This delay in hatching of exposed embryos may have significant implications in the survival of the hatchlings. If conditions are not optimal as far as food availability, availability of shelter, presence or absence of predators or water temperature are concerned when *S. apama* hatch then the survival of the hatchlings will be compromised.

The weight and mantle length of *S. apama* at hatch was significantly different (P<0.05) with the hatchlings exposed to 6.25% RO brine showing approximately half the body weight of the controls. The toxicity data (Table 5.11) shows that the length, width and weight all had similar EC50 values of 6.4 – 7.5%.

6.7 Post Hatch

The mantle length, mantle width and weight (Table 5.13) had all increased slightly from the data at hatch and all were significantly different (P<0.05) between treatments. Again the EC50 data for these parameters were similar to each other ranging from 7.11 – 7.38% RO brine.

The survival post hatch showed that this is a critical life stage, as only 12 of the 15 controls survived to 30 days post hatch (Table 5.12). The EC50 of ~4% RO brine shows that survival post hatch is the most sensitive end point to *S. apama* exposed to the RO brine (Table 5.14). In this instance post hatch survival is a chronic test and is not categorised as an acute test as it is the result of a long term exposure over several life stages. Therefore, no acute to chronic ratio should be applied to this data.

7. SPECIES PROTECTION LEVELS

7.1 Introduction

The BurrliOZ (CSIRO 2000) is commonly used to determine ecosystem protection values. In the past the NOEC has been used to derive these values simply because NOEC data was readily available. However, using NOECs as valid test points and as regulatory endpoints has been extensively criticised because of it dependence on the concentrations used in the test and its variability (Chapman 2005). It has been suggested that “the NOEC is neither a consistent summary statistic nor an indicator of safe concentrations of toxic chemicals” (Crane and Newman 2000). Therefore, a point estimation is preferred that considers the dose response relationship and is preferable to hypothesis testing as is used in determining the NOEC (Chapman 2005). A point estimation of EC10 has been recommended and is used commonly throughout Australia (Dr M. Warne CSIRO, Pers Comm., Chapman 2005). Therefore, these Protection Values for giant cuttlefish have been calculated using the EC10 values.

7.2 Methodology

Following the protocol outlined in ANZECC (2000) interim 99%, 95%, 90% and 80% protection values were calculated using data from eleven chronic endpoints in the *S. apama* exposure. Values in Table 7.1 were placed in the BurrliOZ software to calculate a value designed to protect 99%, 95%, 90% and 80% of the *S. apama* from brine discharged from the proposed desalination plant at Point Lowly. The value calculated is an interim value, which will change when further testing is performed after commissioning of the plant and additional data becomes available.

12.2.a Statistical Methodology

The BurrliOZ software is designed to estimate the protecting concentrations of chemicals such that a given percentage of species will survive. The estimations of the protecting concentrations are computed by fitting a certain distribution to the input data. The distribution, called the Burr III distribution, is that required by the Environment Protection Authority. There are other distributions fitted to the data, the normal and the log-logistic distributions. However, these two latter distributions are provided only as a reference guide and are not used for the estimation of the protecting concentrations.

After the Burr III distribution has been fitted to the data, the protecting concentration (for preserving, for example, 90% of the species) is estimated using the estimated distribution parameters to compute the concentration such that the probability of there being a greater concentration (according to the fitted distribution) is 90%.

Once the protecting concentration has been computed, an estimate for the lower confidence limit of 50% can be computed. This value can be used as a very conservative (lower) estimate for the protecting concentration.

The EC10 values shown in Table 7.1 are representative of the important endpoints in each of the life stages exposed during the 5 month test. The Stage at Day 90 data was omitted due to skewing by the removal of dead embryos.

7.3 Results

Table 7.1 EC10 Data used in BurrliOZ calculation

End Point	EC10 %
Embryo Development	2.37
Day 30 Stage	4.61
Day 60 Stage	3.16
Days to Hatch	3.16
Length at Hatch	6.51
Width at Hatch	6.24
Weight at Hatch	5.74
Length 30 Days Post Hatch	6.30
Width 30 Days Post Hatch	6.18
Weight 30 Days Post Hatch	6.24
Survival Post Hatch	1.86

Table 7.2 BurrliOZ Protection Levels

Protection Level	Protection Value %	Dilution Factor
99	0.97	103
95	1.89	53
90	2.52	40
80	3.35	30

7.4 Discussion

Due to the reasons discussed above the use of the Interim Protection Values derived from EC10s is recommended. The Protection Values based on EC10 data would theoretically protect X% of species from experiencing inhibitory impacts greater than 10%. Therefore, to protect 99% of the *S. apama* from experiencing inhibitory impacts greater than 10%, RO brine would need to be diluted approximately 100 times.

New Protection Values should be generated using results from the actual brine discharged from the desalination plant after completion.

The use of sub-lethal testing is always recommended to remove the correction factors that are required when using LC50 data.

7.4.1 Seawater Diluent

The seawater from Spencer Gulf that was used in this project had a salinity of 45 ppt, as measured by refractometer, which is higher than typically recorded at Point Lowly. This may have been due to a range of factors e.g. evaporation from containers during transport, or lack of precision in the instrument used to measure salinity, and may have been compounded by an initial salinity at the extreme of its natural range.

If the *S. apama* embryos are growing at the upper limit of their salinity tolerance at 45 ppt, then any increase in salinity may have a greater impact on them than if they were growing at a lower salinity within the range of salinities found at Point Lowly. Therefore, the dilution values calculated in Table 7.2 may be 2 – 3 times higher than actually required (based on recalculated EC50 data, Table 7.4). Table 7.3 shows the concentrations of RO brine that are required to meet the salinities used in this project with a diluent seawater of 42 ppt.

Table 7.3 Concentrations Tested

Original Concentrations Tested %	Adjusted Concentrations for Control = 42 ppt %	Salinity ppt
0	8.33	45
1.5	9.71	45.5
3.1	11.18	46
6.25	14.06	47
12.5	19.79	49.125
25	31.25	53.25
50	54.17	61.5
100	100	78

Table 7.4 Recalculated EC50 Results

End Point	EC50 % 42 ppt Diluent	EC50 % 45 ppt Diluent	Difference Factor
Days to Hatch	12.2	5.42	2.25
Post Hatch Survival	12.6	4.00	3.15
Embryo Development	14.4	5.81	2.5

8. REFERENCES

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APPENDIX 1

SUMMARY SHEETS FOR TOXICITY DATA

Embryo Development

					Giant Cuttlefish Embryo Development		
Start Date:	8/06/2006	Test ID:	ENV06-128			Sample ID:	Brine RO
End Date:	13/10/2006	Lab ID:	Freo			Sample Type:	Brine
Sample Date:		Protocol:	Geotech WI			Test Species:	Sepia apama
Comments:							

Conc-%	1	2	3	4	5
Control	1.0000	1.0000	1.0000	1.0000	1.0000
1.5	0.8182	0.9091	0.9091	0.9091	0.9091
3.125	0.8182	0.7273	0.8182	1.0000	0.8182
6.25	0.8182	0.6364	0.9091	0.5455	0.8182
12.5	0.0000	0.0000	0.0000	0.0000	0.0000
25	0.0000	0.0000	0.0000	0.0000	0.0000
50	0.0000	0.0000	0.0000	0.0000	0.0000
100	0.0000	0.0000	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
Control	1.0000	1.0000	1.4195	1.4195	1.4195	0.000	5			0	55
*1.5	0.8909	0.8909	1.2377	1.1303	1.2645	4.850	5	15.00	17.00	6	55
3.125	0.8364	0.8364	1.1663	1.0213	1.4195	12.789	5	17.50	17.00	9	55
*6.25	0.7455	0.7455	1.0559	0.8309	1.2645	16.589	5	15.00	17.00	14	55
12.5	0.0000	0.0000	0.1513	0.1513	0.1513	0.000	5			55	55
25	0.0000	0.0000	0.1513	0.1513	0.1513	0.000	5			55	55
50	0.0000	0.0000	0.1513	0.1513	0.1513	0.000	5			55	55
100	0.0000	0.0000	0.1513	0.1513	0.1513	0.000	5			55	55

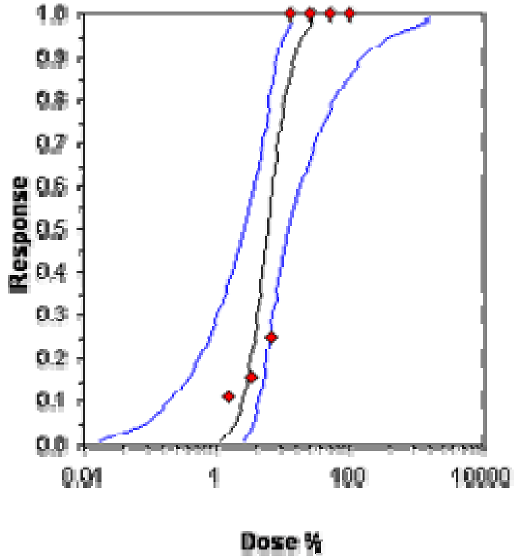
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.92795	0.868	0.3631	1.31152
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	<1.5	1.5		

Parameter	Value	SE	95% Fiducial Limits		Maximum Likelihood-Probit Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	3.28268	0.9079	0.94884	5.61652	0	42.6229	11.0705	4.4E-08	0.76443	0.30463	7
Intercept	2.49061	0.76551	0.52279	4.45842							
TSCR											

Point	Probits	%	95% Fiducial Limits	
EC01	2.674	1.137	0.01689	2.54948
EC05	3.355	1.83385	0.08457	3.5203
EC10	3.718	2.36611	0.19651	4.24586
EC15	3.964	2.81	0.34316	4.87345
EC20	4.158	3.22145	0.5289	5.49489
EC25	4.326	3.62213	0.75832	6.15716
EC40	4.747	4.86695	1.74297	8.84656
EC50	5.000	5.81344	2.64242	11.9722
EC60	5.253	6.94401	3.68411	17.6181
EC75	5.674	9.33045	5.46776	39.2024
EC80	5.842	10.491	6.17127	55.8016
EC85	6.036	12.0271	6.99937	85.4993
EC90	6.282	14.2834	8.07349	148.572
EC95	6.645	18.4291	9.77984	343.747
EC99	7.326	29.724	13.5563	1713.97

Significant heterogeneity detected (p = 4.41E-08)



Day 30 Stage

Giant Cuttlefish Day 30 Stage										
Start Date:	8/06/2009		Test ID:	ENV06-128			Sample ID:	BHP RO		
End Date:	8/07/2006		Lab ID:	Freo			Sample Type:	Brine		
Sample Date:			Protocol:	GEOTECH WI			Test Species:	Sepia Apama		
Comments:										
Conc-%	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Control	0.9390	1.0000	0.8450	1.0000	0.9390	0.9860	1.0000	0.8450	1.0000	0.8450
1.5	1.0000	1.0000	1.0000	0.9390	1.0000	1.0000	1.0000	1.0000	1.0000	0.8450
3.13	0.8450	0.8450	0.9390	0.8920	0.9860	0.9390	0.8450	0.9860	0.9860	1.0000
6.25	0.8450	0.8450	0.8450	0.9390	0.8450	0.8450	0.8450	0.9390	0.8450	0.8450
12.5	0.0000	0.0000	0.0000	0.0000	0.8450	0.8450	0.0000	0.0000	0.0000	0.0000
25	0.0000	0.0000	0.0000	0.0000	0.0000	0.8450	0.8450	0.0000	0.0000	0.0000
50	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
100	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root						1-Tailed		
Conc-%	**Mean**	**N-Mean**	**Mean**	**Min**	**Max**	**CV%**	**N**	**Rank Sum**	**Critical**	**Number Resp**	**Total Number**
Control	0.9399	1.0000	1.3676	1.1661	1.5208	11.614	10			60	1000
1.5	0.9784	1.0410	1.4654	1.1661	1.5208	8.354	10	124.50	75.00	22	1000
3.13	0.9263	0.9855	1.3254	1.1661	1.5208	10.385	10	95.00	75.00	74	1000
*6.25	0.8638	0.9190	1.1972	1.1661	1.3212	5.462	10	75.00	75.00	140	1000
*12.5	0.1690	0.1798	0.2732	0.0500	1.1661	172.226	10	58.00	75.00	832	1000
*25	0.1690	0.1798	0.2732	0.0500	1.1661	172.226	10	58.00	75.00	832	1000
50	0.0000	0.0000	0.0500	0.0500	0.0500	0.000	10			1000	1000
100	0.0000	0.0000	0.0500	0.0500	0.0500	0.000	10			1000	1000

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates non-normal distribution (p <= 0.01)	1.62766	1.035	2.15269	5.02664
Bartlett's Test indicates unequal variances (p = 4.93E-09)	47.3001	15.0863		

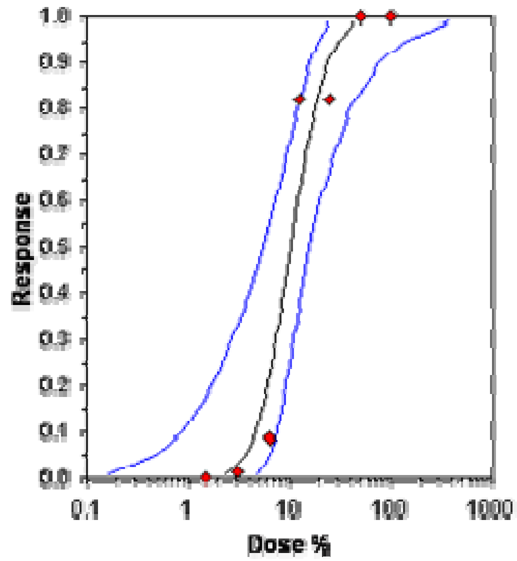
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	3.13	6.25	4.42295	31.9489

Parameter	Value	SE	95% Fiducial Limits	
Slope	3.65284	0.87224	1.41068	5.895
Intercept	1.2935	0.95687	-1.1662	3.75322
TSCR	0.03892	0.03509	-0.0513	0.12912

Maximum Likelihood-Probit Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
0.06	357.535	11.0705	4.2E-75	1.01469	0.27376	5

Point	Probits	%	95% Fiducial Limits	
EC01	2.674	2.38687	0.16305	4.79
EC05	3.355	3.6677	0.48433	6.40033
EC10	3.718	4.6116	0.85905	7.52475
EC15	3.964	5.38214	1.25809	8.43604
EC20	4.158	6.08537	1.69613	9.27975
EC25	4.326	6.76147	2.18189	10.1155
EC40	4.747	8.81724	3.99303	12.9568
EC50	5.000	10.344	5.54469	15.5773
EC60	5.253	12.1351	7.38265	19.531
EC75	5.674	15.8248	10.6703	31.6767
EC80	5.842	17.5829	11.9733	39.5847
EC85	6.036	19.8803	13.4923	52.0958
EC90	6.282	23.202	15.4366	74.7612
EC95	6.645	29.1731	18.4789	130.232
EC99	7.326	44.828	25.0926	380.669

Significant heterogeneity detected (p = 4.17E-75)



Day 30 Length

Day 30 Length										
Start Date:	8/06/2009	Test ID:	ENV06-128			Sample ID:	BHP RO			
End Date:	8/07/2006	Lab ID:	Freo			Sample Type:	Brine			
Sample Date:		Protocol:	GEOTECH WI			Test Species:	Sepia Apama			
Comments:										

Conc-%	1	2	3	4	5	6	7	8	9	10
Control	1.0000	1.0000	1.0000	0.9130	1.0000	1.0000	1.0000	0.9210	0.8820	1.0000
1.5	1.0000	1.0000	0.9820	1.0000	0.7510	1.0000	1.0000	0.9640	1.0000	0.9950
3.13	0.9100	0.8740	1.0000	0.8330	1.0000	0.8770	1.0000	0.9410	0.9920	0.8740
6.25	0.8620	0.8620	0.9080	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
12.5	0.8670	0.8670	0.8310	0.9740	0.9920	1.0000	1.0000	0.8230	1.0000	1.0000
25	1.0000	0.9130	1.0000	1.0000	0.7380	1.0000	1.0000	1.0000	1.0000	1.0000
50	0.8670	1.0000	0.9380	1.0000	1.0000	1.0000	0.8280	0.9280	1.0000	0.9950
100	0.9490	0.8720	1.0000	0.8740	0.9950	0.9640	1.0000	1.0000	0.8820	0.8690

			Transform: Arcsin Square Root						1-		
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Rank Sum	Tailed Critical	Number Resp	Total Number
Control	0.9716	1.0000	1.4423	1.2201	1.5208	8.835	10			29	1000
1.5	0.9692	0.9975	1.4489	1.0484	1.5208	10.262	10	103.00	74.00	32	1000
3.13	0.9301	0.9573	1.3413	1.1498	1.5208	11.414	10	81.50	74.00	71	1000
6.25	0.9632	0.9914	1.4288	1.1902	1.5208	10.451	10	101.50	74.00	37	1000
12.5	0.9354	0.9627	1.3652	1.1366	1.5208	12.646	10	87.00	74.00	65	1000
25	0.9651	0.9933	1.4471	1.0334	1.5208	11.411	10	108.50	74.00	35	1000
50	0.9556	0.9835	1.4063	1.1432	1.5208	10.764	10	96.50	74.00	44	1000
100	0.9405	0.9680	1.3619	1.2004	1.5208	10.669	10	84.00	74.00	61	1000

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates non-normal distribution (p <= 0.01)	2.50207	1.035	-0.8815	-0.1859
Bartlett's Test indicates equal variances (p = 1.00)	0.95928	18.4753		

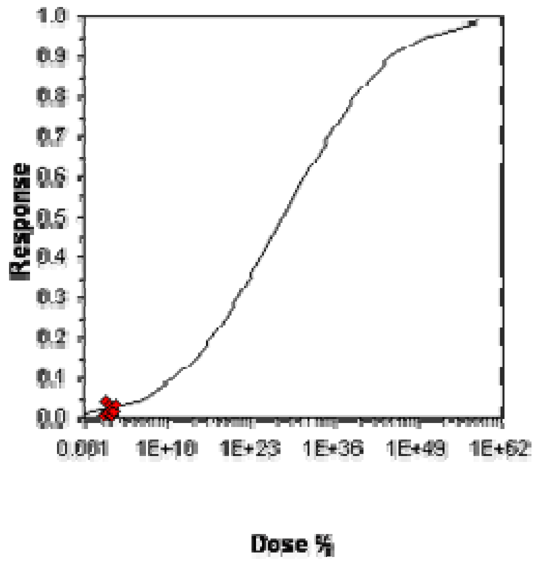
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	100	>100		1

Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	0.07597	0.22981	-0.5148	0.66672	0.029	32.7108	11.0705	4.3E-06	27.9159	13.1638	4
Intercept	2.87934	0.40693	1.8333	3.92538							
TSCR	0.02899	0.01357	-0.0059	0.06387							

Point	Probits	%	95% Fiducial Limits
EC01	2.674	0.00196	
EC05	3.355	1833825	
EC10	3.718	1.1E+11	
EC15	3.964	1.9E+14	
EC20	4.158	6.9E+16	
EC25	4.326	1.1E+19	
EC40	4.747	3.8E+24	
EC50	5.000	8.2E+27	
EC60	5.253	1.8E+31	
EC75	5.674	6.2E+36	
EC80	5.842	9.9E+38	
EC85	6.036	3.6E+41	
EC90	6.282	6.1E+44	
EC95	6.645	3.7E+49	
EC99	7.326	3.5E+58	

Significant heterogeneity detected (p = 4.30E-06)



Day 30 Width

						Giant Cuttlefish Day 30 Width				
Start Date:	8/06/2009		Test ID:	ENV06-128			Sample ID:	BHP RO		
End Date:	8/07/2006		Lab ID:	Freo			Sample Type:	Brine		
Sample Date:			Protocol:	GEOTECH WI			Test Species:	Sepia Apama		
Comments:										

Conc-%	1	2	3	4	5	6	7	8	9	10
Control	1.0000	1.0000	0.9810	0.9120	1.0000	1.0000	1.0000	1.0000	0.9810	0.9060
1.5	0.9940	1.0000	0.9870	0.9870	0.7990	0.9120	0.8990	0.9940	1.0000	1.0000
3.13	0.8990	0.9810	0.9870	0.9870	0.9750	0.8990	1.0000	0.9870	1.0000	1.0000
6.25	0.9060	0.8930	0.9750	1.0000	1.0000	1.0000	1.0000	0.9120	0.9060	1.0000
12.5	0.9060	0.9120	0.8990	0.9430	0.9940	0.9810	0.8240	0.8930	1.0000	0.9810
25	0.6920	1.0000	0.9060	0.9120	0.8740	1.0000	1.0000	0.9810	1.0000	0.9120
50	0.8870	0.9870	0.9120	1.0000	0.9940	0.9750	0.9940	0.9940	0.9060	0.8990
100	0.7990	0.9940	0.9810	0.9810	0.9120	0.9940	0.9120	0.9120	0.9120	1.0000

			Transform: Arcsin Square Root								
								Rank	1-Tailed	Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
Control	0.9780	1.0000	1.4518	1.2592	1.5208	7.245	10			22	1000
1.5	0.9572	0.9787	1.4085	1.1059	1.5208	10.413	10	93.50	74.00	43	1000
3.13	0.9715	0.9934	1.4271	1.2474	1.5208	7.153	10	93.00	74.00	27	1000
6.25	0.9592	0.9808	1.4041	1.2376	1.5208	9.375	10	94.50	74.00	40	1000
12.5	0.9333	0.9543	1.3360	1.1379	1.5208	9.489	10	76.00	74.00	68	1000
25	0.9277	0.9486	1.3504	0.9825	1.5208	13.517	10	89.50	74.00	73	1000
50	0.9548	0.9763	1.3873	1.2280	1.5208	8.734	10	82.00	74.00	45	1000
100	0.9397	0.9608	1.3557	1.1059	1.5208	10.106	10	82.00	74.00	62	1000

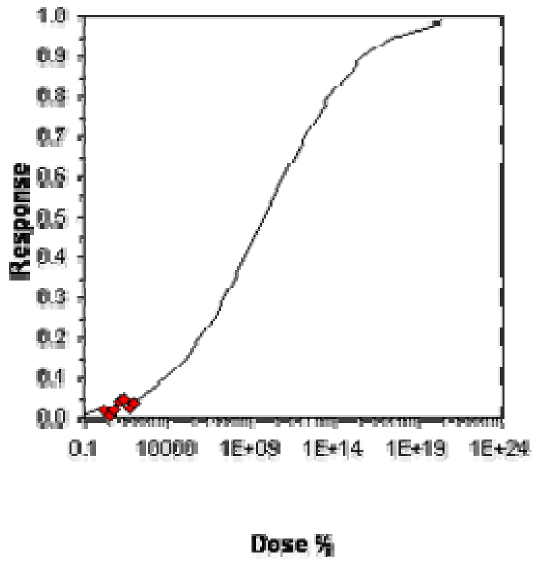
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates non-normal distribution (p <= 0.01)	1.62797	1.035	-0.6144	-0.4619
Bartlett's Test indicates equal variances (p = 0.74)	4.37383	18.4753		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	100	>100		1

Parameter	Value	SE	95% Fiducial Limits		Maximum Likelihood-Probit Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	0.21745	0.1634	-0.2026	0.63748	0.022	24.4556	11.0705	1.8E-04	9.79697	4.59868	4
Intercept	2.86961	0.27001	2.17552	3.5637							
TSCR	0.02155	0.01013	-0.0045	0.0476							

Point	Probits	%	95% Fiducial Limits
EC01	2.674	0.12556	
EC05	3.355	170.931	
EC10	3.718	8008.12	
EC15	3.964	107337	
EC20	4.158	844555	
EC25	4.326	4956914	
EC40	4.747	4.3E+08	
EC50	5.000	6.3E+09	
EC60	5.253	9.2E+10	
EC75	5.674	7.9E+12	
EC80	5.842	4.6E+13	
EC85	6.036	3.7E+14	
EC90	6.282	4.9E+15	
EC95	6.645	2.3E+17	
EC99	7.326	3.1E+20	

Significant heterogeneity detected (p = 1.77E-04)



Day 30 Weight

						Giant Cuttlefish Day 30 Weight						
Start Date:	8/06/2009		Test ID:	ENV06-128			Sample ID:	BHP RO				
End Date:	8/07/2006		Lab ID:	Freo			Sample Type:	Brine				
Sample Date:			Protocol:	GEOTECH WI			Test Species:	Sepia Apama				
Comments:												
Conc-%	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**		
Control	1.0000	1.0000	0.8500	0.8000	1.0000	0.9500	1.0000	1.0000	0.8500	1.0000		
1.5	0.9250	1.0000	0.9000	1.0000	0.6000	0.9000	0.9750	0.9250	1.0000	1.0000		
3.13	0.8500	0.7750	0.9000	0.7750	0.9000	0.7250	1.0000	0.8500	1.0000	0.9750		
6.25	0.6750	0.7500	0.7250	1.0000	1.0000	0.9500	1.0000	0.9750	1.0000	1.0000		
12.5	0.7500	0.7500	0.7500	0.9750	0.8500	0.9250	0.8750	0.7250	1.0000	0.9000		
25	1.0000	1.0000	0.8750	0.9250	0.5750	1.0000	0.8750	0.9250	1.0000	0.9500		
50	0.7500	0.9750	0.8250	1.0000	0.9750	0.8250	0.9250	0.9000	0.8250	0.9750		
100	0.6250	0.9250	0.9500	0.7750	0.9000	1.0000	0.9250	0.8750	0.9250	1.0000		

			Transform: Arcsin Square Root						1-		
								Rank	Tailed	Number	Total
Conc-%	**Mean**	**N-Mean**	**Mean**	**Min**	**Max**	**CV%**	**N**	**Sum**	**Critical**	**Resp**	**Number**
Control	0.9450	1.0000	1.3923	1.1071	1.5208	12.638	10			55	1000
1.5	0.9225	0.9762	1.3466	0.8861	1.5208	14.902	10	99.00	74.00	78	1000
3.13	0.8750	0.9259	1.2470	1.0188	1.5208	14.598	10	83.00	74.00	126	1000
6.25	0.9075	0.9603	1.3391	0.9642	1.5208	17.579	10	97.50	74.00	92	1000
12.5	0.8500	0.8995	1.2018	1.0188	1.5208	14.214	10	77.00	74.00	150	1000
25	0.9125	0.9656	1.3295	0.8607	1.5208	15.814	10	98.50	74.00	87	1000
50	0.8975	0.9497	1.2764	1.0472	1.5208	12.386	10	83.00	74.00	104	1000
100	0.8900	0.9418	1.2714	0.9117	1.5208	14.430	10	87.50	74.00	112	1000

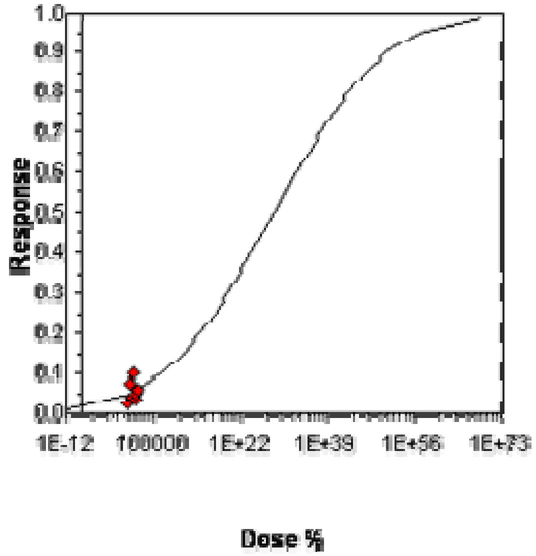
Auxiliary Tests	**Statistic**	**Critical**	**Skew**	**Kurt**
Kolmogorov D Test indicates non-normal distribution (p <= 0.01)	1.21379	1.035	-0.5159	-0.3389
Bartlett's Test indicates equal variances (p = 0.96)	2.00884	18.4753		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	100	>100		1

Parameter	Value	SE	95% Fiducial Limits		Maximum Likelihood-Probit Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	0.05814	0.16179	-0.3578	0.47405	0.055	38.2275	11.0705	3.4E-07	28.5852	17.1996	3
Intercept	3.33803	0.27503	2.63104	4.04502							
TSCR	0.05494	0.01993	0.00372	0.10616							

Point	Probits	%	95% Fiducial Limits
EC01	2.674	3.7E-12	
EC05	3.355	1.96966	
EC10	3.718	3491717	
EC15	3.964	5.7E+10	
EC20	4.158	1.3E+14	
EC25	4.326	9.6E+16	
EC40	4.747	1.7E+24	
EC50	5.000	3.8E+28	
EC60	5.253	8.8E+32	
EC75	5.674	1.5E+40	
EC80	5.842	1.2E+43	
EC85	6.036	2.6E+46	
EC90	6.282	4.2E+50	
EC95	6.645	7.5E+56	
EC99	7.326	4E+68	

Significant heterogeneity detected (p = 3.40E-07)



Day 60 Stage

					Giant Cuttlefish Day 60 Stage					
Start Date:	8/06/2009	Test ID:	ENV06-128			Sample ID:	BHP RO			
End Date:	8/08/2006	Lab ID:	Freo			Sample Type:	Brine			
Sample Date:		Protocol:	GEOTECH WI			Test Species:	Sepia Apama			
Comments:										
Conc-%	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Control	0.9930	0.9930	0.9930	0.9930	1.0000	0.9560	0.9930	0.9930	1.0000	1.0000
1.5	0.9930	0.9930	0.9930	0.9930	0.9930	0.9930	0.6620	0.9930	0.9560	0.9930
3.13	0.9930	0.9930	0.9190	0.9930	0.9930	0.9930	0.9930	0.9930	0.0000	0.9930
6.25	0.9560	0.9190	0.9560	0.9190	0.9190	0.7720	0.9190	0.9190	0.9190	0.0000
12.5	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
25	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
50	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
100	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root						1-Tailed	Number	Total
Conc-%	**Mean**	**N-Mean**	**Mean**	**Min**	**Max**	**CV%**	**N**	**Rank Sum**	**Critical**	**Resp**	**Number**
Control	0.9914	1.0000	1.4844	1.3595	1.5208	3.145	10			10	1000
1.5	0.9562	0.9645	1.4206	0.9504	1.4870	11.968	10	87.50	77.00	46	1000
3.13	0.8863	0.8940	1.3228	0.0500	1.4870	34.156	10	87.00	77.00	116	1000
*6.25	0.8198	0.8269	1.1535	0.0500	1.3595	34.294	10	56.00	77.00	179	1000
12.5	0.0000	0.0000	0.0500	0.0500	0.0500	0.000	10			1000	1000
25	0.0000	0.0000	0.0500	0.0500	0.0500	0.000	10			1000	1000
50	0.0000	0.0000	0.0500	0.0500	0.0500	0.000	10			1000	1000
100	0.0000	0.0000	0.0500	0.0500	0.0500	0.000	10			1000	1000

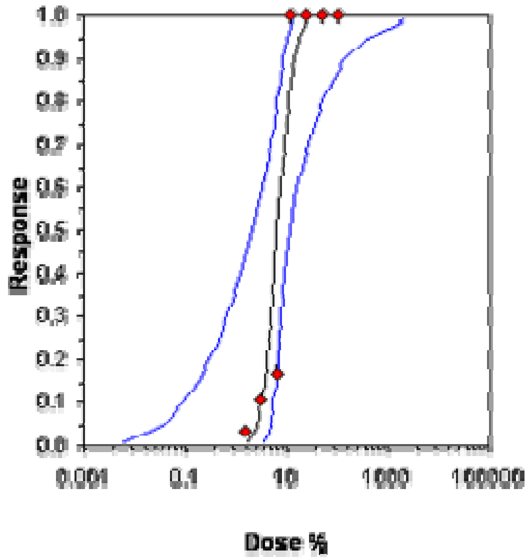
Auxiliary Tests	**Statistic**	**Critical**	**Skew**	**Kurt**
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.5394	0.919	-3.4205	12.0088
Bartlett's Test indicates unequal variances (p = 3.42E-07)	32.8748	11.3449		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	3.13	6.25	4.42295	31.9489

					Maximum Likelihood-Probit						
Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	3.98973	1.22109	0.85082	7.12864	0.01	850.257	11.0705	1.6E-181	0.82043	0.25064	8
Intercept	1.72671	1.05831	-0.9938	4.44717							
TSCR											

Point	Probits	%	95% Fiducial Limits	
EC01	2.674	1.72721	0.00592	3.70018
EC05	3.355	2.55951	0.03627	4.762
EC10	3.718	3.15658	0.09432	5.50401
EC15	3.964	3.63625	0.17842	6.11384
EC20	4.158	4.06895	0.29417	6.69047
EC25	4.326	4.48097	0.44868	7.27778
EC40	4.747	5.71385	1.23699	9.4544
EC50	5.000	6.61346	2.13435	11.8043
EC60	5.253	7.65471	3.37209	16.0958
EC75	5.674	9.76079	5.65663	34.3647
EC80	5.842	10.7492	6.49629	49.6461
EC85	6.036	12.0283	7.41752	78.4491
EC90	6.282	13.8561	8.52286	143.463
EC95	6.645	17.0884	10.1363	362.587
EC99	7.326	25.3228	13.3667	2166.94

Significant heterogeneity detected (p = 1.55E-181)



Day 60 Length

						Giant Cuttlefish Day 60 Length					
Start Date:	8/06/2009		Test ID:	ENV06-128			Sample ID:	BHP RO			
End Date:	8/08/2006		Lab ID:	Freo			Sample Type:	Brine			
Sample Date:			Protocol:	GEOTECH WI			Test Species:	Sepia Apama			
Comments:											
Conc-%	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	
Control	1.0000	1.0000	0.9750	1.0000	1.0000	1.0000	1.0000	1.0000	0.8930	0.8960	
1.5	1.0000	1.0000	0.8410	1.0000	1.0000	0.7660	0.7260	0.8080	0.8530	0.8980	
3.13	0.9450	1.0000	0.8480	0.9730	1.0000	1.0000	0.9300	1.0000	1.0000	1.0000	
6.25	1.0000	0.9630	1.0000	0.9750	1.0000	1.0000	0.9050	0.9900	0.8830	0.9750	
12.5	0.9300	1.0000	0.9430	1.0000	0.8560	0.8510	1.0000	0.8960	0.8510	0.8960	
25	0.9300	1.0000	0.8410	0.8530	1.0000	1.0000	0.8860	0.9680	0.8080	0.7740	
50	1.0000	0.8980	0.8180	1.0000	0.6390	1.0000	0.8580	1.0000	0.8980	0.9700	
100	0.8510	1.0000	1.0000	1.0000	0.9680	0.5550	1.0000	0.9000	0.8980	1.0000	

			Transform: Arcsin Square Root						1-Tailed		
Conc-%	**Mean**	**N-Mean**	**Mean**	**Min**	**Max**	**CV%**	**N**	**Rank Sum**	**Critical**	**Number Resp**	**Total Number**
Control	0.9764	1.0000	1.4537	1.2376	1.5208	8.093	10			23	1000
1.5	0.8892	0.9107	1.2870	1.0199	1.5208	16.327	10	83.00	74.00	110	1000
3.13	0.9696	0.9930	1.4338	1.1703	1.5208	8.781	10	100.00	74.00	31	1000
6.25	0.9691	0.9925	1.4234	1.2217	1.5208	7.794	10	93.00	74.00	32	1000
12.5	0.9223	0.9446	1.3211	1.1745	1.5208	11.138	10	81.50	74.00	77	1000
25	0.9060	0.9279	1.3013	1.0754	1.5208	13.511	10	78.50	74.00	94	1000
50	0.9081	0.9300	1.3212	0.9263	1.5208	15.708	10	87.00	74.00	91	1000
100	0.9172	0.9394	1.3505	0.8405	1.5208	16.722	10	93.50	74.00	82	1000

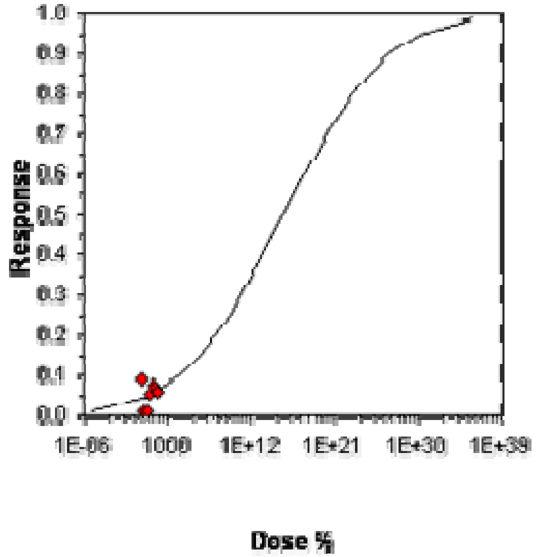
Auxiliary Tests	**Statistic**	**Critical**	**Skew**	**Kurt**
Kolmogorov D Test indicates non-normal distribution (p <= 0.01)	1.21618	1.035	-0.4901	-0.0804
Bartlett's Test indicates equal variances (p = 0.23)	9.29435	18.4753		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	100	>100		1

Parameter	Value	SE	95% Fiducial Limits		Maximum Likelihood-Probit Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	0.1132	0.20985	-0.4262	0.65263	0.023	84.6414	11.0705	9.0E-17	15.5051	8.83386	3
Intercept	3.24481	0.32912	2.39879	4.09083							
TSCR	0.02321	0.01958	-0.0271	0.07354							

Point	Probits	%	95% Fiducial Limits
EC01	2.674	9E-06	
EC05	3.355	9.43399	
EC10	3.718	15277.7	
EC15	3.964	2235620	
EC20	4.158	1.2E+08	
EC25	4.326	3.5E+09	
EC40	4.747	1.8E+13	
EC50	5.000	3.2E+15	
EC60	5.253	5.5E+17	
EC75	5.674	2.9E+21	
EC80	5.842	8.7E+22	
EC85	6.036	4.6E+24	
EC90	6.282	6.7E+26	
EC95	6.645	1.1E+30	
EC99	7.326	1.1E+36	

Significant heterogeneity detected (p = 8.95E-17)



Day 60 Width

Giant Cuttlefish Day 60 Egg Width

Start Date:	8/06/2009	Test ID:	ENV06-128	Sample ID:	BHP RO
End Date:	8/08/2006	Lab ID:	Freo	Sample Type:	Brine
Sample Date:		Protocol:	GEOTECH WI	Test Species:	Sepia Apama
Comments:					

Conc-%	1	2	3	4	5	6	7	8	9	10
Control	0.9780	0.9890	1.0000	0.9780	1.0000	0.9040	0.9160	0.9830	1.0000	0.9720
1.5	0.8880	1.0000	0.9160	0.9940	1.0000	0.9150	0.8430	0.9040	0.8760	0.9940
3.13	0.8990	0.9780	0.9040	0.9780	1.0000	0.9830	0.9890	0.9890	0.8820	0.9940
6.25	1.0000	0.9100	0.9270	0.9040	1.0000	0.9040	0.9830	0.9890	1.0000	0.9160
12.5	0.8820	0.9150	0.0803	0.8090	0.9040	0.8710	0.8150	0.8710	0.7920	0.8880
25	0.8760	0.8930	0.7980	0.8710	0.8030	0.8150	0.9720	0.9780	0.8090	0.8200
50	0.8930	0.8820	0.7920	0.8760	0.7080	0.9100	0.7980	0.8150	0.8820	0.8880
100	0.8090	0.8880	0.8150	0.9660	0.8880	0.7190	0.8200	0.7920	0.9100	0.8800

			Transform: Arcsin Square Root						1-		
								Rank	Tailed	Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
Control	0.9720	1.0000	1.4247	1.2558	1.5208	6.640	10			28	1000
1.5	0.9330	0.9599	1.3439	1.1634	1.5208	10.741	10	89.00	74.00	67	1000
3.13	0.9596	0.9872	1.3953	1.2201	1.5208	7.949	10	97.50	74.00	41	1000
6.25	0.9533	0.9808	1.3820	1.2558	1.5208	8.754	10	98.00	74.00	47	1000
*12.5	0.7827	0.8053	1.1016	0.2873	1.2750	26.533	10	56.50	74.00	217	1000
*25	0.8635	0.8884	1.2069	1.1047	1.4219	9.766	10	61.50	74.00	136	1000
*50	0.8444	0.8687	1.1712	0.9999	1.2661	7.225	10	56.00	74.00	155	1000
*100	0.8487	0.8731	1.1814	1.0121	1.3853	8.914	10	58.00	74.00	150	1000

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates normal distribution (p > 0.01)	0.7905	1.035	-2.3986	12.8125
Bartlett's Test indicates unequal variances (p = 1.15E-03)	23.9819	18.4753		

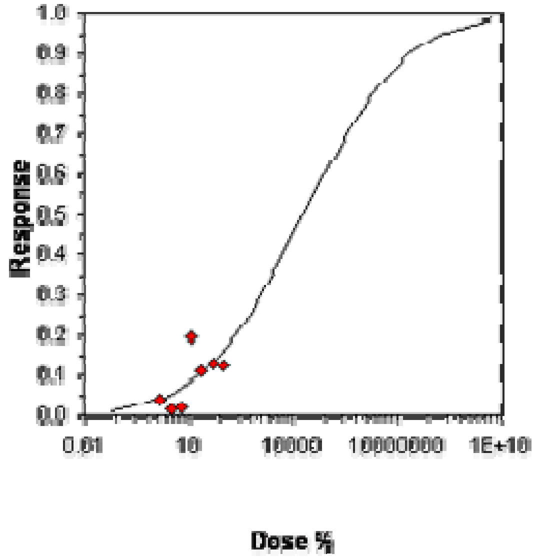
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	6.25	12.5	8.83883	16

Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	0.42402	0.27196	-0.2751	1.12311	0.028	168.345	11.0705	1.7E-34	4.30993	2.35836	5
Intercept	3.17249	0.41751	2.09924	4.24574							
TSCR	0.02549	0.02877	-0.0485	0.09945							

Point	Probits	%	95% Fiducial Limits
EC01	2.674	0.06661	
EC05	3.355	2.6963	
EC10	3.718	19.3895	
EC15	3.964	73.3911	
EC20	4.158	211.388	
EC25	4.326	523.886	
EC40	4.747	5157.55	
EC50	5.000	20414	
EC60	5.253	80800	
EC75	5.674	795459	
EC80	5.842	1971397	
EC85	6.036	5678212	
EC90	6.282	2.1E+07	
EC95	6.645	1.5E+08	
EC99	7.326	6.3E+09	

Significant heterogeneity detected (p = 1.65E-34)



Day 60 Weight

Giant Cuttlefish Day 60 Egg Weight

Start Date:	8/06/2009	Test ID:	ENV06-128	Sample ID:	BHP RO
End Date:	8/08/2006	Lab ID:	Freo	Sample Type:	Brine
Sample Date:		Protocol:	GEOTECH WI	Test Species:	Sepia Apama
Comments:					

Conc-%	1	2	3	4	5	6	7	8	9	10
Control	0.4210	1.0000	1.0000	0.8990	1.0000	0.9980	0.8840	0.8450	1.0000	0.9980
1.5	0.8620	1.0000	0.8990	1.0000	1.0000	0.7170	0.6890	0.7570	0.7790	1.0000
3.13	0.8840	0.9960	0.7920	1.0000	1.0000	0.9470	1.0000	1.0000	0.7520	1.0000
6.25	1.0000	0.9190	1.0000	0.8050	1.0000	0.9500	0.9340	1.0000	1.0000	0.9450
12.5	0.8420	0.7700	0.5960	0.7650	0.7940	0.6620	0.8640	0.8330	0.6320	0.8270
25	0.7370	0.8600	0.6640	0.7000	0.7570	0.7630	0.9690	0.9340	0.6510	0.6560
50	0.9740	0.8490	0.7410	0.7700	0.4340	1.0000	0.6340	0.8030	0.6910	0.8110
100	0.6580	0.8400	0.7700	0.9120	0.8090	0.4780	0.8110	0.7110	0.8570	0.8770

			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
Control	0.9045	1.0000	1.3478	0.7061	1.5261	19.995	10				96	1000
1.5	0.8703	0.9622	1.2646	0.9792	1.5208	18.489	10	0.966	2.383	0.2052	129	1000
3.13	0.9371	1.0360	1.3820	1.0495	1.5208	13.872	10	-0.397	2.383	0.2052	63	1000
6.25	0.9553	1.0562	1.3990	1.1134	1.5208	10.226	10	-0.594	2.383	0.2052	46	1000
*12.5	0.7585	0.8386	1.0633	0.8820	1.1931	10.306	10	3.304	2.383	0.2052	242	1000
*25	0.7691	0.8503	1.0869	0.9388	1.3938	14.665	10	3.031	2.383	0.2052	231	1000
*50	0.7707	0.8521	1.1062	0.7192	1.5208	20.739	10	2.806	2.383	0.2052	231	1000
*100	0.7723	0.8538	1.0847	0.7634	1.2696	13.656	10	3.056	2.383	0.2052	227	1000

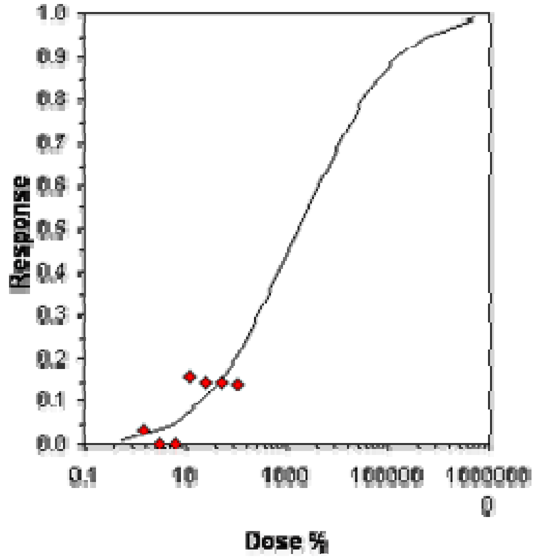
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates normal distribution (p > 0.01)	0.86886	1.035	-0.5512	0.7937
Bartlett's Test indicates equal variances (p = 0.16)	10.533	18.4753		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	6.25	12.5	8.83883	16	0.1235	0.12985	0.21434	0.03706	2.4E-05	7, 72

Parameter	Value	SE	95% Fiducial Limits		Maximum Likelihood-Probit Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	0.66943	0.51418	-0.6523	1.99118	0.096	181.7	11.0705	2.3E-37	3.23385	1.4938	15
Intercept	2.83515	0.84503	0.66295	5.00736							
TSCR	0.08397	0.04915	-0.0424	0.2103							

Point	Probits	%	95% Fiducial Limits
EC01	2.674	0.57379	
EC05	3.355	5.9809	
EC10	3.718	20.8673	
EC15	3.964	48.487	
EC20	4.158	94.7623	
EC25	4.326	168.382	
EC40	4.747	716.803	
EC50	5.000	1713.36	
EC60	5.253	4095.43	
EC75	5.674	17434.2	
EC80	5.842	30978.8	
EC85	6.036	60544.5	
EC90	6.282	140680	
EC95	6.645	490832	
EC99	7.326	5116235	

Significant heterogeneity detected (p = 2.32E-37)



Day 90 Stage

					Giant Cuttlefish Day 90 Stage					
Start Date:	8/06/2009	Test ID:	ENV06-128			Sample ID:	BHP RO			
End Date:	8/09/2006	Lab ID:	Freo			Sample Type:	Brine			
Sample Date:		Protocol:	GEOTECH WI			Test Species:	Sepia Apama			
Comments:										
Conc-%	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Control	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9730	1.0000	0.9730
1.5	0.0000	1.0000	0.0000	0.9730	0.0000	1.0000	0.0000	1.0000	0.0000	0.9730
3.13	0.9730	0.9730	0.9730	0.0000	0.0000	0.9730	0.9730	0.9730	0.0000	0.9730
6.25	0.0000	0.0000	0.0000	0.0000	0.0000	0.9060	0.0000	0.6040	0.0000	0.9730
12.5	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
25	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
50	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
100	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root						1-		
								Rank	Tailed	Number	Total
Conc-%	**Mean**	**N-Mean**	**Mean**	**Min**	**Max**	**CV%**	**N**	**Sum**	**Critical**	**Resp**	**Number**
Control	0.9946	1.0000	1.4978	1.4057	1.5208	3.239	10			6	1000
*1.5	0.4946	0.4973	0.7624	0.0500	1.5208	98.647	10	75.00	77.00	506	1000
*3.13	0.6811	0.6848	0.9990	0.0500	1.4057	65.551	10	62.00	77.00	321	1000
*6.25	0.2483	0.2496	0.3905	0.0500	1.4057	144.007	10	56.00	77.00	752	1000
12.5	0.0000	0.0000	0.0500	0.0500	0.0500	0.000	10			1000	1000
25	0.0000	0.0000	0.0500	0.0500	0.0500	0.000	10			1000	1000
50	0.0000	0.0000	0.0500	0.0500	0.0500	0.000	10			1000	1000
100	0.0000	0.0000	0.0500	0.0500	0.0500	0.000	10			1000	1000

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.94238	0.919	-0.0826	-0.9538
Bartlett's Test indicates unequal variances (p = 8.18E-08)	35.8186	11.3449		

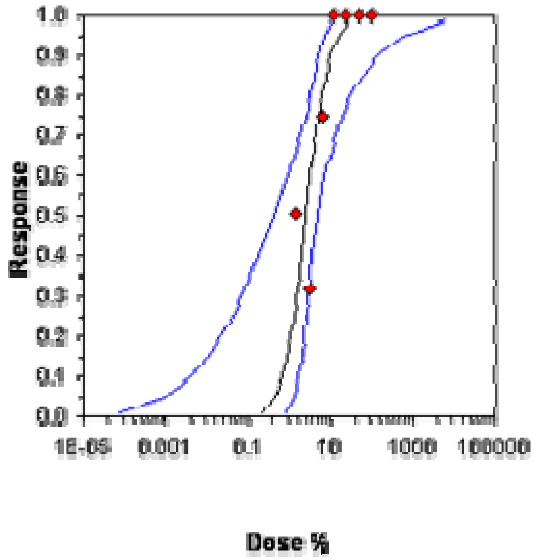
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	<1.5	1.5		

Parameter	Value	SE	95% Fiducial Limits	
Slope	2.18386	0.61997	0.59018	3.77754
Intercept	4.10853	0.44752	2.95814	5.25893
TSCR	0.00753	0.0287	-0.0662	0.08132

Maximum Likelihood-Probit Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
0.006	554.229	11.0705	1.6E-117	0.40821	0.45791	6

Point	Probits	%	95% Fiducial Limits	
EC01	2.674	0.22027	7.2E-05	0.83465
EC05	3.355	0.45188	0.001	1.30199
EC10	3.718	0.66279	0.00403	1.66483
EC15	3.964	0.85826	0.01025	1.97728
EC20	4.158	1.05396	0.02141	2.27924
EC25	4.326	1.25706	0.04006	2.58907
EC40	4.747	1.95974	0.18656	3.71657
EC50	5.000	2.55981	0.44533	4.88215
EC60	5.253	3.34362	0.97177	7.01568
EC75	5.674	5.21267	2.49894	18.2369
EC80	5.842	6.21714	3.20564	30.2151
EC85	6.036	7.63477	4.0455	57.654
EC90	6.282	9.88637	5.14242	137.042
EC95	6.645	14.5009	6.93933	522.948
EC99	7.326	29.7478	11.3026	6946.02

Significant heterogeneity detected (p = 1.56E-117)



Day 90 Length

Day 90 Length					
Start Date:	8/06/2006	Test ID:	ENV06-128	Sample ID:	Effluent RO
End Date:	13/10/2006	Lab ID:	Freo	Sample Type:	Brine
Sample Date:		Protocol:	Geotech WI	Test Species:	Sepia apama
Comments:					

Conc-%	1	2	3	4	5
Control	1.0000	1.0000	1.0000	1.0000	0.9810
1.5	1.0000	1.0000	0.9650	1.0000	0.9140
3.125	0.7590	0.6730	0.9080	0.9240	0.7750
6.25	0.5050	0.6000	0.6600	0.6540	0.6730
25	0.0000	0.0000	0.0000	0.0000	0.0000
50	0.0000	0.0000	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
Control	0.9962	1.0000	1.5031	1.4325	1.5208	2.626	5				2	500
1.5	0.9758	0.9795	1.4436	1.2732	1.5208	7.794	5	0.950	2.230	0.1396	13	500
*3.125	0.8078	0.8109	1.1301	0.9621	1.2915	12.512	5	5.958	2.230	0.1396	96	500
*6.25	0.6184	0.6208	0.9057	0.7904	0.9621	7.803	5	9.541	2.230	0.1396	192	500
25	0.0000	0.0000	0.0500	0.0500	0.0500	0.000	5				500	500
50	0.0000	0.0000	0.0500	0.0500	0.0500	0.000	5				500	500

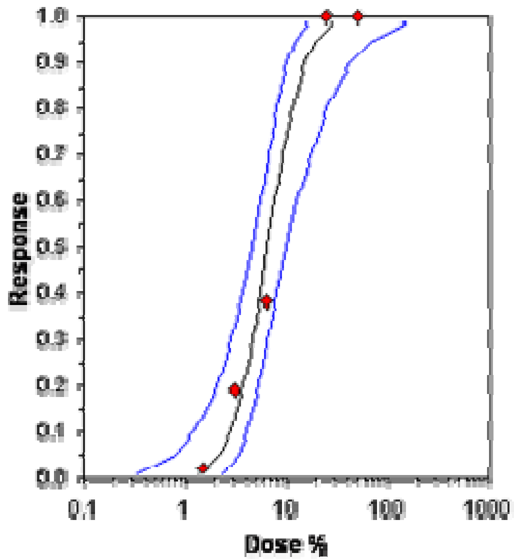
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.96224	0.868	-0.3209	-0.3554
Bartlett's Test indicates equal variances (p = 0.13)	5.5948	11.3449		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	1.5	3.125	2.16506	66.6667	0.03779	0.03796	0.39062	0.0098	1.2E-07	3, 16

					Maximum Likelihood-Probit						
Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	3.48619	0.53313	1.78953	5.18286	0.004	41.5931	7.81472	4.9E-09	0.81208	0.28685	7
Intercept	2.16892	0.42488	0.81677	3.52106							
TSCR	0.00729	0.01291	-0.0338	0.04835							

Point	Probits	%	95% Fiducial Limits	
EC01	2.674	1.39567	0.3355	2.40014
EC05	3.355	2.1891	0.78191	3.3503
EC10	3.718	2.78277	1.21362	4.04829
EC15	3.964	3.27181	1.61904	4.63836
EC20	4.158	3.72108	2.02082	5.20648
EC25	4.326	4.15538	2.42648	5.79078
EC40	4.747	5.48799	3.6855	7.90334
EC50	5.000	6.48761	4.56488	9.89308
EC60	5.253	7.66932	5.49808	12.7352
EC75	5.674	10.1288	7.15581	20.2839
EC80	5.842	11.311	7.8624	24.6544
EC85	6.036	12.8642	8.73251	31.1001
EC90	6.282	15.1249	9.91262	41.8774
EC95	6.645	19.2267	11.8745	65.5639
EC99	7.326	30.157	16.4413	154.049

Significant heterogeneity detected (p = 4.89E-09)



Day 90 Width

				Giant Cuttlefish Day 90 Width			
Start Date:	8/06/2006	Test ID:	ENV06-128			Sample ID:	Effluent RO
End Date:	13/10/2006	Lab ID:	Freo			Sample Type:	Brine
Sample Date:		Protocol:	Geotech WI			Test Species:	Sepia apama
Comments:							

Conc-%	1	2	3	4	5
Control	1.0000	1.0000	1.0000	0.9860	1.0000
1.5	0.9670	0.9100	0.9060	0.9720	0.9060
3.125	0.9860	0.9670	0.9060	0.9010	0.8870
6.25	0.6040	0.6790	0.6840	0.5850	0.6750
25	0.0000	0.0000	0.0000	0.0000	0.0000
50	0.0000	0.0000	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
Control	0.9972	1.0000	1.5071	1.4522	1.5208	2.035	5				1	500
*1.5	0.9322	0.9348	1.3151	1.2592	1.4027	5.595	5	4.464	2.230	0.0959	33	500
*3.125	0.9294	0.9320	1.3156	1.2280	1.4522	7.504	5	4.450	2.230	0.0959	34	500
*6.25	0.6454	0.6472	0.9335	0.8708	0.9738	5.247	5	13.335	2.230	0.0959	178	500
25	0.0000	0.0000	0.0500	0.0500	0.0500	0.000	5				500	500
50	0.0000	0.0000	0.0500	0.0500	0.0500	0.000	5				500	500

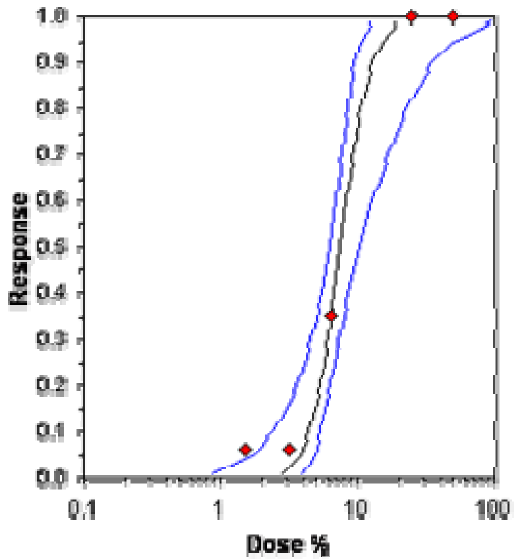
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.91838	0.868	0.49359	-0.6431
Bartlett's Test indicates equal variances (p = 0.18)	4.87701	11.3449		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	<1.5	1.5			0.02122	0.0213	0.28922	0.00463	4.7E-09	3, 16

Parameter	Value	SE	95% Fiducial Limits		Maximum Likelihood-Probit Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	5.53612	1.01588	2.30315	8.7691	0.002	13.6239	7.81472	3.5E-03	0.86955	0.18063	14
Intercept	0.18607	0.81704	-2.4141	2.78627							
TSCR	0.03753	0.01197	-0.0006	0.07564							

Point	Probits	%	95% Fiducial Limits	
EC01	2.674	2.81408	0.8647	3.92157
EC05	3.355	3.73625	1.68432	4.75898
EC10	3.718	4.3457	2.38564	5.31503
EC15	3.964	4.81211	2.99716	5.76475
EC20	4.158	5.21825	3.56725	6.19381
EC25	4.326	5.5939	4.10671	6.64389
EC40	4.747	6.66479	5.50466	8.43466
EC50	5.000	7.4054	6.24156	10.2423
EC60	5.253	8.22832	6.89822	12.7598
EC75	5.674	9.80354	7.92901	18.8904
EC80	5.842	10.5093	8.34108	22.175
EC85	6.036	11.3962	8.8326	26.7793
EC90	6.282	12.6194	9.47468	34.0176
EC95	6.645	14.6778	10.4875	48.6144
EC99	7.326	19.4877	12.634	95.3915

Significant heterogeneity detected (p = 3.46E-03)



Day 90 Weight

					Giant Cuttlefish Day 90 Weight					
Start Date:	8/06/2009		Test ID:	ENV06-128		Sample ID:		BHP RO		
End Date:	8/09/2006		Lab ID:	Freo		Sample Type:		Brine		
Sample Date:			Protocol:	GEOTECH WI		Test Species:		Sepia Apama		
Comments:										
Conc-%	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Control	0.7400	1.0000	1.0000	0.9600	0.9410	1.0000	0.9720	0.8780	1.0000	0.8740
1.5	0.5120	0.9420	0.5880	0.8400	0.4090	1.0000	0.4540	0.6660	0.4850	0.8460
3.13	1.0000	1.0000	0.9450	0.3650	0.7050	0.5770	1.0000	1.0000	0.5030	0.6740
6.25	0.3150	0.2720	0.3840	0.4940	0.2310	0.5130	0.4170	0.4790	0.5360	0.8590
12.5	0.5930	0.5280	0.6130	0.6510	0.6590	0.4570	0.5490	0.4810	0.5650	0.5370
25	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
50	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
100	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root						1-Tailed		
Conc-%	**Mean**	**N-Mean**	**Mean**	**Min**	**Max**	**CV%**	**N**	**Rank Sum**	**Critical**	**Number Resp**	**Total Number**
Control	0.9365	1.0000	1.3638	1.0357	1.5208	12.365	10			64	1000
*1.5	0.6742	0.7199	1.0005	0.6939	1.5208	27.982	10	69.00	76.00	325	1000
3.13	0.7769	0.8296	1.1677	0.6487	1.5208	29.990	10	91.00	76.00	225	1000
*6.25	0.4500	0.4805	0.7366	0.5014	1.1859	26.066	10	56.00	76.00	550	1000
*12.5	0.5633	0.6015	0.8494	0.7423	0.9472	7.997	10	55.00	76.00	436	1000
25	0.0000	0.0000	0.0500	0.0500	0.0500	0.000	10			1000	1000
50	0.0000	0.0000	0.0500	0.0500	0.0500	0.000	10			1000	1000
100	0.0000	0.0000	0.0500	0.0500	0.0500	0.000	10			1000	1000

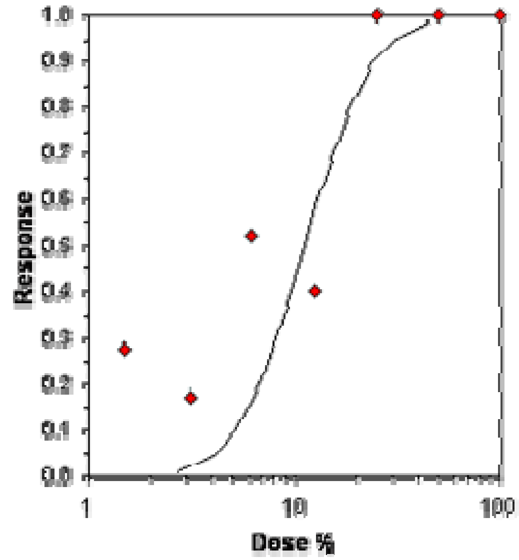
Auxiliary Tests	**Statistic**	**Critical**	**Skew**	**Kurt**
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.98164	0.93	0.1943	-0.0921
Bartlett's Test indicates unequal variances (p = 5.27E-04)	19.8813	13.2767		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	<1.5	1.5		

Parameter	Value	SE	95% Fiducial Limits		Maximum Likelihood-Probit Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	3.81134	1.55081	-0.1751	7.79782	0.064	557.582	11.0705	3.0E-118	1.04803	0.26237	26
Intercept	1.0056	1.82396	-3.683	5.69421							
TSCR	0.2218	0.08525	0.00264	0.44095							

Point	Probits	%	95% Fiducial Limits
EC01	2.674	2.7394	
EC05	3.355	4.13488	
EC10	3.718	5.14972	
EC15	3.964	5.97168	
EC20	4.158	6.71754	
EC25	4.326	7.43125	
EC40	4.747	9.58426	
EC50	5.000	11.1694	
EC60	5.253	13.0167	
EC75	5.674	16.788	
EC80	5.842	18.5717	
EC85	6.036	20.8913	
EC90	6.282	24.2258	
EC95	6.645	30.1716	
EC99	7.326	45.5413	

Significant heterogeneity detected (p = 2.95E-118)



Days To Hatch

							Giant Cuttlefish Days to Hatch		
Start Date:	8/06/2006		Test ID:	ENV06-128			Sample ID:	Brine RO	
End Date:	13/10/2006		Lab ID:	Freo			Sample Type:	Brine	
Sample Date:			Protocol:	GEOTECH WI			Test Species:	Sepia apama	
Comments:									

Conc-%	1	2	3	4	5
Control	0.8440	0.7330	0.8990	0.9660	1.0000
1.5	0.4100	0.6440	0.5330	0.8330	0.6990
3.125	0.2430	0.6100	0.5440	1.0000	0.6110
6.25	0.2880	0.2990	0.4210	0.1210	0.5110
12.5	0.0000	0.0000	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
Control	0.8884	1.0000	1.2692	1.0278	1.5208	15.076	5				56	500
*1.5	0.6238	0.7022	0.9169	0.6949	1.1498	18.794	5	2.336	2.230	0.3363	189	500
*3.125	0.6016	0.6772	0.9319	0.5155	1.5208	39.177	5	2.237	2.230	0.3363	200	500
*6.25	0.3280	0.3692	0.6006	0.3553	0.7964	27.775	5	4.434	2.230	0.3363	336	500
12.5	0.0000	0.0000	0.0500	0.0500	0.0500	0.000	5				500	500

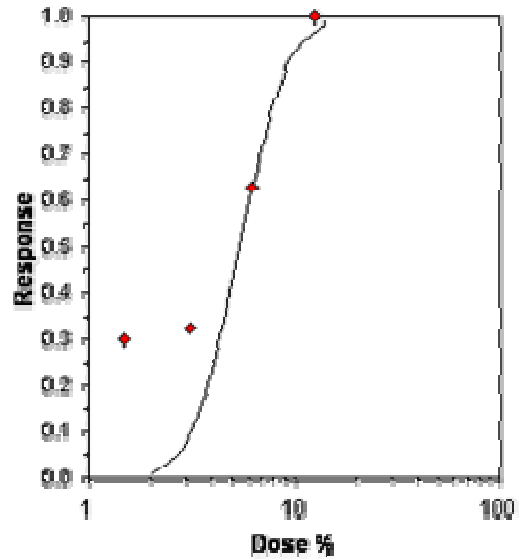
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.95244	0.868	0.71537	1.7825
Bartlett's Test indicates equal variances (p = 0.33)	3.44931	11.3449		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	<1.5	1.5			0.26638	0.29216	0.37295	0.05685	0.00423	3, 16

Parameter	Value	SE	95% Fiducial Limits		Maximum Likelihood-Probit Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	5.47728	2.1573	-3.8048	14.7594	0.112	61.2547	5.99148	5.0E-14	0.73378	0.18257	50
Intercept	0.9809	1.78541	-6.7011	8.66291							
TSCR	0.26273	0.0752	-0.0608	0.5863							

Point	Probits	%	95% Fiducial Limits
EC01	2.674	2.03727	
EC05	3.355	2.71313	
EC10	3.718	3.16082	
EC15	3.964	3.5039	
EC20	4.158	3.80294	
EC25	4.326	4.07974	
EC40	4.747	4.86992	
EC50	5.000	5.41721	
EC60	5.253	6.02601	
EC75	5.674	7.19315	
EC80	5.842	7.71672	
EC85	6.036	8.37529	
EC90	6.282	9.28435	
EC95	6.645	10.8163	
EC99	7.326	14.4046	

Significant heterogeneity detected (p = 5.00E-14)



Length at Hatch

Giant Cuttlefish Length at Hatch										
Start Date:	8/06/2006		Test ID:	ENV06-128			Sample ID:		BHP RO	
End Date:	13/10/2006		Lab ID:	Freo			Sample Type:		Brine	
Sample Date:			Protocol:	GEOTECH WI			Test Species:		Sepia Apama	
Comments:										

Conc-%	1	2	3	4	5	6	7	8	9	10
Control	0.8730	0.9440	1.0000	0.8810	1.0000	0.9760	1.0000	0.9760	1.0000	0.9830
1.5	0.8570	0.8650	0.9750	0.9750	0.8890	0.8180	0.9990	0.8810	0.9910	1.0000
3.125	0.8810	0.8730	1.0000	0.8260	1.0000	0.8490	0.9990	0.8890	0.9750	0.9830
6.25	0.8812	0.8655	0.9750	0.8812	0.8890	0.8960	0.8570	0.8260	0.9760	0.8570
12.5	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	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
Control	0.9633	1.0000	1.4111	1.2064	1.5208	8.654	10				37	1000
1.5	0.9250	0.9602	1.3317	1.1301	1.5392	11.658	10	1.307	2.137	0.1297	74	1000
3.125	0.9275	0.9628	1.3401	1.1405	1.5392	12.001	10	1.169	2.137	0.1297	72	1000
*6.25	0.8904	0.9243	1.2440	1.1405	1.4153	7.555	10	2.751	2.137	0.1297	108	1000
12.5	0.0000	0.0000	0.0500	0.0500	0.0500	0.000	10				1000	1000

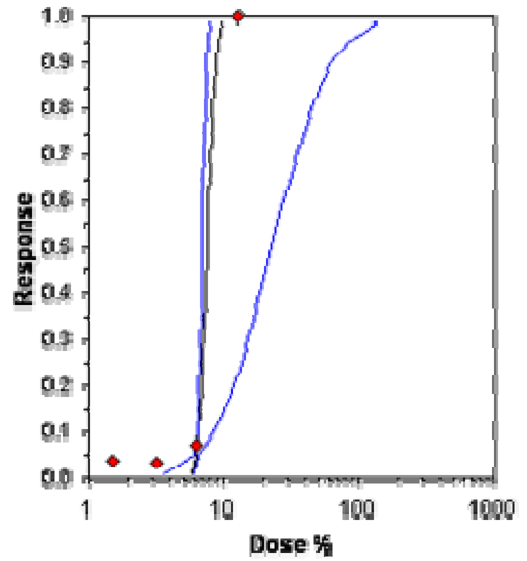
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.92713	0.919	0.03866	-1.2968
Bartlett's Test indicates equal variances (p = 0.40)	2.92091	11.3449		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	3.125	6.25	4.41942	32	0.05617	0.05763	0.04684	0.01843	0.07161	3, 36

Parameter	Value	SE	95% Fiducial Limits	
Slope	20.6267	9.03327	2.92151	38.3319
Intercept	-13.061	7.19401	-27.161	1.03953
TSCR	0.061	0.00437	0.05244	0.06956

Maximum Likelihood-Probit Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
0.037	5.06294	5.99148	0.08	0.8756	0.04848	50

Point	Probits	%	95% Fiducial Limits	
EC01	2.674	5.79183	3.61067	6.02823
EC05	3.355	6.24964	5.94287	6.52873
EC10	3.718	6.50831	6.33832	8.33072
EC15	3.964	6.68885	6.45357	10.0729
EC20	4.158	6.83591	6.53742	11.7303
EC25	4.326	6.96465	6.60769	13.3732
EC40	4.747	7.29989	6.78342	18.6198
EC50	5.000	7.50929	6.88976	22.7272
EC60	5.253	7.72469	6.99714	27.7433
EC75	5.674	8.09652	7.17849	38.653
EC80	5.842	8.249	7.25156	44.0913
EC85	6.036	8.43036	7.33757	51.4039
EC90	6.282	8.66422	7.44711	62.3528
EC95	6.645	9.02283	7.61229	83.0158
EC99	7.326	9.73603	7.93164	142.023



Width at Hatch

						Giant Cuttlefish Width at Hatch					
Start Date:	8/06/2006	Test ID:	ENV06-128				Sample ID:	BHP RO			
End Date:	27/10/2006	Lab ID:	Freo				Sample Type:	Brine			
Sample Date:		Protocol:	Geotech Work Instructions				Test Species:	Sepia apama			
Comments:											

Conc-%	1	2	3	4	5	6	7	8	9	10
Control	0.8850	1.0000	0.9990	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	
1.5	0.8660	0.9990	1.0000	0.9990	0.9990	0.8080	1.0000	0.8750	0.9890	1.0000
3.13	0.8850	0.8750	0.9040	0.8470	0.9990	0.8750				
6.25	0.8650	0.8460	0.8850	0.8460	0.8370	0.8270	0.8850	0.8460		
12.5	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
25	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
Control	0.9871	1.0000	1.4899	1.2248	1.5392	6.685	9			12	900
1.5	0.9535	0.9660	1.4168	1.1172	1.5392	12.025	10	95.00	73.00	45	1000
3.13	0.8975	0.9092	1.2679	1.1689	1.5392	10.711	6	31.00	29.00	62	600
*6.25	0.8546	0.8658	1.1805	1.1418	1.2248	2.634	8	37.00	49.00	115	800
12.5	0.0000	0.0000	0.0500	0.0500	0.0500	0.000	10			1000	1000
25	0.0000	0.0000	0.0500	0.0500	0.0500	0.000	10			1000	1000

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.91007	0.906	-0.7707	1.45365
Bartlett's Test indicates unequal variances (p = 1.88E-03)	14.9278	11.3449		

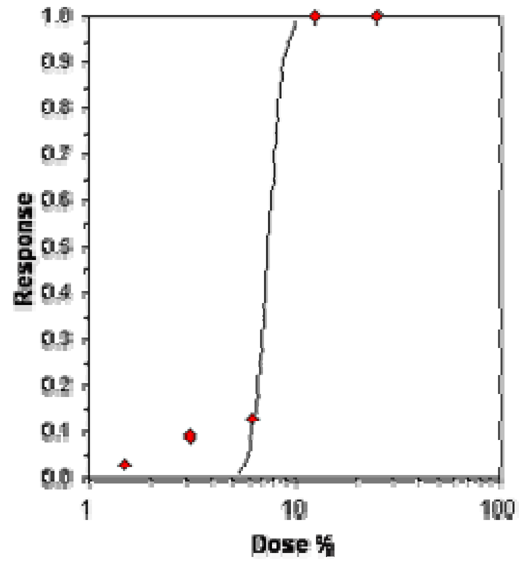
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Wilcoxon Rank Sum Test	3.13	6.25	4.42295	31.9489

Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	17.1563	8.71189	-10.569	44.8815	0.01333	41.2608	7.81472	5.8E-09	0.87026	0.05829	50
Intercept	-9.9305	6.96793	-32.106	12.2446							
TSCR	0.0476	0.01579	-0.0027	0.09786							

Point	Probits	%	95% Fiducial Limits
EC01	2.674	5.42832	
EC05	3.355	5.94823	
EC10	3.718	6.24545	
EC15	3.964	6.45433	
EC20	4.158	6.62531	
EC25	4.326	6.7756	
EC40	4.747	7.16961	
EC50	5.000	7.41758	
EC60	5.253	7.67413	
EC75	5.674	8.12039	
EC80	5.842	8.30459	
EC85	6.036	8.52459	
EC90	6.282	8.80969	
EC95	6.645	9.24989	
EC99	7.326	10.1358	

Significant heterogeneity detected (p = 5.76E-09)



Weight at Hatch

						Giant Cuttlefish Weight at Hatch					
Start Date:	8/06/2006		Test ID:	ENV06-128			Sample ID:	BHP RO			
End Date:	13/10/2006		Lab ID:	Freo			Sample Type:	Brine			
Sample Date:			Protocol:	GEOTECH WI			Test Species:	Sepia Apama			
Comments:											
Conc-%	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	
Control	0.7140	0.9460	1.0000	0.9960	1.0000	0.8970	0.6650	1.0000	1.0000	1.0000	
1.5	0.6310	0.6810	0.9130	1.0000	0.7140	0.3820	1.0000	0.4980	1.0000	1.0000	
3.125	0.9300	0.8470	1.0000	0.9630	1.0000	0.6970	1.0000	0.7800	0.9460	0.3650	
6.25	0.6810	0.6640	0.3320	0.4650	0.4980	0.4150	0.5310	0.3650	0.8970	0.3320	
12.5	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	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**
Control	0.9218	1.0000	1.3652	0.9535	1.5208	16.468	10				78	1000
1.5	0.7819	0.8482	1.1699	0.6663	1.5208	28.995	10	1.639	2.137	0.2546	219	1000
3.125	0.8528	0.9251	1.2467	0.6487	1.5208	22.548	10	0.994	2.137	0.2546	146	1000
*6.25	0.5180	0.5619	0.8094	0.6141	1.2441	24.493	10	4.664	2.137	0.2546	481	1000
12.5	0.0000	0.0000	0.0500	0.0500	0.0500	0.000	10				1000	1000

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.96054	0.919	-0.3838	-0.5044
Bartlett's Test indicates equal variances (p = 0.40)	2.92873	11.3449		

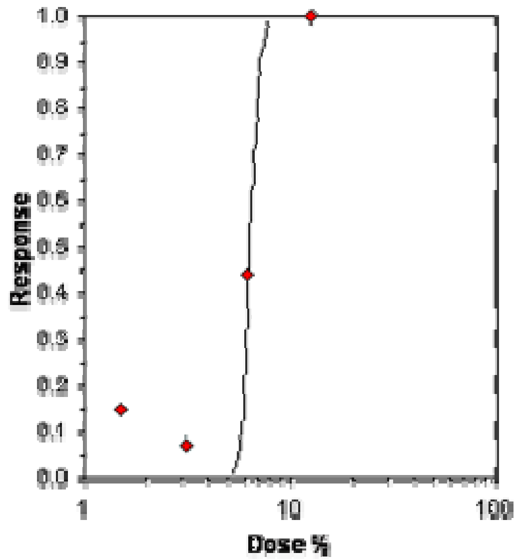
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	3.125	6.25	4.41942	32	0.15556	0.16233	0.57343	0.07098	3.0E-04	3, 36

Parameter	Value	SE	95% Fiducial Limits	
Slope	27.5939	2727626	-1E+07	1.2E+07
Intercept	-17.238	2170863	-9E+06	9340459
TSCR	0.14767	0.02913	0.02233	0.273

Maximum Likelihood-Probit Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
0.078	40.4511	5.99148	1.6E-09	0.8059	0.03624	38

Point	Probits	%	95% Fiducial Limits
EC01	2.674	5.26737	
EC05	3.355	5.57559	
EC10	3.718	5.74721	
EC15	3.964	5.86597	
EC20	4.158	5.96211	
EC25	4.326	6.04584	
EC40	4.747	6.26209	
EC50	5.000	6.39588	
EC60	5.253	6.53253	
EC75	5.674	6.76618	
EC80	5.842	6.86121	
EC85	6.036	6.97365	
EC90	6.282	7.11776	
EC95	6.645	7.33685	
EC99	7.326	7.76617	

Significant heterogeneity detected (p = 1.64E-09)



Survival Post Hatch

			30 Day Post Hatch Survival			
Start Date:	8/06/2006	Test ID:	ENV06-128		Sample ID:	BHP RO
End Date:	27/10/2006	Lab ID:	Freo		Sample Type:	Brine
Sample Date:		Protocol:	Geotech WI		Test Species:	Sepia apama
Comments:						

Conc-%	1	2	3	4	5
Control	0.6667	0.6667	0.6667	1.0000	1.0000
1.5	0.6667	1.0000	1.0000	1.0000	1.0000
3.125	0.6667	0.3333	1.0000	0.0000	0.0000
6.25	0.6667	1.0000	0.0000	0.0000	0.0000
12.5	0.0000	0.0000	0.0000	0.0000	0.0000
25	0.0000	0.0000	0.0000	0.0000	0.0000

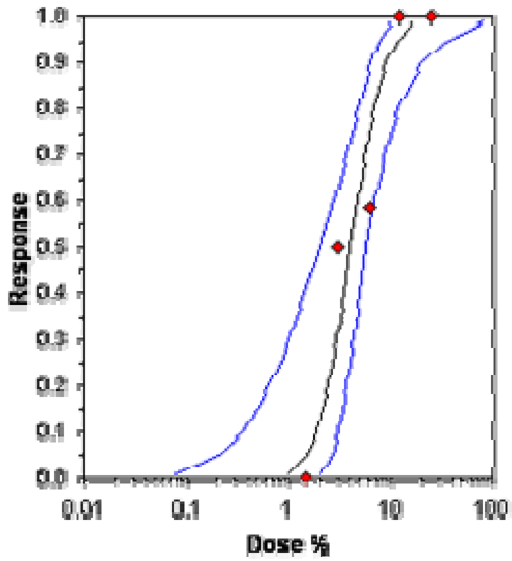
			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
Control	0.8000	1.0000	1.0844	0.9553	1.2780	16.297	5				3	15
1.5	0.9333	1.1667	1.2134	0.9553	1.2780	11.891	5	-0.606	2.230	0.4746	1	15
3.125	0.4000	0.5000	0.6869	0.2928	1.2780	62.493	5	1.868	2.230	0.4746	9	15
6.25	0.3333	0.4167	0.6224	0.2928	1.2780	74.781	5	2.171	2.230	0.4746	10	15
12.5	0.0000	0.0000	0.2928	0.2928	0.2928	0.000	5				15	15
25	0.0000	0.0000	0.2928	0.2928	0.2928	0.000	5				15	15

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.93243	0.868	0.60856	-0.2336
Bartlett's Test indicates equal variances (p = 0.08)	6.68741	11.3449		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	6.25	12.5	8.83883	16	0.45349	0.58031	0.42453	0.11323	0.03257	3, 16

Parameter	Value	SE	95% Fiducial Limits		Maximum Likelihood-Probit Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	3.79623	1.14004	1.56176	6.0307	0.2	5.20634	7.81472	0.16	0.60816	0.26342	17
Intercept	2.69127	0.84767	1.02984	4.35271							
TSCR	0.14461	0.08002	-0.0122	0.30145							

Point	Probits	%	95% Fiducial Limits	
EC01	2.674	0.98937	0.07717	1.99532
EC05	3.355	1.49581	0.20738	2.63076
EC10	3.718	1.86457	0.34969	3.06218
EC15	3.964	2.16345	0.49602	3.40262
EC20	4.158	2.4348	0.65323	3.70921
EC25	4.326	2.69457	0.82528	4.00383
EC40	4.747	3.47878	1.46406	4.93187
EC50	5.000	4.05661	2.02865	5.69627
EC60	5.253	4.73042	2.74203	6.74457
EC75	5.674	6.10712	4.15449	9.7275
EC80	5.842	6.75868	4.73764	11.6328
EC85	6.036	7.6064	5.41135	14.6212
EC90	6.282	8.82568	6.25393	19.9402
EC95	6.645	11.0014	7.53277	32.4933
EC99	7.326	16.6329	10.2285	84.7822



Length at 30 Days Post Hatch

					30 Day Post Hatch Length					
Start Date:	8/06/2006		Test ID:	ENV06-128		Sample ID:	BHP RO			
End Date:	27/10/2006		Lab ID:	Freo		Sample Type:	Brine			
Sample Date:			Protocol:	Geotech WI		Test Species:	Sepia apama			
Comments:										
Conc-%	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Control	0.9200	1.0000	1.0000	1.0000	0.9350	0.9560	0.9270	1.0000	0.9350	1.0000
Control	1.0000	0.9050	1.0000	0.9200	1.0000					
1.5	0.9350	0.8200	0.9350	0.9420	0.9350	0.9350	0.9490	1.0000	0.9060	1.0000
1.5	1.0000	0.9280	1.0000	0.9200	0.9560					
3.125	1.0000	0.9200	0.8550	0.9780	0.9420	0.9350	0.9050	0.9420	0.9280	0.9280
3.125	0.9420	0.9050	0.9420	0.9130	0.9436					
6.25	0.9058	0.8190	0.9200	0.9130	0.8260	0.8120	0.8910	0.8480	0.8770	0.8690
6.25	0.8180	0.8990	0.8400	0.8620	0.8840					
12.5	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
12.5	0.0000	0.0000	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**
Control	0.9665	1.0000	1.4183	1.2575	1.5208	8.132	15				50	1500
1.5	0.9441	0.9768	1.3560	1.1326	1.5208	8.470	15	1.814	2.104	0.0723	81	1500
*3.125	0.9319	0.9642	1.3164	1.1801	1.5208	5.842	15	2.968	2.104	0.0723	103	1500
*6.25	0.8656	0.8956	1.1984	1.1223	1.2840	4.544	15	6.404	2.104	0.0723	201	1500
12.5	0.0000	0.0000	0.0500	0.0500	0.0500	0.000	15				1500	1500

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates normal distribution (p > 0.01)	0.80987	1.035	0.15887	-0.3124
Bartlett's Test indicates equal variances (p = 0.03)	9.33507	11.3449		

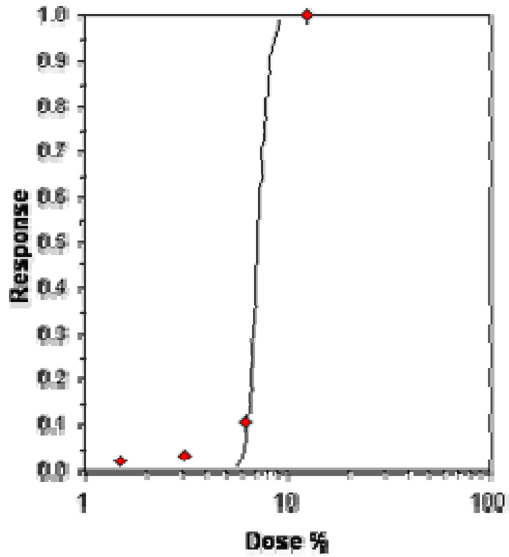
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	1.5	3.125	2.16506	66.6667	0.02659	0.02722	0.12868	0.00884	4.0E-07	3, 56

Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits	
Slope	22.6789	131.909	-544.88	590.239
Intercept	-14.412	104.984	-466.12	437.299
TSCR	0.052	0.00685	0.02251	0.08149

Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
0.03333	8.57406	5.99148	0.01	0.85596	0.04409	50

Point	Probits	%	95% Fiducial Limits
EC01	2.674	5.66743	
EC05	3.355	6.07346	
EC10	3.718	6.30167	
EC15	3.964	6.46046	
EC20	4.158	6.58952	
EC25	4.326	6.70229	
EC40	4.747	6.99508	
EC50	5.000	7.17735	
EC60	5.253	7.36436	
EC75	5.674	7.68608	
EC80	5.842	7.81762	
EC85	6.036	7.97378	
EC90	6.282	8.17471	
EC95	6.645	8.48187	
EC99	7.326	9.08953	



Width at 30 Days Post Hatch

30 Day Post Hatch Width										
Start Date:	8/06/2006	Test ID:	ENV06-128			Sample ID:	BHP RO			
End Date:	27/10/2006	Lab ID:	Freo			Sample Type:	Brine			
Sample Date:		Protocol:	Geotech WI			Test Species:	Sepia apama			
Comments:										
Conc-%	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Control	0.9900	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9900	1.0000
Control	1.0000	0.7270	1.0000	0.9820	1.0000					
1.5	0.9640	0.8460	0.8640	0.9090	0.8820	0.9900	0.9900	1.0000	0.8730	1.0000
1.5	0.9900	1.0000	1.0000	0.9180	0.9820					
3.125	0.9820	0.9550	0.8900	1.0000	1.0000	1.0000	0.8360	0.9730	0.9820	0.9550
3.125	1.0000	0.9730	0.9900	0.9550	1.0000					
6.25	0.8550	0.8640	0.8730	0.8550	0.8550	0.8450	0.8550	0.8360	0.8360	0.8550
6.25	0.8180	0.8450	0.8270	0.8550	0.8550					
12.5	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
12.5	0.0000	0.0000	0.0000	0.0000	0.0000					

			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-%	**Mean**	**N-Mean**	**Mean**	**Min**	**Max**	**CV%**	**N**	**Sum**	**Critical**	**Resp**	**Number**
Control	0.9793	1.0000	1.4751	1.0210	1.5208	8.702	15			31	1500
*1.5	0.9472	0.9673	1.3762	1.1675	1.5208	10.014	15	175.50	182.00	80	1500
*3.125	0.9661	0.9865	1.4144	1.1539	1.5208	7.828	15	180.50	182.00	50	1500
*6.25	0.8486	0.8666	1.1715	1.1301	1.2064	1.690	15	135.00	182.00	226	1500
12.5	0.0000	0.0000	0.0500	0.0500	0.0500	0.000	15			1500	1500

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates non-normal distribution (p <= 0.01)	1.44699	1.035	-1.7549	4.84967
Bartlett's Test indicates unequal variances (p = 8.22E-08)	35.8096	11.3449		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	<1.5	1.5		

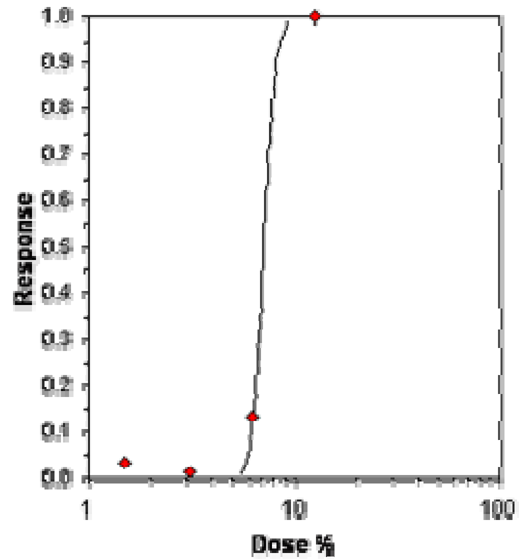
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits	
Slope	21.1395	80.5033	-325.24	367.517
Intercept	-13.004	64.0717	-288.68	262.675
TSCR	0.03578	0.00724	0.00464	0.06691

Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
0.02067	13.6606	5.99148	1.1E-03	0.85166	0.0473	50

Point	Probits	%	95% Fiducial Limits
EC01	2.674	5.5159	
EC05	3.355	5.94093	
EC10	3.718	6.18073	
EC15	3.964	6.34798	
EC20	4.158	6.48412	
EC25	4.326	6.60324	
EC40	4.747	6.9132	
EC50	5.000	7.10663	
EC60	5.253	7.30547	
EC75	5.674	7.64839	
EC80	5.842	7.78891	
EC85	6.036	7.95595	
EC90	6.282	8.17123	
EC95	6.645	8.50106	
EC99	7.326	9.15611	

Significant heterogeneity detected (p = 1.08E-03)



Weight at 30 Days Post Hatch

						30 Day Post Hatch Weight						
Start Date:	8/06/2006		Test ID:	ENV06-128			Sample ID:		BHP RO			
End Date:	27/10/2006		Lab ID:	Freo			Sample Type:		Brine			
Sample Date:			Protocol:	Geotech WI			Test Species:		Sepia apama			
Comments:												

Conc-%	1	2	3	4	5	6	7	8	9	10
Control	0.9520	1.0000	1.0000	1.0000	0.8090	0.9520	0.9680	1.0000	0.9360	1.0000
Control	1.0000	0.6350	0.8890	0.8250	1.0000					
1.5	0.9210	0.7620	0.8410	0.8090	1.0000	0.9680	0.9840	1.0000	0.8730	1.0000
1.5	1.0000	0.9840	1.0000	0.9200	1.0000					
3.125	1.0000	0.9680	0.8250	0.9050	0.8730	0.9680	0.7940	0.9360	0.8890	0.8570
3.125	0.9520	0.9050	0.9050	0.9365	0.9680					
6.25	0.8090	0.9680	0.9410	0.9050	0.7620	0.8090	0.7940	0.8090	0.8410	0.8250
6.25	0.7780	0.8570	0.7930	0.7930	0.8250					
12.5	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
12.5	0.0000	0.0000	0.0000	0.0000	0.0000					

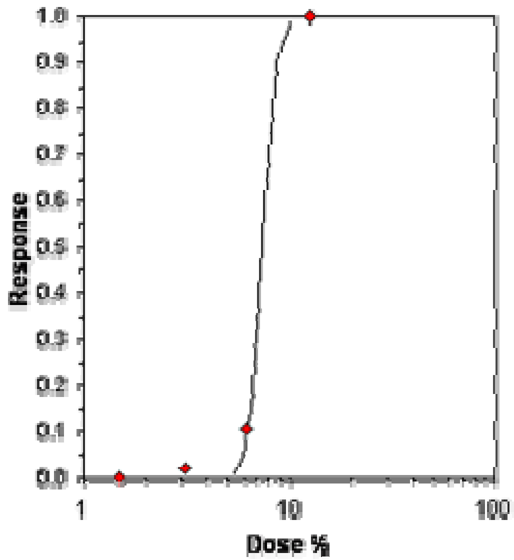
			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
Control	0.9311	1.0000	1.3641	0.9221	1.5208	13.844	15				102	1500
1.5	0.9375	1.0069	1.3680	1.0612	1.5208	12.222	15	-0.073	2.104	0.1119	95	1500
3.125	0.9121	0.9796	1.2871	1.0997	1.5208	8.627	15	1.449	2.104	0.1119	131	1500
*6.25	0.8339	0.8957	1.1593	1.0612	1.3909	8.122	15	3.853	2.104	0.1119	250	1500
12.5	0.0000	0.0000	0.0500	0.0500	0.0500	0.000	15				1500	1500

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates normal distribution (p > 0.01)	0.98763	1.035	-0.64	0.39723
Bartlett's Test indicates equal variances (p = 0.04)	8.39905	11.3449		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	3.125	6.25	4.41942	32	0.05598	0.05844	0.14296	0.0212	5.8E-04	3, 56

					Maximum Likelihood-Probit						
Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	17.6428	4.64575	-2.3463	37.6319	0.068	6.65806	5.99148	0.04	0.86815	0.05668	50
Intercept	-10.317	3.70739	-26.268	5.63506							
TSCR	0.07289	0.00707	0.04247	0.10331							

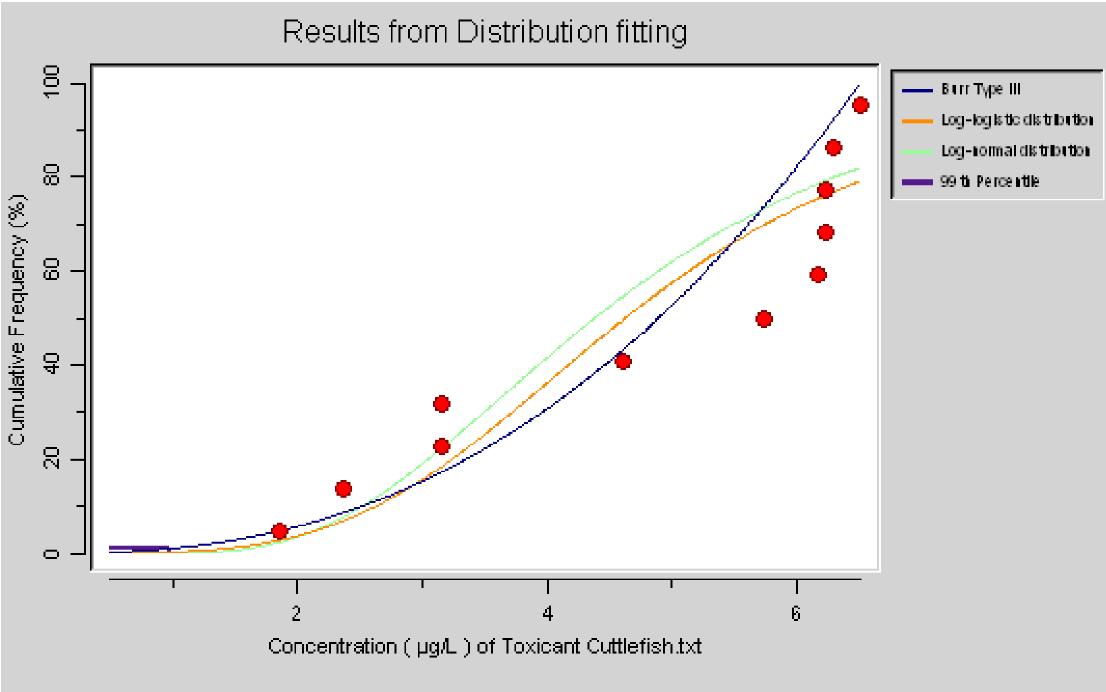
Point	Probits	%	95% Fiducial Limits	
EC01	2.674	5.44867		
EC05	3.355	5.9555		
EC10	3.718	6.24468		
EC15	3.964	6.44768		
EC20	4.158	6.61371		
EC25	4.326	6.75956		
EC40	4.747	7.14149		
EC50	5.000	7.38157		
EC60	5.253	7.62972		
EC75	5.674	8.06082		
EC80	5.842	8.23858		
EC85	6.036	8.45073		
EC90	6.282	8.72545		
EC95	6.645	9.14913		
EC99	7.326	10.0002		



APPENDIX 2

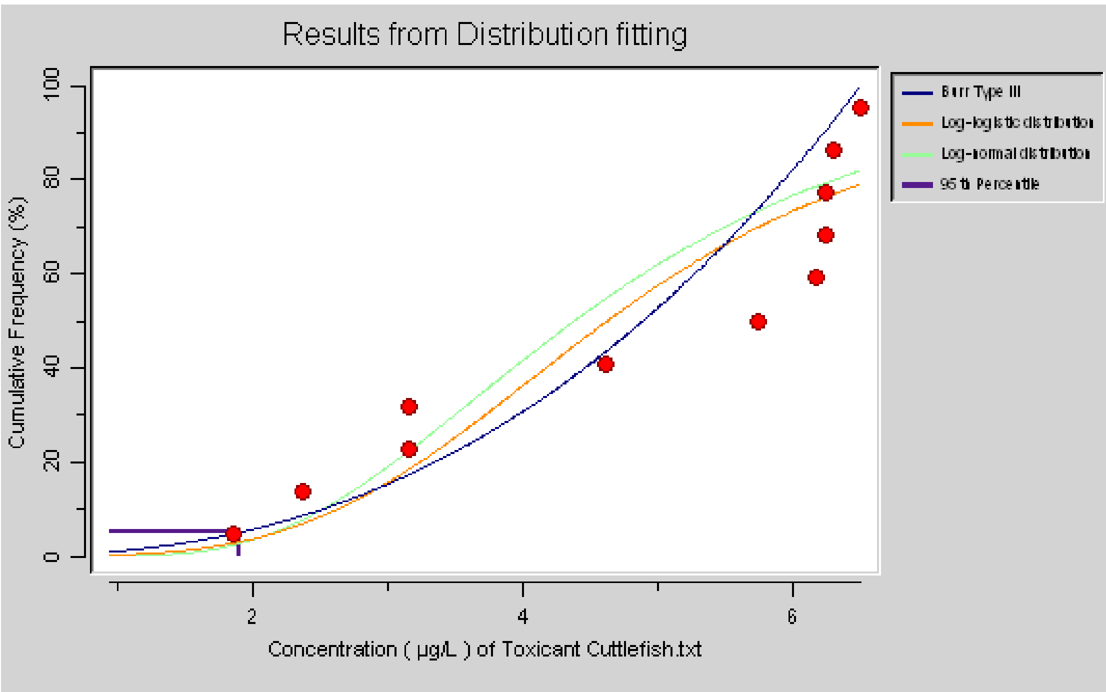
BURRLIOZ GRAPHS

Graph 1. EC10 99% Protection Value for *S. apama*



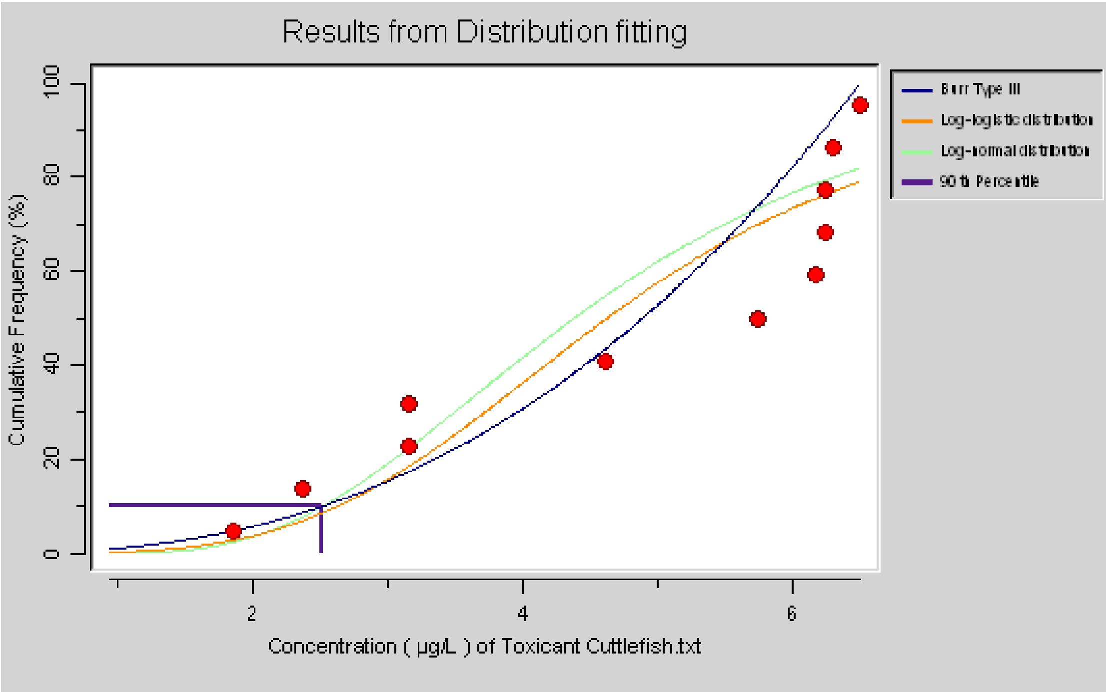
PC99 50% = 0.97 (501 Bootstrap Samples)
Burr Type III distribution fitted to 11 observations

Graph 2. EC10 95% Protection Value for *S. apama*



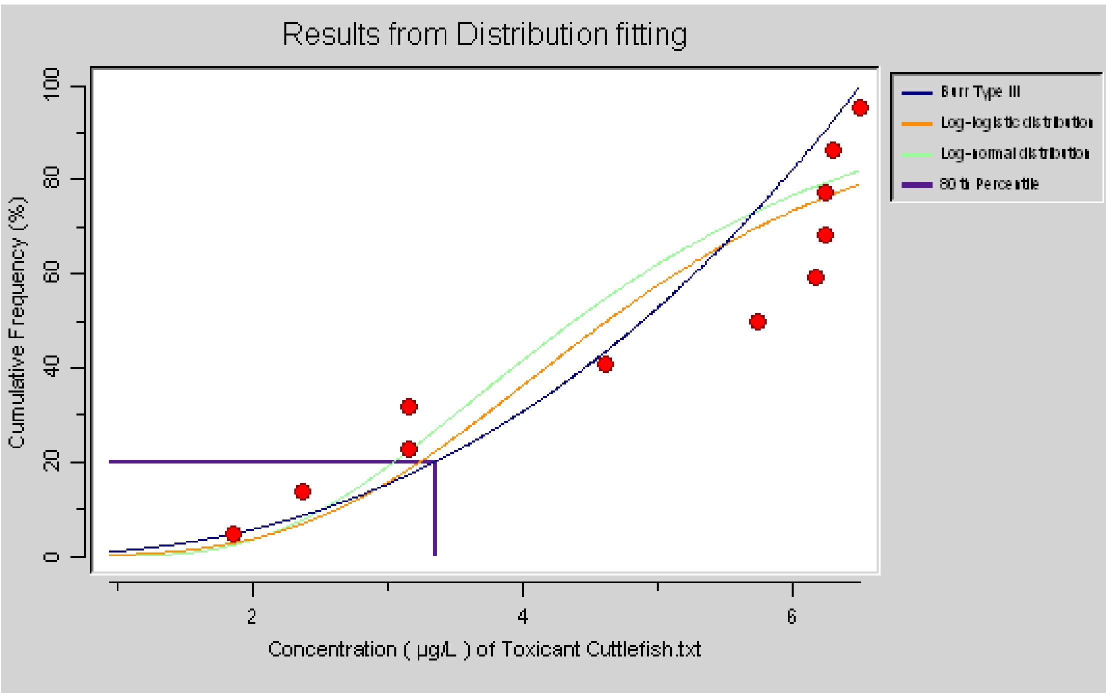
PC95 50% = 1.89 (501 Bootstrap Samples)
Burr Type III distribution fitted to 11 observations

Graph 3. EC10 90% Protection Value for *S. apama*



PC90 50% = 2.52 (501 Bootstrap Samples)
Burr Type III distribution fitted to 11 observations

Graph 4. EC10 80% Protection Value for *S. apama*



PC80 50% = 3.35 (501 Bootstrap Samples)
Burr Type III distribution fitted to 11 observations

APPENDIX 3

RO Water Processing Report

CITOR Pty Ltd

Manufacturers of Reverse Osmosis
and associated Water Purification Equipment

A.B.N. 44 064 083 [illegible]

BHP BILLITON ECOTOXICOLOGY PROJECT

RO WATER PROCESSING REPORT 29/05/06 – 02/06/06

Conducted by Paul Fildes on 29/05/2006 to 01/06/06.
At SAAM Pty Ltd Pt Lowly Whyalla

Containers 1 – 8 had been filled with ambient Seawater on Friday 26 May by SAAM Pty Ltd.
Processing was commenced @ 1000 hrs Mon 29/05/06. TDS (Seawater): 44.2 PPT
The Containers were each dosed with NALCO Anti-scalant PC-1020T
Dosing Rate: 3.6 mg/l

Method

Five (5) x Containers (1000 L) used in the processing of Brine.
Each Container had the Brine recirculated from the RO Discharge and the Permeate discarded until the desired Salinity level was achieved.

Four (4) Containers were finished on Wed 31/05 @ 1600 hrs.
Each Containers holding approx. 500 L Brine
TDS Readings varied i.e. of the range 79.1 – 79.5 PPT
These Containers were then 'blended' to achieve a uniform TDS of 79.2 PPT in Containers 1 & 2.

Container 3 was successfully processed on Thurs 01/06.
Resulting in a final TDS reading of 79.4 PPT @ 1100 hrs.
200 L Brine was then decanted into a 200 L Drum with a final TDS result of 79.3 PPT

The 1000 L Containers were collected by Tell Logistics on Thurs 01/06 at approx. 1400 hrs.
Containers 1 & 2 held 1000 L each of Brine. Container 3 held approx. 300 L.
The remaining Containers 4 – 10 held 1000 L each of ambient Seawater.

The 200 L Drums – one holding Brine, the other ambient Seawater – were delivered to A. Kumar of the University of Adelaide, SA @ 1100 hrs Fri 02/06 by P. Fildes.

Note

All readings varied over the four (4) days of Processing and the Readings given are the final ones taken on Thurs 01/06.

Paul Fildes

08/06/2006

HEAD OFFICE AND PRODUCTION PLANT:
[illegible] ROAD, [illegible] WESTERN AUSTRALIA 6160
BOX [illegible] WESTERN AUSTRALIA 6959
PH: 61 08 9410 5566 FAX: 61 08 9336-1851
WEBSITE: www.citor.com.au
EMAIL: [illegible]@citor.com.au

APPENDIX O10.4

The provision of reverse osmosis brine toxicity testing (report by Geotechnical Services, 2008)

See overleaf for report.

The Provision of Reverse Osmosis Brine Toxicity Testing

Prepared for

ARUP

Report ECX07-1805

Marine Toxicity Tests

18th August 2008

Prepared by

Dr Jill Woodworth

GEOTECHNICAL
SERVICES PTY LTD
41-45 Furnace Road, Welshpool, Western Australia 6106
Telephone : (08) 9458 8877 Facsimile : (08) 9458 8857
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Quality System
Quality Endorsed Company

Contents

Glossary

EC50	Concentration that causes an effect on 50% of the population Eg. Growth: Concentration that results in 50% less growth when compared to controls Reproduction: Concentration that results in 50% less fecundity when compared to controls Germination: Concentration that results in 50% germination of zoospores Larval development: Concentration that results in 50% of larva deformed Calculated statistically
IC50	Concentration that causes an inhibition of growth of 50% when compared with controls (Unicellular alga bioassay) Calculated statistically
EC10 / IC10	Above except the result is a 10% impact on the test species
LOEC	Lowest Observed Effect Concentration Function of concentration tested
NOEC	No Observed Effect Concentration Function of concentration tested
‰ / ppt	Parts per thousand

1. Executive Summary

This report presents a study initiated by Arup/HLA on behalf of BHP Billiton to determine the toxicity of the reverse osmosis brine (RO brine) to be discharged into Spencer Gulf from a proposed desalination plant located at Point Lowly. The toxicity of the RO brine was assessed using species indigenous to, or representative of, the receiving ecosystem. The results from these toxicity tests were then used to determine the species protection values for the RO brine in Spencer Gulf.

The toxicity of the RO brine was assessed using the following tests some of which are NATA accredited (N) which are shown in Table 1.1. Selected results were used to calculate the species protection trigger values.

Table 1.1 Tests Performed

Category	Species	Test End Point	Exposure
Bacteria Microtox (N)	*Vibrio fischeri*	Growth	15 minutes
Microalgal (N)	*Isochrysis galbana*	Growth inhibition	72 hours
Macroalgal Brown kelp(N)	*Ecklonia radiata*	Germination	72 hours
Mollusc Pacific oyster	*Crassostrea gigas*	Larval development	48 hours
Crustacean Copepod (N)	*Gladioferens imparipes*	Reproduction	2 day pulse
Western king prawn (juvenile)	*Melicertus latisulcatus*	Growth	21 days
Western king prawn (adult)	*Melicertus latisulcatus*	Growth	28 days
Fish Mulloway	*Argyrosomus japonicus*	Larval growth	7 days
Pink snapper (N)	*Pagrus auratus*	Larval growth	7 days
Yellowtail kingfish	*Seriola lalandi*	Larval growth	7 days
Cephalopod	*Sepia apama*	Embryo development	4 months
Australian giant cuttlefish	*Sepia apama*	Days to hatch	4 months
	Sepia apama	Length at hatch	4 months
	Sepia apama	Weight at hatch	4 months
	Sepia apama	Width at hatch	4 months
	Sepia apama	Survival post hatch	5 months

Category	Species	Test End Point	Exposure
	Sepia apama	Length post hatch	5 months
	Sepia apama	Weight post hatch	5 months
	Sepia apama	Width post hatch	5 months

The EC/IC50, EC/IC10, NOEC and LOEC values for each test were calculated using the Toxcalc (Tidepool Scientific) statistics program and the results from the RO brine are summarised in Tables 1.2 and 1.3.

All toxicity tests, with the exception of the Pacific oyster, were undertaken at Geotechnical Services' (Geotech) Ecotoxicology Laboratory at Fremantle using filtered seawater obtained from Spencer Gulf as the dilution water (SA Control). The Pacific oyster test was performed by Ecotox Services in Sydney using RO brine and filtered Spencer Gulf seawater as the diluent as supplied by Geotech.

The yellowtail kingfish larvae were tested using filtered Rottnest Island seawater as the diluent due to poor quality eggs and salinity effects of the SA control in the first test. In the first test yellowtail kingfish eggs were transported from South Australia to Fremantle in 35 ppt salinity seawater. Upon hatch they were placed in the SA control seawater at 40 ppt and were unable to acclimatise to the higher salinity. This resulted in high mortality in the controls, thus confounding the test and rendering the test unable to meet its quality criteria. A second test was performed using control seawater of a similar salinity to that of the seawater that the yellowtail kingfish eggs were transported in.

Table 1.2 Summary of Toxicity Values for RO Brine

Test	EC/IC50 %	EC/IC10 %	LOEC %	NOEC %
15 Minute Microtox	81 - >100	-	-	-
72 Hour Algal	>84.4	>84.4	>84.4	84.4
72 Hour Macroalgal	59.1	27.6	50.6	25.3
48 Hour Oyster	4.2	3.3	6.3	3.2
Copepod Reproduction 2 Day Pulse	14.1	10.9	21.1	10.5
21 Day Juvenile Prawn	75.5	53.9	101.3	50.6

Table 1.2 cont Test	EC/IC50 %	EC/IC10 %	LOEC %	NOEC %
28 Day Adult Prawn	21.4	11.8	25.3	12.7
7 Day Larval Mulloway	15.8	11.6	1.6	<1.6
7 Day Larval Snapper	30.1	22.2	25.3	12.7
7 Day Larval Kingfish	16.4	11.1	6.3	3.2

Table 1.3 Cuttlefish Toxicity Results

End Point	2006 EC50 %	2006 EC10 %	2007 EC50 %	2007 EC10 %
Embryo Development	5.81	2.37	9.11	6.38
Days to Hatch	5.42	3.16	8.36	6.39
Length at Hatch	7.51	6.51	8.81	6.3*
Weight at Hatch	6.40	5.74	8.67	6.3*
Width at Hatch	7.42	6.24	8.94	6.3*
Length 30 Days Post Hatch	7.18	6.30	8.52	6.3*
Weight 30 Days Post Hatch	7.38	6.24	8.25	6.3*
Width 30 Days Post Hatch	7.10	6.18	8.25	6.3*
Survival Post Hatch	4.06	1.86	8.68	6.3*

* Toxcalc does not include the EC10 values when using the Trimmed Spearman-Karber non-parametric analysis. The NOEC value is used to replace the EC10 value.

The toxicity of the RO brine may have been masked in the microalgal test (Table 1.2) due to constituents acting as nutrients.

Table 1.4 shows all the concentrations that were used in each test for the WET tests. Concentrations vary due to different methodologies, small test volumes and the addition of food to several of the tests

Geotech has used a pulse exposure for the copepod reproduction assay which takes into account the fact that copepods are planktonic species that will drift through the plume in a short period.

The concentrations of RO brine used in the tests were calculated on the proposed salinity of 78 ppt. The salinity of the RO brine used in the tests was measured at 79 ppt. Therefore, the test concentrations were recalculated to concentrations equivalent to a brine salinity of 78 ppt where 79 ppt = 101.3% RO brine.

Table 1.4 Concentrations of RO Brine Tested

Test	Conc 1 %	Conc 2 %	Conc 3 %	Conc 4 %	Conc 5 %	Conc 6 %	Conc 7 %	Conc 8 %	Conc 9 %
Microtox	6.3	12.7	25.3	50.6	101.3				
Micro-algae	0.33	0.65	1.3	2.6	5.2	10.5	21.1	42.2	84.4
Macro-algae	0.79	1.6	3.2	6.3	12.7	25.3	50.6	101.3	
Oyster	0.79	1.6	6.3	12.7	25.3	50.6			
Copepod	0.7	1.3	2.6	5.25	10.5	21.1	42.2		
Fish	0.79	1.6	3.2	6.3	12.7	25.3	50.6	101.3	
Prawns	0.79	1.6	3.2	6.3	12.7	25.3	50.6	101.3	
Cuttle-fish	0.4	0.79	1.6	3.2	6.3	12.7	25.3	50.6	101.3

Protection Values

The EC10 results in Table 1.5 were used in the BurrliOZ statistics program (Campbell *et al.* 2000) to calculate the protection values at 99%, 95%, 90% and 80% species protection.

Table 1.5 EC10 Results Used to Calculate Species Protection Trigger Values

Test	EC/IC10 %	Test	EC/IC10 %
Microalgal	>84.4	**Kingfish**	11.1
Macroalgal	27.6	**Mulloway**	11.6
Oyster	3.3	**Cuttlefish Post Hatch Survival**	3.7*
Copepod 2 Day Pulse	10.9	**Adult Prawn**	11.8
Larval Snapper	22.2		

*Lowest geometric mean of 2006 and 2007 cuttlefish tests with adjustment factor of 2 to account for the toxicity being determined in 45 ppt diluent.

Table 1.6 BurrliOZ Species Protection Trigger Values

Protection Level	Protection Value % Brine	Dilution
99	1.76	57
95	3.13	32
90	4.20	24
80	5.96	17

Table 1.6 shows that a dilution factor of 57 is required to meet the 99% species protection level. The dilution factor calculated for the 99% species protection level using the EC10 values will theoretically result in only 1% of the exposed species showing a 10% reduction in growth or reproduction if those levels are exceeded outside the mixing zone.

2. Introduction

Geotechnical Services were requested by ARUP/HLA to study the environmental impacts of the RO brine produced by the proposed BHP Billiton desalination plant to be located at Point Lowly in Spencer Gulf. The aim of this project was to determine species protection trigger values for the site based on testing of indigenous species, or species representative of, the receiving temperate marine ecosystem.

As part of the proposed BHP Billiton Olympic Dam mine expansion, a desalination plant located at Point Lowly, 20 km north of Whyalla is planned in order to provide 120 ML per day of fresh water for the mine expansion and possibly also water for Eyre Peninsula communities currently reliant on Murray River and ground water. A discharge of 196 ML per day of concentrated seawater up to 78 ppt is proposed.

2.1 Selected WET Tests and Laboratory

All WET tests were performed at Geotechnical Services' (Geotech) Fremantle Ecotoxicology Laboratory with the exception of the Pacific oyster test which was performed by Ecotox Services based in Sydney. Most of the bioassays performed by Geotech are NATA accredited (N). As Geotech works to a Quality Management System and is a private company all protocols are commercial-in-confidence and are controlled documents, which precludes publication in a public document. However, summaries of the protocols have been included in this document in Appendix 1.

The toxicity of the RO brine was assessed using the following tests, listed in Table 2.1.

Table 2.1 Tests Performed

Category	Species	Test End Point	Exposure
Bacteria Microtox (N)	*Vibrio fischeri*	Growth	15 minutes
Microalgal (N)	*Isochrysis galbana*	Growth inhibition	72 hours
Macroalgal Brown kelp(N)	*Ecklonia radiata*	Germination	72 hours
Mollusc Pacific oyster	*Crassostrea gigas*	Larval development	48 hours
Crustacean Copepod (N)	*Gladioferens imparipes*	Reproduction	2 day pulse
Western king prawn (juvenile)	*Melicertus latisulcatus*	Growth	21 days
Western king prawn (adult)	*Melicertus latisulcatus*	Growth	28 days

Category	Species	Test End Point	Exposure
Fish Mulloway	*Argyrosomus japonicus*	Larval growth	7 days
Pink snapper (N)	*Pagrus auratus*	Larval growth	7 days
Yellowtail kingfish	*Seriola lalandi*	Larval growth	7 days
Cephalopod	*Sepia apama*	Embryo development	4 months
Australian giant cuttlefish	*Sepia apama*	Days to hatch	4 months
	Sepia apama	Length at hatch	4 months
	Sepia apama	Weight at hatch	4 months
	Sepia apama	Width at hatch	4 months
	Sepia apama	Length post hatch	5 months
	Sepia apama	Weight post hatch	5 months
	Sepia apama	Width post hatch	5 months
	Sepia apama	Survival post hatch	5 months

2.2 Rationale of Selected WET tests

The use of living test organisms is the only reliable way to measure the potential biological impacts of a sample. For maximum relevance to the receiving ecosystem, the organisms selected for WET testing must be relevant to or indigenous to the receiving ecosystem or appropriate surrogates following ANZECC and ARMCANZ (2000). Geotech only performs chronic WET tests for calculating species protection trigger levels. Summaries of the following tests are located in Appendix 1.

2.2.1 Microtox

The 15 minute Microtox test is used by Geotech as a range finding test to ensure that the concentrations selected for the chronic bioassays will bracket the EC50. In this case it was used to establish the variability between batches and the stability of the RO brine. The marine bacteria *Vibrio fischeri* is a ubiquitous bacteria, found in marine ecosystems throughout the world. *V. fischeri* displays a high sensitivity to a broad range of chemicals and is used throughout the world for determining toxicity of water, soil and sediment samples.

2.2.2 Microalgae

Unicellular algae form the base of the food chain in the marine system. These algae are primary producers in the marine system and provide food for larval, juvenile and adult crustaceans and molluscs. The marine microalgal species *Isochrysis galbana* was selected as the microalgal species to assess the toxicity of the RO Brine. *Isochrysis* spp. has been commonly used in toxicity tests throughout Australia for the past 15 years and, therefore, a large amount of information is available (Evans *et al*. 2000).

2.2.3 Macroalgae

The marine macroalga *Ecklonia radiata* provides both food and habitat for a range of other organisms in near-shore coastal areas. *E. radiata* is common along the temperate southern Australian coast. Toxicity tests using *E. radiata* have been performed on marine discharges throughout temperate Australia for over 10 years (Bidwell *et al.* 1998, Burridge *et al*. 1999).

2.2.4 Copepod

Copepods are a major part of the marine food chain as they represent a first order consumer and they, in turn, provide food for larval fish and crustaceans. The estuarine copepod *Gladioferens imparipes* was selected to represent copepod species in the marine environment as it has been cultured in the laboratory for over 35 years and has been shown to reproduce successfully at salinities up to 60 ppt after acclimation (Rippingale and Hodgkin 1974). *G. imparipes* is common in estuaries throughout south-west Western Australia (Rippingale and Hodgkin 1974). Toxicity testing has been performed on this species for the last 15 years, therefore, a large amount of information is available (Evans *et al.* 2000).

As marine copepods are normally in the surface water and moved by the currents the amount of exposure that they will get to a RO brine plume will be minimal. The reproduction test is a worst case scenario where the copepods are exposed for the duration of their life. A pulse exposure test provides a more environmentally realistic representation of exposure risk to planktonic copepod species.

2.2.4 Oyster

The Pacific oyster, *Crassostrea gigas*, is a first order consumer, filtering bacteria, microalgae and other small particles from the water column. *C. gigas* is found in temperate waters throughout the world and Australia. *C. gigas* has been used in toxicity tests throughout the world since 1980 and methodology follows the ASTM E724-98 (ASTM 1998).

2.2.6 Larval Fish

The pink snapper, *Pagrus auratus*, yellowtail kingfish, *Seriola lalandi,* mulloway, *Argyrosomus japonicus*, are temperate marine fish commonly found associated with reefs throughout temperate and subtropical Australia. These fish species are commonly found in sheltered waters where they spawn in spring when the larvae and juveniles find appropriate habitat and food within the seagrass beds. Methodology for the larval fish growth tests follows that of USEPA Method 1004.0 Sheepshead Minnow Larval Survival and Growth Test (USEPA. 2003b).

2.2.7 Western King Prawn

The western king prawn, *Melicertus latisulcatus,* is present along the west, south and east coasts of Australia, from Cape Leeuwin in Western Australia to Ballina in northern New South Wales. They also inhabit the gulfs and associated waters in South Australia west to Ceduna. There has been no sampling to determine if the distribution is continuous across the Great Australian Bight. Populations of western king prawns tend to occur in concentrated pockets which are often associated with hypersaline waters or marine embayments such as Shark Bay, Exmouth Gulf, the Gulf of Carpentaria, Gulf St Vincent and Spencer Gulf.

Juvenile and adult western king prawns have not previously been used in toxicity tests. The test methodology for this project was based on work performed by Sang and Fotedar (2004).

2.2.8 Australian Giant Cuttlefish

The Australian Giant Cuttlefish, *Sepia apama,* aggregates annually for breeding in the Point Lowly area and has become a major tourist attraction in South Australia, being visited by divers from all over world.

Testing was performed in 2006 to determine impacts on embryo development of the giant cuttlefish and the methodology developed by Geotech in 2006 was used for testing in 2007. This methodology is described in detail in Geotech's 2006 report "Effects of RO Brine on the Development of Giant Cuttlefish (*Sepia apama*) embryos Report ENV06-128".

This study was instigated to determine the potential impacts of the prototype desalination plant effluent (RO brine) on the local marine ecosystem, including the Australian giant cuttlefish, and to derive species protection trigger values for the discharge site.

3. RO Brine and Point Lowly Diluent

The RO brine and Point Lowly seawater were delivered to Geotech's Welshpool Laboratory on 5th June 2007. The RO brine was processed on site with 3.6 mg/l of NALCO PC-1020T antiscalant added to the seawater prior to processing which concentrated the antiscalant to 7.0 ppm. Salinity, temperature and pH were tested on arrival at the laboratory prior to testing. Sample details are given in Table 3.1. Both the RO brine and diluent seawater were transported in 1000 litre containers in a refrigerated truck at 4°C from South Australia to Perth, Western Australia. The RO brine and seawater were refrigerated at 4°C until use. Prior to use in the bioassays the seawater was filtered to 0.45 micron in 100 – 150 L batches as required and transported to Geotech's Fremantle Ecotoxicology Laboratory in 25 L HDPE containers. The RO brine sample was tested as received. Rottnest Island seawater was used in the yellowtail kingfish larval growth assay. The physicochemical data for the Rottnest Island diluent are shown in Table 3.2. The physicochemical data for the RO brine tests are shown in Table 3.3.

Table 3.1 Sample Information Sheet of RO Brine ECX07-1805

Contact Company	ARUP
Contact Person	David Wiltshire
Contact Phone	08 8104 8310
Contact Address	Level 2 431 – 439 King William St Adelaide SA 5000
Number of Samples	1
Sample Type	RO brine and Point Lowly seawater
Date Sampled	31 May 2007
Location Collected	Point Lowly, South Australia
Sampled by	Paul Fields
Sample pH	Brine 8.04 Seawater 7.62
Sample Salinity	Brine 79.2 ppt Seawater 39.9 ppt
Transport Conditions	Transported at 4ºC
Date of Arrival at Geotech	5th June 2007
Time of Arrival at Geotech	3 pm

Sample Temp on Arrival	4ºC
Sample Received by	Ken Traynor
Tests Requested	Microtox Algal Growth Macroalgal Germination Mollusc Larval Development Copepod Reproduction Bioassay Larval Fish Growth Prawn Growth (Adult and Juvenile) Sardine Larval Growth Crab Larval Growth Cuttlefish Tests

Note: *Crab and sardine larval growth tests were not performed due to mortalities of adult sardines and crab larvae.*

Table 3.2 Rottnest Island Diluent

Concentration 78 ppt RO Brine %	DO ppm	Salinity ppt	pH
0	6.0	35.2	8.22
0.79	6.1	35.2	8.20
1.6	6.0	35.2	8.19
3.2	5.9	36.7	8.19
6.3	5.8	38.1	8.18
12.7	5.8	40.0	8.09
25.3	5.7	44.8	8.05
50.6	5.7	56.3	8.06
101.3	5.6	78.9	8.04

Table 3.3 Physicochemical Parameters for RO Brine Testing (n = 15)

Concentration 78 ppt RO Brine %	DO ppm	Salinity ppt	pH
0	5.8 ± 1.1	40.2 ± 0.8	8.07 ± 0.23
0.40	5.8 ± 1.1	40.8 ± 1.1	8.07 ± 0.24
0.79	5.8 ± 1.1	41.3 ± 1.0	8.06 ± 0.23
1.6	5.8 ± 1.1	41.8 ± 0.9	8.08 ± 0.21
3.2	5.8 ± 1.1	42.3 ± 0.9	8.08 ± 0.23
6.3	5.7 ± 1.2	43.5 ± 0.8	8.09 ± 0.21
12.7	5.0 ± 0.6	46.5 ± 1.2	8.09 ± 0.26
25.3	4.7 ± 0.6	52.1 ± 1.6	7.99 ± 0.33
50.6	4.3 ± 0.6	62.6 ± 1.8	8.01 ± 0.35
101.3	3.2 ± 0.1	79.2 ± 1.3	8.04 ± 0.20

The concentrations of RO brine used in the toxicity tests and calculations were based on the proposed salinity of 78 ppt. The salinity of the RO brine used in the tests was measured at 79 ppt. Therefore, the test concentrations were recalculated to concentrations equivalent to a brine salinity of 78 ppt where 79 ppt = 101.3% RO brine.

The RO brine was tested with Microtox on arrival and at 30 day intervals to determine the stability of the brine. A second batch of RO brine was produced on the 28th August 2007 by Citor. This was also tested with Microtox to monitor the toxicity over the duration of the tests. The Microtox results are shown in Table 3.4.

Table 3.4 Microtox Results for RO Brine

Date	Day	EC50 %
13/06/07	Day 7 Batch 1	>100
12/07/07	Day 30 Batch 1	>100
16/08/07	Day 60 Batch 1	> 100
30/08/07	Day 1 Batch 2	83
06/09/07	Day 7 Batch 2	81
28/09/07	Day 30 Batch 2	81

The Microtox results in Table 3.4 showed that the toxicity of the RO brine from Batch 1 was stable until day 60. Batch 2 of the RO brine also showed stability over the testing period.

4. Results

The results for all the toxicity tests on the RO brine follow in this section and all raw data are shown in Appendix 2.

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Ecotoxicology Laboratory Test Report
Report Date: 11 October 2007

Sample Details

Lab ID No. ECX07-1805	**Sample:** RO Brine
Client: ARUP	**Date Sampled:** 19/06/07
Attn: David Wiltshire	**Date Received:** 19/06/07
Level 2	**Sampled By:** D. Bourke
431 – 439 King William St	**pH:** 8.1
Adelaide SA 5000	**Salinity:** 39.9 – 79
Phone No. 08 8104 8310	**Test Started:** 19/06/07
Mobile:	**Test Finished:** 22/06/07
Order No.: Contract	**Test Temperature:** 20.0 ± 1.0°C

Test Performed	Microalgal
Test Protocol	WIENV-45
Reference	Stauber et al. 1994
Test Species	*Isochrysis galbana*
Deviations from Protocol	Nil

Algal Test Results

Concentration %	% Growth of Control n = 4
SA Control	99.9 ± 6.0
0.33	101.0 ± 11.1
0.65	111.4 ± 5.8
1.3	123.5 ± 8.3
2.6	128.9 ± 12.4
5.2	150.7 ± 5.8
10.5	109.3 ± 7.3
21.1	112.5 ± 9.9
42.2	113.9 ± 10.9
84.4	109.9 ± 8.9

Sample	EC50 %	EC10 %	LOEC %	NOEC %
RO Brine	>84.4	>84.4	>84.4	84.4

Results apply to the sample in the condition as received by Geotech

Quality Assurance Limits for the Algal Toxicity Test.

	EC50	Cusum Chart Limits	Coefficient of Variation
Copper	38.6 ppb	32.4 – 113 ppb	27.7 %

Authorised Signatory: Dr Jill Woodworth Laboratory Manager	NATA ACCREDITED FOR TECHNICAL COMPETENCE

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Ecotoxicology Laboratory Test Report

Report Date: 11th October 2007

Sample Details

Lab ID No. ECX07-1805	**Sample:** RO Brine
Client: ARUP	**Date Sampled:** 06/07/07
Attn: David Wiltshire	**Date Received:** 06/07/07
Level 2	**Sampled By:** D. Bourke
431 – 439 King William St	**pH:** 8.1
Adelaide SA 5000	**Salinity:** 39.9 – 79
Phone No. 08 8104 8310	**Test Started:** 06/07/07
Mobile:	**Test Finished:** 09/07/07
Order No.: Contract	**Test Temperature:** 20.0 ± 1.0°C

Test Performed	Macroalgal Germination
Test Protocol	WIENV-67
Reference	Burridge *et al.* 1999
Test Species	*Ecklonia Radiata*
Deviations from Protocol	Nil

Macroalgal Test Results

Concentration Tested %	**% Germination n = 90**
SA Control	92.2 ± 3.8
0.79	96.7 ± 5.8
1.6	90.0 ± 3.3
3.2	92.2 ± 5.1
6.3	88.8 ± 7.7
12.7	85.6 ± 8.4
25.3	90.0 ± 3.3
50.6	56.7 ± 3.4
101.3	0.0 ± 0.0

Sample	**EC50 %**	**EC10 %**	**LOEC %**	**NOEC %**
RO Brine	59.1	27.6	50.6	25.3

Results apply to the sample in the condition as received by Geotech

Quality Assurance Limits for the Macroalgal Toxicity Test.

	EC50	**Cusum Chart Limits**	**Coefficient of Variation**
Copper	202.6 ppb	44.6 – 205.2 ppb	32.1%

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Ecotoxicology Laboratory Test Report

Report Date: 12th October 2007

Sample Details

Lab ID No. ECX07-1805	**Sample:** RO Brine
Client: ARUP	**Date Sampled:** 13th June 2007
Attn: David Wiltshire	**Date Received:** 14th June 2007
Level 2	**Sampled By:** D. Bourke
31 – 39 King William St	**pH:** 7.9 – 8.1
Adelaide SA 5000	**Salinity:** 39.9 -79
Phone No. 08 8104 8310	**Test Started:** 16th June 2007
Mobile:	**Test Finished:** 19th June 2007
Order No.: Contract	**Test Temperature:** 25°C

Test Performed	Pacific Oyster Larval Development
Test Protocol	ESA SOP 106
Reference	APHA (1998)
Test Species	*Crassostrea gigas*
Deviations from Protocol	Nil

Mussel Test Results

Concentration Tested %	% Normal n = 120
Laboratory Control	68.1 ± 7.7
SA Control	67.0 ± 8.7
0.79	68.1 ± 3.4
1.6	65.9 ± 5.0
3.2	61.4 ± 17.0
6.3	0.0 ± 0.0
12.7	0.0 ± 0.0
25.3	0.0 ± 0.0
50.6	0.0 ± 0.0

Sample	EC50 %	EC10 %	LOEC %	NOEC %
RO Brine	4.22	3.27	6.3	3.2

Results apply to the sample in the condition as received by ESA

Quality Assurance Limits for the Mussel Toxicity Test.

	EC50	Cusum Chart Limits	Coefficient of Variation
Copper	17.8 ppb	13.9 – 24.6 ppb	Not Stated

Authorised Signatory: Dr Jill Woodworth [signature] Laboratory Manager	

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Ecotoxicology Laboratory Test Report

Report Date: 11 October 2007

Sample Details

Lab ID No. ECX07-1805	**Sample:** RO Brine
Client: ARUP	**Date Sampled:** 10/08/07
Attn: David Wiltshire	**Date Received:** 10/08/07
Level 2,	**Sampled By:** D. Bourke
431 – 439 King William St	**pH:** 8.1
Adelaide SA 5000	**Salinity:** 39.9 – 79
Phone No. 08 8104 8310	**Test Started:** 10/08/07
Mobile:	**Test Finished:** 08/09/07
Order No.: Contract	**Test Temperature:** 20.0 ± 1.0°C

Test Performed	Copepod Reproduction
Test Protocol	WIENV-62
Reference	USEPA 1002.0 Cladoceran 7 Day Reproduction Test
Test Species	*Gladioferens imparipes*
Deviations from Protocol	2 day pulse exposure

Copepod Reproduction Test Results

Concentration Tested %	Av. Neonates / female n = 4	Av. Production %
SA Control	42.2 ± 9.2	100 ± 21.8
0.7	55.7 ± 31.2	131.9 ± 73.9
1.3	40.5 ± 31.8	95.8 ± 75
2.6	44.0 ± 32.5	86.3 ± 95.4
5.25	36.5 ± 40.3	86.4 ± 95.4
10.5	37.5 ± 23.3	88.9 ± 55.0
21.1	0.0 ± 0.0	0.0 ± 0.0
42.2	0.0 ± 0.0	0.0 ± 0.0

Sample	EC50 %	EC10 %	LOEC %	NOEC %
RO Brine	14.1	10.9	21.1	10.5

Results apply to the sample in the condition as received by Geotech

Quality Assurance Limits for the Copepod Reproduction Toxicity Test.

	EC50	Cusum Chart Limits	Coefficient of Variation
Chromium	285 ppb	113 – 325 ppb	24%

Authorised Signatory: Dr Jill Woodworth Laboratory Manager	NATA ACCREDITED FOR TECHNICAL COMPETENCE

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Ecotoxicology Laboratory Test Report

Report Date: 16th October 2007

Sample Details

Lab ID No. ECX07-1805	**Sample:** RO Brine
Client: ARUP	**Date Sampled:** 31 st July 2007
Attn: David Wiltshire	**Date Received:** 31st July 2007
Level 2	**Sampled By:** D. Bourke
431 – 439 King William St	**pH:** 8.1
Adelaide SA 5000	**Salinity:** 39.9 – 79
Phone No. 08 8104 8310	**Test Started:** 31st July 2007
Mobile:	**Test Finished:** 7th August 2007
Order No.: Contract	**Test Temperature:** 21.0 ± 1.0 °C

Test Performed	Fish Larval Growth
Test Protocol	WIENV-64
Reference	USEPA 1004.0 Larval Fish Growth Test
Test Species	*Pagrus auratus*
Deviations from Protocol	Nil

Larval Fish Test Results

Concentration Tested %	Av. Length (mm) n=30	% Growth n = 30
Initial	2.24 ± 0.07	
SA Control	3.24 ± 0.16	100.0 ± 15.7
0.79	3.17 ± 0.10	93.3 ± 10.5
1.6	3.21 ± 0.14	97.0 ± 14.2
3.2	3.24 ± 0.17	100.0 ± 16.9
6.3	3.25 ± 0.09	101.0 ± 9.0
12.7	3.18 ± 0.22	94.3 ± 22.1
25.3	2.98 ± 0.06	74.0 ± 5.5
50.6	0.0 ± 0.0	0.0 ± 0.0
101.3	0.0 ± 0.0	0.0 ± 0.0

Sample	EC50 %	EC10 %	LOEC %	NOEC %
RO Brine	30.1	22.2	25.3	12.7

Results apply to the sample in the condition as received by Geotech

Quality Assurance Limits for the Larval Fish Toxicity Test.

	EC50	Cusum Chart Limits	Coefficient of Variation
Chromium	3.37 ppm	2.29 – 3.77 ppm	12.1

Authorised Signatory: Dr Jill Woodworth JWood Laboratory Manager	NATA ACCREDITED FOR TECHNICAL COMPETENCE

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Ecotoxicology Laboratory Test Report

Report Date: 18th December 2007

Sample Details

Lab ID No. ECX07-1805	**Sample:** RO Brine
Client: ARUP	**Date Sampled:** 13th November 2007
Attn: David Wiltshire	**Date Received:** 13th November 2007
Level 2	**Sampled By:** D. Bourke
431 – 439 King William St	**pH:** 8.1
Adelaide SA 5000	**Salinity:** 35.2 – 79
Phone No. 08 8104 8310	**Test Started:** 13th November 2007
Mobile:	**Test Finished:** 21st November 2007
Order No.: Contract	**Test Temperature:** 21.0 ± 1.0°C

Test Performed	Fish Larval Growth
Test Protocol	WIENV-64
Reference	USEPA 1004.0 Larval Fish Growth Test
Test Species	Yellowtail Kingfish
Deviations from Protocol	Rottnest Island Seawater Diluent

Larval Fish Test Results

Concentration Tested %	Av. Length mm	% Growth n = 30
Initial	3.93 ± 0.09	
RI Control	4.45 ± 0.07	100 ± 12.7
0.79	4.31 ± 0.12	73.6 ± 22.6
1.6	4.31 ± 0.05	74.1 ± 8.9
3.2	4.32 ± 0.16	76.6 ± 29.9
6.3	4.25 ± 0.04	62.4 ± 8.6
12.7	4.25 ± 0.04	61.1 ± 6.7
25.3	3.89 ± 0.11	-6.5 ± 22.3
50.6	0.0 ± 0.0	0.0 ± 0.0
101.3	0.0 ± 0.0	0.0 ± 0.0

Sample	EC50 %	EC10 %	LOEC %	NOEC %
RO Brine	16.4	11.1	6.3	3.2

Results apply to the sample in the condition as received by Geotech

Quality Assurance Limits for the Larval Fish Toxicity Test.

	EC50	Cusum Chart Limits	Coefficient of Variation
Chromium	3.9ppm	2.3 – 3.9 ppm	13.0%

Authorised Signatory: Dr Jill Woodworth Laboratory Manager	

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Ecotoxicology Laboratory Test Report

Report Date: 12th October 2007

Sample Details

Lab ID No. ECX07-1805	**Sample:** RO Brine
Client: ARUP	**Date Sampled:** 27th October 2007
Attn: David Wiltshire	**Date Received:** 27th October 2007
Level 2	**Sampled By:** D. Bourke
431 – 439 King William St	**pH:** 8.1
Adelaide SA 5000	**Salinity:** 39.9 – 79
Phone No. 08 8104 8310	**Test Started:** 27th October 2007
Mobile:	**Test Finished:** 3rd November 2007
Order No.: Contract	**Test Temperature:** 21.0 ± 1.0°C

Test Performed	Fish Larval Growth
Test Protocol	WIENV-64
Reference	USEPA 1004.0 Larval Fish Growth Test
Test Species	Mulloway
Deviations from Protocol	Nil

Larval Fish Test Results

Concentration Tested %	Av. Length mm	% Growth n = 30
Initial	2.34 ± 0.03	
SA Control	2.79 ± 0.08	101.5 ± 19.6
0.79	2.58 ± 0.04	52.4 ± 10.4
1.6	2.69 ± 0.04	77.6 ± 9.0
3.2	2.64 ± 0.04	67.9 ± 8.3
6.3	2.66 ± 0.03	71.8 ± 6.3
12.7	2.62 ± 0.05	63.5 ± 12.5
25.3	0.0 ± 0.0	0.0 ± 0.0
50.6	0.0 ± 0.0	0.0 ± 0.0
101.3	0.0 ± 0.0	0.0 ± 0.0

Sample	EC50 %	EC10 %	LOEC %	NOEC %
RO Brine	15.8	11.6	1.6	<1.6

Results apply to the sample in the condition as received by Geotech

Quality Assurance Limits for the Larval Fish Toxicity Test.

	EC50	Cusum Chart Limits	Coefficient of Variation
Chromium	2.9 ppm	2.29 – 3.77 ppm	12.1%

Authorised Signatory: Dr Jill Woodworth Laboratory Manager	

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Ecotoxicology Laboratory Test Report

Report Date: 16th October 2007

Sample Details

Lab ID No. ECX07-1805	**Sample:** RO Brine
Client: ARUP	**Date Sampled:** 22 June 2007
Attn: David Wiltshire	**Date Received:** 22 June 2007
Level 2	**Sampled By:** D. Bourke
431 – 439 King William St	**pH:** 8.1
Adelaide SA 5000	**Salinity:** 39.9 – 79
Phone No. 08 8104 8310	**Test Started:** 22 June 2007
Mobile:	**Test Finished:** 13 July 2007
Order No.: Contract	**Test Temperature:** 17.0 ± 1.0 °C

Test Performed	Juvenile Prawn Growth
Test Protocol	Appendix 1
Reference	Sang and Fotedar 2004
Test Species	Western King Prawn
Deviations from Protocol	NA

Juvenile Prawn Test Results

Concentration Tested %	Initial Weight (g) n = 30	21 Day Weight (g) n=30	% Growth n = 30
SA Control	0.239 ± 0.047	0.246 ± 0.062	102.4 ± 14.8
0.79	0.323 ± 0.020	0.333 ± 0.039	102.9 ± 5.8
1.6	0.228 ± 0.060	0.228 ± 0.069	100.0 ± 14.9
3.2	0.222 ± 0.038	0.222 ± 0.027	100.4 ± 7.1
6.3	0.287 ± 0.028	0.259 ± 0.044	91.1 ± 18.3
12.7	0.254 ± 0.019	0.262 ± 0.051	102.8 ± 15.7
25.3	0.282 ± 0.021	0.339 ± 0.045	119.7 ± 12.2
50.6	0.233 ± 0.037	0.243 ± 0.023	106.0 ± 21.7
101.3	0.271 ± 0.030	0.0 ± 0.0	0.0 ± 0.0

Sample	EC50 %	EC10 %	LOEC %	NOEC %
RO Brine	75.5	53.9	101.3	50.6

Results apply to the sample in the condition as received by Geotech

Authorised Signatory: Dr Jill Woodworth Laboratory Manager	

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Ecotoxicology Laboratory Test Report

Report Date: 16th October 2007

Sample Details

Lab ID No. ECX07-1805	**Sample:** RO Brine
Client: ARUP	**Date Sampled:** 17 July 2007
Attn: David Wiltshire	**Date Received:** 17 July 2007
Level 2	**Sampled By:** D. Bourke
431 – 439 King William St	**pH:** 8.1
Adelaide SA 5000	**Salinity:** 39.9 – 79
Phone No. 08 8104 8310	**Test Started:** 17 July 2007
Mobile:	**Test Finished:** 14 August 2007
Order No.: Contract	**Test Temperature:** 17.0 ± 1.0 °C

Test Performed	Adult Prawn Growth
Test Protocol	Appendix 1
Reference	Sang and Fotedar 2004
Test Species	Western King Prawn
Deviations from Protocol	NA

Adult Prawn Test Results

Concentration Tested %	Initial Weight (g) n = 30	Av. Weight (g) n=30	% Growth n = 30
SA Control	28.3 ± 2.3	35.1 ± 0.4	100.3 ± 40.4
0.79	27.7 ± 9.0	32.8 ± 8.2	74.0 ± 11.9
1.6	23.1 ± 2.6	27.9 ± 3.6	74.8 ± 8.9
3.2	27.3 ± 1.9	30.8 ± 1.1	51.2 ± 11.3
6.3	33.5 ± 3.4	39.7 ± 3.7	92.1 ± 4.0
12.7	28.5 ± 0.5	32.1 ± 0.7	52.9 ± 9.6
25.3	30.5 ± 3.7	0.0 ± 0.0	0.0 ± 0.0
50.6	28.5 ± 3.5	0.0 ± 0.0	0.0 ± 0.0
101.3	32.0 ± 3.7	0.0 ± 0.0	0.0 ± 0.0

Sample	EC50 %	EC10 %	LOEC %	NOEC %
RO Brine	21.4	11.8	25.3	12.7

Results apply to the sample in the condition as received by Geotech

Authorised Signatory: Dr Jill Woodworth JWood Laboratory Manager	

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Ecotoxicology Laboratory Test Report

Report Date: 12th November 2007

Sample Details

Lab ID No. ECX07-1805	**Sample:** RO Brine
Client: ARUP	**Date Sampled:** 06/07 – 11/ 07
Attn: David Wiltshire	**Date Received:** 06/07 – 11/ 07
Level 2	**Sampled By:** D. Bourke
431 – 439 King William St	**pH:** 8.1
Adelaide SA 5000	**Salinity:** 39.9 – 79
Phone No. 08 8104 8310	**Test Started:** 7th June 2007
Mobile:	**Test Finished:** 29th October 2007
Order No.: Contract	**Test Temperature:** 15.0 ± 0.5°C

Test Performed	Giant Cuttlefish
Test Protocol	Appendix 1
Reference	Geotech Report ENV06-128 Nov 2006
Test Species	*Sepia apama*
Deviations from Protocol	Nil

Embryo Development Test Results

Concentration Tested %	Embryo Development n=11
SA Control	61.8 ± 7.6
0.4	67.2 ± 4.9
0.79	59.9 ± 8.1
1.6	59.9 ± 12.2
3.2	65.4 ± 14.9
6.3	56.3 ± 4.1
12.7	0.0 ± 0.0
25.3	0.0 ± 0.0
50.6	0.0 ± 0.0
101.3	0.0 ± 0.0

Sample	EC50 %	EC10 %	LOEC %	NOEC %
RO Brine	9.1	6.4	12.7	6.3

Results apply to the sample in the condition as received by Geotech

Authorised Signatory: Dr Jill Woodworth Laboratory Manager	

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Ecotoxicology Laboratory Test Report

Report Date: 12th November 2007

Sample Details

Lab ID No. ECX07-1805	**Sample:** RO Brine
Client: ARUP	**Date Sampled:** 06/07 – 11/ 07
Attn: David Wiltshire	**Date Received:** 06/07 – 11/ 07
Level 2	**Sampled By:** D. Bourke
431 – 439 King William St	**pH:** 8.1
Adelaide SA 5000	**Salinity:** 39.9 – 79
Phone No. 08 8104 8310	**Test Started:** 7th June 2007
Mobile:	**Test Finished:** 29th October 2007
Order No.: Contract	**Test Temperature:** 15.0 ± 0.5°C

Test Performed	Giant Cuttlefish
Test Protocol	Appendix 1
Reference	Geotech Report ENV06-128 Nov 2006
Test Species	*Sepia apama*
Deviations from Protocol	Nil

Days to Hatch Test Results

Concentration Tested %	% Days to Hatch n=11
SA Control	95.5 ± 7.1
0.4	110.4 ± 1.5
0.79	96.8 ± 5.8
1.6	101.5 ± 5.9
3.2	101.3 ± 5.4
6.3	101.1 ± 5.7
12.7	0.0 ± 0.0
25.3	0.0 ± 0.0
50.6	0.0 ± 0.0
101.3	0.0 ± 0.0

Sample	EC50 %	EC10 %	LOEC %	NOEC %
RO Brine	8.4	6.4	12.7	6.3

Results apply to the sample in the condition as received by Geotech

Authorised Signatory: Dr Jill Woodworth [signature] Laboratory Manager	

GEOTECHNICAL SERVICES

Ecotoxicology Laboratory Test Report

Report Date: 12th November 2007

Sample Details

Lab ID No. ECX07-1805	**Sample:** RO Brine
Client: ARUP	**Date Sampled:** 06/07 – 11/ 07
Attn: David Wiltshire	**Date Received:** 06/07 – 11/ 07
Level 2	**Sampled By:** D. Bourke
431 – 439 King William St	**pH:** 8.1
Adelaide SA 5000	**Salinity:** 39.9 – 79
Phone No. 08 8104 8310	**Test Started:** 7th June 2007
Mobile:	**Test Finished:** 29th October 2007
Order No.: Contract	**Test Temperature:** 15.0 ± 0.5°C

Test Performed	Giant Cuttlefish
Test Protocol	Appendix 1
Reference	Geotech Report ENV06-128 Nov 2006
Test Species	*Sepia apama*
Deviations from Protocol	Nil

Length at Hatch Results

Concentration Tested %	Length at Hatch cm n=3
SA Control	1.27 ± 0.02
0.4	1.16 ± 0.05
0.79	1.27 ± 0.07
1.6	1.19 ± 0.03
3.2	1.23 ± 0.05
6.3	1.24 ± 0.04
12.7	0.0 ± 0.0
25.3	0.0 ± 0.0
50.6	0.0 ± 0.0
101.3	0.0 ± 0.0

Sample	EC50 %	EC10 %	LOEC %	NOEC %
RO Brine	8.81	NC	12.7	6.3

NC = Not calculated by Toxcalc
Results applying to the sample in the condition as received by Geotech

Authorised Signatory: Dr Jill Woodworth Laboratory Manager	

GEOTECHNICAL SERVICES

Ecotoxicology Laboratory Test Report

Report Date: 12th November 2007

Sample Details

Lab ID No. ECX07-1805	**Sample:** RO Brine
Client: ARUP	**Date Sampled:** 06/07 – 11/ 07
Attn: David Wiltshire	**Date Received:** 06/07 – 11/ 07
Level 2	**Sampled By:** D. Bourke
431 – 439 King William St	**pH:** 8.1
Adelaide SA 5000	**Salinity:** 39.9 – 79
Phone No. 08 8104 8310	**Test Started:** 7th June 2007
Mobile:	**Test Finished:** 29th October 2007
Order No.: Contract	**Test Temperature:** 15.0 ± 0.5°C

Test Performed	Giant Cuttlefish
Test Protocol	Appendix 1
Reference	Geotech Report ENV06-128 Nov 2006
Test Species	*Sepia apama*
Deviations from Protocol	Nil

Weight at Hatch Results

Concentration Tested %	Weight at Hatch g n=3
SA Control	0.66 ± 0.03
0.4	0.55 ± 0.04
0.79	0.60 ± 0.08
1.6	0.59 ± 0.06
3.2	0.62 ± 0.05
6.3	0.65 ± 0.10
12.7	0.0 ± 0.0
25.3	0.0 ± 0.0
50.6	0.0 ± 0.0
101.3	0.0 ± 0.0

Sample	EC50 %	EC10 %	LOEC %	NOEC %
RO Brine	8.68	NC	12.7	6.3

NC = Not calculated by Toxcalc
Results apply to the sample in the condition as received by Geotech

Authorised Signatory: Dr Jill Woodworth  Laboratory Manager	

GEOTECHNICAL SERVICES

Ecotoxicology Laboratory Test Report

Report Date: 12th November 2007

Sample Details

Lab ID No. ECX07-1805	**Sample:** RO Brine
Client: ARUP	**Date Sampled:** 06/07 – 11/ 07
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Level 2	**Sampled By:** D. Bourke
431 – 439 King William St	**pH:** 8.1
Adelaide SA 5000	**Salinity:** 39.9 – 79
Phone No. 08 8104 8310	**Test Started:** 7th June 2007
Mobile:	**Test Finished:** 29th October 2007
Order No.: Contract	**Test Temperature:** 15.0 ± 0.5°C

Test Performed	Giant Cuttlefish
Test Protocol	Appendix 1
Reference	Geotech Report ENV06-128 Nov 2006
Test Species	*Sepia apama*
Deviations from Protocol	Nil

Width at Hatch Results

Concentration Tested %	Width at Hatch cm n=3
SA Control	0.99 ± 0.10
0.4	0.88 ± 0.09
0.79	0.99 ± 0.07
1.6	0.97 ± 0.07
3.2	1.01 ± 0.05
6.3	1.02 ± 0.08
12.7	0.0 ± 0.0
25.3	0.0 ± 0.0
50.6	0.0 ± 0.0
101.3	0.0 ± 0.0

Sample	EC50 %	EC10 %	LOEC %	NOEC %
RO Brine	8.94	NC	12.7	6.3

NC = Not calculated by Toxcalc
Results apply to the sample in the condition as received by Geotech

Authorised Signatory: Dr Jill Woodworth Laboratory Manager	

GEOTECHNICAL SERVICES

Ecotoxicology Laboratory Test Report

Report Date: 12th November 2007

Sample Details

Lab ID No. ECX07-1805	**Sample:** RO Brine
Client: ARUP	**Date Sampled:** 06/07 – 11/ 07
Attn: David Wiltshire	**Date Received:** 06/07 – 11/ 07
Level 2	**Sampled By:** D. Bourke
431 – 439 King William St	**pH:** 8.1
Adelaide SA 5000	**Salinity:** 39.9 – 79
Phone No. 08 8104 8310	**Test Started:** 7th June 2007
Mobile:	**Test Finished:** 29th October 2007
Order No.: Contract	**Test Temperature:** 15.0 ± 0.5°C

Test Performed	Giant Cuttlefish
Test Protocol	Appendix 1
Reference	Geotech Report ENV06-128 Nov 2006
Test Species	*Sepia apama*
Deviations from Protocol	Nil

Post Hatch Length Results

Concentration Tested %	Post Hatch Length cm n=4
SA Control	1.28 ± 0.05
0.4	1.29 ± 0.04
0.79	1.23 ± 0.05
1.6	1.25 ± 0.05
3.2	1.27 ± 0.08
6.3	1.31 ± 0.11
12.7	0.0 ± 0.0
25.3	0.0 ± 0.0
50.6	0.0 ± 0.0
101.3	0.0 ± 0.0

Sample	EC50 %	EC10 %	LOEC %	NOEC %
RO Brine	8.52	NC	12.7	6.3

NC = Not calculated by Toxcalc
Results apply to the sample in the condition as received by Geotech

Authorised Signatory: Dr Jill Woodworth Laboratory Manager	

GEOTECH

GEOTECHNICAL SERVICES

Ecotoxicology Laboratory Test Report

Report Date: 12th November 2007

Sample Details

Lab ID No. ECX07-1805	**Sample:** RO Brine
Client: ARUP	**Date Sampled:** 06/07 – 11/ 07
Attn: David Wiltshire	**Date Received:** 06/07 – 11/ 07
Level 2	**Sampled By:** D. Bourke
431 – 439 King William St	**pH:** 8.1
Adelaide SA 5000	**Salinity:** 39.9 – 79
Phone No. 08 8104 8310	**Test Started:** 7th June 2007
Mobile:	**Test Finished:** 29th October 2007
Order No.: Contract	**Test Temperature:** 15.0 ± 0.5°C

Test Performed	Giant Cuttlefish
Test Protocol	Appendix 1
Reference	Geotech Report ENV06-128 Nov 2006
Test Species	*Sepia apama*
Deviations from Protocol	Nil

Post Hatch Weight Results

Concentration Tested %	Post Hatch Weight g n=4
SA Control	0.56 ± 0.05
0.4	0.53 ± 0.06
0.79	0.53 ± 0.04
1.6	0.59 ± 0.06
3.2	0.59 ± 0.03
6.3	0.68 ± 0.14
12.7	0.0 ± 0.0
25.3	0.0 ± 0.0
50.6	0.0 ± 0.0
101.3	0.0 ± 0.0

Sample	EC50 %	EC10 %	LOEC %	NOEC %
RO Brine	8.25	NC	12.7	6.3

NC = Not calculated by Toxcalc

Results apply to the sample in the condition as received by Geotech

Authorised Signatory: Dr Jill Woodworth [signature] Laboratory Manager	

GEOTECH

GEOTECHNICAL SERVICES

Ecotoxicology Laboratory Test Report

Report Date: 12th November 2007

Sample Details

Lab ID No. ECX07-1805	**Sample:** RO Brine
Client: ARUP	**Date Sampled:** 06/07 – 11/ 07
Attn: David Wiltshire	**Date Received:** 06/07 – 11/ 07
Level 2	**Sampled By:** D. Bourke
431 – 439 King William St	**pH:** 8.1
Adelaide SA 5000	**Salinity:** 39.9 – 79
Phone No. 08 8104 8310	**Test Started:** 7th June 2007
Mobile:	**Test Finished:** 29th October 2007
Order No.: Contract	**Test Temperature:** 15.0 ± 0.5°C

Test Performed	Giant Cuttlefish
Test Protocol	Appendix 1
Reference	Geotech Report ENV06-128 Nov 2006
Test Species	*Sepia apama*
Deviations from Protocol	Nil

Post Hatch Width Results

Concentration Tested %	Post Hatch Width cm n=4
SA Control	0.93 ± 0.06
0.4	0.94 ± 0.05
0.79	0.87 ± 0.05
1.6	0.87 ± 0.03
3.2	0.91 ± 0.07
6.3	0.97 ± 0.13
12.7	0.0 ± 0.0
25.3	0.0 ± 0.0
50.6	0.0 ± 0.0
101.3	0.0 ± 0.0

Sample	EC50 %	EC10 %	LOEC %	NOEC %
RO Brine	8.25	NC	12.7	6.3

NC = Not calculated by Toxcalc

Results apply to the sample in the condition as received by Geotech

Authorised Signatory: Dr Jill Woodworth Laboratory Manager	

GEOTECH
GEOTECHNICAL SERVICES

Ecotoxicology Laboratory Test Report

Report Date: 12th November 2007

Sample Details

Lab ID No. ECX07-1805	**Sample:** RO Brine
Client: ARUP	**Date Sampled:** 06/07 – 11/ 07
Attn: David Wiltshire	**Date Received:** 06/07 – 11/ 07
Level 2	**Sampled By:** D. Bourke
431 – 439 King William St	**pH:** 8.1
Adelaide SA 5000	**Salinity:** 39.9 – 79
Phone No. 08 8104 8310	**Test Started:** 7th June 2007
Mobile:	**Test Finished:** 29th October 2007
Order No.: Contract	**Test Temperature:** 15.0 ± 0.5°C

Test Performed	Giant Cuttlefish
Test Protocol	Appendix 1
Reference	Geotech Report ENV06-128 Nov 2006
Test Species	*Sepia apama*
Deviations from Protocol	Nil

Post Hatch Survival Test Results

Concentration Tested %	No Dead	No Live	Total
SA Control	5	14	19
0.4	12	5	17
0.79	4	14	18
1.6	6	12	18
3.2	6	15	21
6.3	2	14	16
12.7	0	0	0
25.3	0	0	0
50.6	0	0	0
101.3	0	0	0

Sample	EC50 %	EC10 %	LOEC %	NOEC %
RO Brine	8.7	NC	12.7	6.3

NC = Not Calculated by Toxcalc
Results apply to the sample in the condition as received by Geotech

Authorised Signatory: Dr Jill Woodworth [signature] Laboratory Manager	

5. Species Protection Trigger Values

5.1. Introduction

Following the protocol outlined in ANZECC and ARMCANZ (2000) 99%, 95%, 90% and 80% species protection trigger values were calculated using the EC10 data from nine chronic bioassays that were performed on species indigenous to, or surrogate to, the receiving ecosystem at Point Lowly. The EC10 data used in the BurrliOz statistics package are shown in Table 5.1.

It is important to note that the use of NOEC values is not recommended for the calculation of species protection trigger values, as has been done in the past when only NOEC values were available. This was pointed out in ANZECC and ARMCANZ (2000) which stated that methods used to derive the trigger values are not data specific as long as only one type of data is used. Therefore, trigger values could be derived using EC10 values if there were sufficient data (Warne 1998). ANZECC and ARMCANZ (2000) also suggest that the use of NOEC data be phased out as EC10 data becomes available.

The use of toxicity values that correspond to a fixed biological effect (eg an LC5 or EC10) that would be calculated using regression analysis is recommended. The NOEC is an inappropriate number to use for regulatory purposes for the reasons discussed in Chapman (2005). Problems with the use of NOEC and LOEC data revolve around the fact that these values are determined using hypothesis based statistical techniques. Specifically the problems are that:

- only tested concentrations can be NOEC or LOEC values (therefore the NOEC and LOEC are, to a large degree, affected by the concentrations used in the toxicity test),

- the NOEC title is misleading. A NOEC is the highest concentration used in a toxicity test that causes an effect not significantly different to the control(s). It therefore does not correspond to ‘no effect’. Typically, the NOEC corresponds to a 10 to 30% effect (Hoekstra and Van Ewijk, 1993, Moore and Caux, 1997, USEPA, 1991).

Further, usually there is a high level of statistical uncertainty associated with the EC/IC5 values making these values inappropriate for use in the BurrliOZ statistics package for calculation of protection/dilution values (Chapman 2005).

The dilution factors calculated for the 99% species protection value using the EC10 values will theoretically result in only 1% of the exposed species showing a 10% reduction in growth or reproduction if those levels are exceeded outside the mixing zone.

Values in Table 5.1 were placed in the BurrliOZ software to calculate a trigger value designed to protect 99%, 95%, 90% and 80% of the species from adverse effects during exposure to the RO brine plume from the proposed desalination plant. The trigger values calculated are shown in Table 5.2. The graph for the species protection trigger values is shown in Appendix 3.

5.2. Methodology

The BurrliOZ software was developed by the CSIRO Environmetrics Group for Environment Australia to implement ANZECC and ARMCANZ (2000) requirements to generate trigger values (ie the maximum concentration of a chemical that should permit the integrity and function of aquatic environments to be maintained) for local conditions within Australia. BurrliOZ uses a flexible family of distributions, the Burr Type III, to estimate the protecting concentrations of chemicals such that a given percentage of species will not be adversely affected (Campbell *et al.* 2000).

5.*3. Results*

Table 5.1 EC10 Results Used to Calculate Species Protection Trigger Values

Test	**EC/IC10 %**	**Test**	**EC/IC10 %**
Microalgal	84.4	**Kingfish Growth**	11.1
Macroalgal	27.6	**Mulloway Growth**	11.6
Oyster	3.3	**Cuttlefish Post Hatch Survival**	3.7*
Copepod Reproduction 2 Day Pulse	10.9	**Adult Prawn Growth**	11.8
Larval Snapper Growth	22.2		

*Lowest geometric mean of 2006 and 2007 cuttlefish tests with adjustment factor of 2 to account for the toxicity being determined in 45 ppt diluent.

Table 5.2 BurrliOZ Species Protection Trigger Levels

Protection Level	Protection Value % Brine	Dilution
99	1.76	57
95	3.13	32
90	4.20	24
80	5.96	17

5.4 Discussion

5.4.1 Microalgal Test
The results from the microalgal tests show that the algae is probably using the RO brine as a nutrient source as there was increased growth in all concentrations of RO brine tested. This absence of any observed toxicity rendered the EC10 unable to be calculated. Therefore, the unbounded NOEC value of 84.4 was used in place of the EC10 to calculate the protection trigger values. This will result in a conservative calculation as the actual NOEC may be up to 100%.

5.4.2 Invertebrate Tests
The oyster larval development test was the most sensitive with an EC10 of 3.3 % (Table 1.1). The adult prawns were shown to be more sensitive to the RO brine than the juvenile prawns with EC10s of 11.8% and 53.9% respectively. The adult prawn EC10 was, therefore, used in the BurrliOZ program to calculate the species protection trigger values. The pulse exposure of the copepods provided a realistic measure of toxicity of the RO brine.

5.4.3 Fish Tests
Pink snapper, mulloway and yellowtail kingfish eggs were obtained from Challenger TAFE hatchery, Fremantle, Western Australia. The adult fish are maintained in seawater of 34 ppt. Eggs from each species were placed in test solutions to acclimatise for 24 hours prior to hatching. Unhatched eggs were removed from the test solutions. Due to the poor quality yellowtail kingfish eggs received from South Australia, eggs from Fremantle were used in a repeat test using Rottnest Island seawater as the diluent. The mulloway and yellowtail kingfish showed similar EC10 values with the pink snapper showing less sensitivity.

5.4.4 Cuttlefish Tests

The cuttlefish results shown in Table 1.2 show that there were slight variations between the 2006 and 2007 cuttlefish tests, probably due to the difference in diluent salinity, 45 ppt in 2006 and 39.9 ppt in 2007. The higher salinity of the diluent in the 2006 tests would account for the lower EC50 values obtained. In particular, the post hatch survival in the 2006 test showed low EC50 and EC10 values, 4.06% and 1.86% RO brine respectively. This could be directly attributed to the higher salinity as the 2007 test showed an EC50 of 8.68% and a NOEC of 6.3% RO brine.

However, the variations between 2007 and 2006 may also be due to the age of the embryos at the start of the test as the development of the 2007 embryos was slightly more advanced than the 2006 embryos at the start of the test. The quality of the embryos received in June 2007 was not as high as the embryos received in June 2006 as the embryos were small and discoloured with a low fertilisation rate (Table 5.3). The embryos received in 2006 were white and round in shape with a high fertilisation rate.

Table 5.3 Percentage hatch

Concentration	2006 % Hatch	2007 % Hatch
Control	100	61.8 ± 7.6
0.4	NT	67.2 ± 4.9
0.79	NT	59.9 ± 8.1
1.6	89.1	59.9 ± 12.2
3.2	83.6	65.4 ± 14.9
6.3	75.5	56.3 ± 4.1
12.7	0	0
25.3	0	0
50.6	0	0
101.3	0	0

NT = Not Tested

Table 5.3 shows the % hatch for the cuttlefish that was related to the quality of the eggs and not to the concentration of brine and may be a result of low fertilisation rate. It is important to note that the test concentrations from 6.3% and below had hatch rates that were not significantly different from the controls. Due to the low hatch rate, the Day 60 and Day 90 data were not measured as was done in 2006. The Day 30 data was recorded but was confounded by the quality of the eggs. As all the parameters measured were calculated on the response of the controls to the RO brine and the number of hatchings was taken into account for all calculations, the hatch rate, though low, did not confound the results. However, as discussed in Warne (2008), neither the 2006 or 2007 tests were ideal. Therefore the results were combined in this report to select an appropriate value to use in the species protection calculations.

Following the ANZECC and ARMCANZ (2000) procedures for selecting toxicity values where there are several toxicity values for different endpoints as in the case of the cuttlefish tests, the endpoint with the lowest geometric mean is taken to represent the species. The lowest geometric mean EC10 value of 1.86% RO brine was the post hatch survival in the 2006 cuttlefish test. This value was given an adjustment factor of 2 to correct the EC10 value to account for the toxicity being determined in diluent water with a salinity of 45 ppt rather than the normal 42 ppt at Point Lowly (Warne 2007). This resulted in a value of 3.7 representing the cuttlefish in the species protection calculations. This value is more conservative than the EC10s of 6.3 -6.4% obtained in the 2007 tests where the diluent salinity was 39.9 ppt and which may have underestimated the toxicity of the brine in the receiving water.

5.4.5 Chronic Tests

As, in all the chronic tests performed, all test organisms in the various test concentrations are compared with the controls, only the toxic effect of the RO brine is assessed. In this suite of tests the salinity effects were not assessed.

The EC10 results of the range of species tested showed a good distribution fit (Appendix 3). Based on these results the species tested are appropriate for calculating the species protection values using the BurrliOZ statistics package, as a wide range of sensitivities resulted. These results can be refined with further testing, if required.

The results listed in Table 5.2 show the concentrations of RO brine in the water column that will meet the species protection trigger level for 99%, 95%, 90% and 80% species protection levels and the dilutions required to meet those concentrations.

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APPENDIX 1

Summary of Toxicity Test Methodologies

Summary 1.1 Test Conditions for the Microtox 15 Minute Cellular Activity Test

Test type	Static
Test organism	Marine bacteria *Vibrio fischeri*
Source of organisms	SDI Reagent Freeze Dried
Age of test organisms	Newly reconstituted reagent
Salinity	30 (control) - 79 ppt
Temperature	15 ± 0.5 °C
Test chamber size	3.0 mL
Test solution volume	1.0 mL
Volume of bacteria per test chamber	10µL
No of replicates per concentration	3
Dilution water	SDI Diluent
Salinity adjustment	***Not required***
Test concentrations	***101.3, 50.6, 25.3, 12.7, 6.3 and 0%***
Test duration	15 Minutes
Endpoints	Cellular activity as measured by luminescence output
Test acceptability criteria	Phenol Reference Toxicant EC50 between Cusum Chart limits
Quality assurance	All SDI products are to be used before they reach their expiry date.

Summary 1.2 Test Conditions for the Unicellular Algal Growth Test

Test type	Static
Test organism	*Isochrysis galbana* (Tahitian isolate)
Source of test organism	Laboratory Culture
Age of test organisms	5 day old culture
Salinity	39.9 – 79 ppt
Temperature	20 ± 1.0 °C
Light	Ambient laboratory illumination
Photoperiod	12 hour light / 12 hour dark
Test chamber size	3.2 mL
Test solution volume	3 mL
Renewal of test solutions	nil
Volume of algae per test chamber	500 µL
No of replicates per concentration	4
Dilution water	Point Lowly filtered seawater
Test concentrations	***0, 0.33, 0.65, 1.3, 2.6, 5.2, 10.5, 21.1, 42.2 and 84.4 %***
Test duration	72 Hours
Endpoints	Inhibition of growth when compared with controls
Test acceptability criteria	Reference Toxicant EC50 between Cusum Chart limits

Summary 1.3 Test Conditions for the Macroalgae Germination Test

Test type	Static
Test organism	*Ecklonia radiata*
Age of test organisms	Newly released gametes
Source of test organisms	Point Peron
Date collected	6th July 2007
Salinity	39.9 - 79 ppt
Temperature	20 ± 1.0 °C
Light	Ambient laboratory illumination
Photoperiod	12 hour light / 12 hour dark
Test chamber size	25 mL
Test solution volume	20 mL
Renewal of test solutions	nil
No zygotes per test chamber	Minimum of 100
No of replicates per concentration	4
Dilution water	Point Lowly filtered seawater
Test concentrations	***0, 0.79, 1.6, 3.2, 6.3, 12.7, 25.3, 50.6, 101.3%***
Test duration	72 hours
Endpoints	Number of zygotes with germination tubes
Test acceptability criteria	80% or greater germination in the controls and Reference Toxicant EC50 between Cusum Chart limits

Summary 1.4 Test Conditions for the Pacific Oyster Larval Development Test

Test type	Static
Species tested	Pacific oyster: *Crassostrea gigas*
Age of test organisms	Fertilized zygotes
Source of test organisms	Shellfish Culture, Tasmania
Salinity	39.9 - 79 ppt
Temperature	25 ± 0.5 °C
Light	Ambient laboratory illumination
Photoperiod	12 hour light / 12 hour dark
Test chamber size	3.5 mL
Test solution volume	3 mL
Renewal of test solutions	nil
No zygotes/larvae per test chamber	Minimum of 100
No of replicates per concentration	4
Dilution water	Point Lowly filtered seawater
Test concentrations	***0, 0.79, 1.6, 3.2, 6.3, 12.7, 25.3, 50.6%***
Test duration	48 hours
Endpoints	Percentage of normal larvae
Test acceptability criteria	80% or greater normal larvae in the controls and Reference Toxicant EC50 between Cusum Chart limits

Summary 1.5 Test Conditions for the Copepod Reproduction 28 Day Test

Test type	Static Renewal
Test organism	*Gladioferens imparipes*
Age of test organisms	Newly hatched neonates (<24 hrs old)
Source of test organisms	Laboratory culture
Salinity	39.9 - 79 ppt
Temperature	20.0 ± 1.0 °C
Light	Ambient laboratory illumination
Photoperiod	12 hour light / 12 hour dark
Test chamber size	3.2 mL
Test solution volume	3 mL
Renewal of test solutions	100 % / day
No Adults per test chamber	2
No of replicates per concentration	4-6
Source of food	*Isochrysis galbana*
Feeding regime	Fed 0.5 mL algae once/day
Cleaning	Siphon daily prior to test solution renewal and feeding
Aeration	None
Dilution water	Point Lowly filtered seawater
Test concentrations	***0, 0.7, 1.3, 2.6, 5.25, 10.5, 21.1 and 42.2 %***
Test duration	28 Days
Endpoints	Number of neonates produced by female copepod per spawn
Test acceptability criteria	80% or greater survival in the controls and Reference Toxicant EC50 between Cusum Chart limits

Summary 1.6 Test Conditions for the 7 Day Larval Fish Growth Test

Test type	Static
Test organism	Pink snapper: *Pagrus auratus* Mulloway: *Argyrosomus japonicus*. Yellowtail kingfish: *Seriola lalandi*
Source of species	Challenger TAFE, Fremantle, WA
Salinity	39.9 – 79 ppt
Salinity of yellowtail kingfish	35 - 79 ppt
Temperature	21 ± 1.0 °C
Light	Ambient laboratory illumination
Photoperiod	12 hour light / 12 hour dark
Test chamber size	500 mL
Test solution volume	400 mL
Renewal of test solutions	Nil
Age of test organisms	Newly hatched larvae (<24 hrs old)
No larvae per test chamber	20
No of replicates per concentration	3
No larvae per concentration	60
Source of food	Rotifers
Feeding regime	Fed once/day @ 40 / mL from day 3
Aeration	None
Dilution water	Point Lowly filtered seawater
Dilution water for yellowtail kingfish	Rottnest Island filtered seawater
Test concentrations	0,0.79, 1.6, 3.2, 6.3, 12.7, 25.3, 50.6 and 101.3%
Test duration	7 Days
Endpoints	Growth – measured as total length
Test acceptability criteria	80% or greater survival in the controls and Reference Toxicant EC50 between Cusum Chart limits

Summary 1.7 Test Conditions for the Adult Prawn Growth Chronic Toxicity Test

Test organism	Western King Prawn *Melicertus latisulcatus*
Source of organism	Spencer Gulf
Reference	Sang and Fotedar 2004
Source of diluent	Spencer Gulf
Test type	Static renewal
Test duration	30 Days
Test end-points	Growth as wet weight
Test temperature	17.0 ± 1.0ºC
Test salinity (Controls)	Ambient Spencer Gulf – 39.9 ppt
Test chamber size / volume	100 Litres
Number of replicates	3
Number of treatments	9
Actual brine concentrations	0%, 0.79, 1.6, 3.2, 6.3, 12.7, 25.3, 50.6, 101.3%
Number of organisms	10 in each treatment
Total number in treatment	30
Food requirements	6% body weight mussel meat once per day
Test acceptability criteria	80% survival in controls

Summary 1.8 Test Conditions for the Juvenile Prawn Growth Chronic Toxicity Test

Test species	Western King Prawn *Melicertus latisulcatus*
Source of organism	Spencer Gulf
Source of diluent	Spencer Gulf
Reference	Sang and Fotedar 2004
Test type	Static renewal
Test duration	21 Days
Test end-points	Growth as wet weight
Test temperature	17.0 ± 1.0ºC
Test salinity (Controls)	Ambient Spencer Gulf – 39.9 ppt
Test chamber size / volume	100 Litres
Number of replicates	3
Number of treatments	9
Actual brine concentrations	0%, 0.79, 1.6, 3.2, 6.3, 12.7, 25.3, 50.6, 101.3%
Number of organisms	10 in each treatment
Total number in treatment	30
Food requirements	6% body weight mussel meat once per day
Test acceptability criteria	80% survival in controls

Summary 1.9 Test Conditions for the Giant Cuttlefish Test

Test organism	Giant Cuttlefish *Sepia apama*
Source of organisms	Point Lowly
Age of test organisms	Newly laid eggs
Test type	Static Renewal
Salinity	39.9 – 79 ppt
Temperature	15.0 ± 0.5 °C
Light	Shaded laboratory illumination
Photoperiod	8 hour muted light / 16 hour dark then in October change to 12 hour muted light / 12 hour dark
Test chamber size	5 L
Test solution volume	5 L
Renewal of test solutions	50% Daily
Number of embryos per test chamber	11
No of replicates per concentration	5
Dilution water	Spencer Gulf
Test concentrations	0%, 0.4, 0.79, 1.6, 3.2, 6.3, 12.7, 25.3, 50.6, 101.3%
Test duration	5 Months
Endpoints (where possible depending on age of eggs at collection)	Time to hatch Length, weight and width at hatch compared to controls Embryo development Survival and growth post hatch
Test acceptability criteria	<20% mortality in controls

APPENDIX 2

Summary of Toxicity Test Data

Phytoplankton Test-Growth-Absorbance

Start Date:	19/06/2007	Test ID:	ECX07-1805	Sample ID:	Brine
End Date:	22/06/2007	Lab ID:	Freo	Sample Type:	SA Desalination Plant
Sample Date:	18/05/2007	Protocol:	Geotech WIENV-45	Test Species:	Isochrysis sp.
Comments:					

Conc-%	1	2	3	4
Control	0.0943	0.1028	0.0957	0.1071
0.66	0.0971	0.1100	0.0871	0.1100
1.3	0.1220	0.1320	0.1120	0.1250
2.6	0.1270	0.1270	0.1150	0.1450
5.3	0.1500	0.1420	0.1550	0.1540
10.55	0.1180	0.1110	0.1040	0.1020
21.1	0.1240	0.1140	0.1000	0.1110
42.2	0.1300	0.1100	0.1050	0.1100
84.4	0.1220	0.1080	0.1020	0.1050

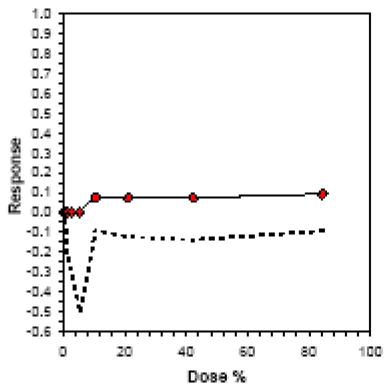
			Transform: Untransformed						1-Tailed		Isot
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean
Control	0.1000	1.0000	0.1000	0.0943	0.1071	6.036	4				0.1205
0.66	0.1011	1.0108	0.1011	0.0871	0.1100	10.996	4	-0.165	2.513	0.0164	0.1205
1.3	0.1228	1.2278	0.1228	0.1120	0.1320	6.763	4	-3.486	2.513	0.0164	0.1205
2.6	0.1285	1.2853	0.1285	0.1150	0.1450	9.626	4	-4.366	2.513	0.0164	0.1205
5.3	0.1503	1.5029	0.1503	0.1420	0.1550	3.933	4	-7.695	2.513	0.0164	0.1205
10.55	0.1088	1.0878	0.1088	0.1020	0.1180	6.689	4	-1.343	2.513	0.0164	0.1116
21.1	0.1123	1.1228	0.1123	0.1000	0.1240	8.800	4	-1.879	2.513	0.0164	0.1116
42.2	0.1138	1.1378	0.1138	0.1050	0.1300	9.747	4	-2.108	2.513	0.0164	0.1116
84.4	0.1093	1.0928	0.1093	0.1020	0.1220	8.097	4	-1.420	2.513	0.0164	0.1093

Auxiliary Tests	Statistic	Critical	Skew
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.964404	0.912	0.298186
Bartlett's Test indicates equal variances (p = 0.94)	2.872124	20.09016	

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob
Dunnett's Test	84.4	>84.4		1.184834	0.016421	0.164252	0.000986	8.54E-05	5.9E-07

Linear Interpolation (200 Resamples)

Point	%	SD	95% CL(Exp)	Skew
IC05	8.8456			
IC10	>84.4			
IC15	>84.4			
IC20	>84.4			
IC25	>84.4			
IC40	>84.4			
IC50	>84.4			



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				Proportion Germinated	
Start Date:	6/07/2007	Test ID:	ECX07-1805	Sample ID:	Brine
End Date:	9/07/2007	Lab ID:	Freo	Sample Type:	SA Desalination Plant
Sample Date:	18/05/2007	Protocol:	Geotech WIENV-67	Test Species:	Ecklonia radiata
Comments:					

Conc-%	1	2	3
Control	0.9667	0.9000	0.9000
0.79	1.0000	1.0000	0.9000
1.6	0.9000	0.9333	0.8667
3.2	0.9667	0.9333	0.8667
6.3	0.9333	0.9333	0.8000
12.7	0.7667	0.8667	0.9333
25.3	0.8667	0.9333	0.9000
50.6	0.5667	0.6000	0.5333
101.3	0.0000	0.0000	0.0000

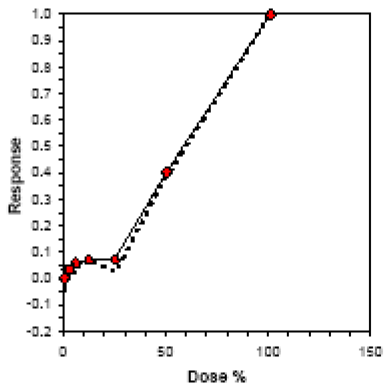
			Transform: Arcsin Square Root						1-Tailed		Isot
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean
Control	0.9222	1.0000	1.2951	1.2490	1.3872	6.159	3				0.9444
0.79	0.9667	1.0482	1.4026	1.2490	1.4794	9.481	3	-1.416	2.560	0.1943	0.9444
1.6	0.9000	0.9759	1.2519	1.1970	1.3096	4.503	3	0.569	2.560	0.1943	0.9111
3.2	0.9222	1.0000	1.2979	1.1970	1.3872	7.368	3	-0.038	2.560	0.1943	0.9111
6.3	0.8889	0.9639	1.2421	1.1071	1.3096	9.412	3	0.698	2.560	0.1943	0.8889
12.7	0.8556	0.9277	1.1911	1.0667	1.3096	10.208	3	1.370	2.560	0.1943	0.8778
25.3	0.9000	0.9759	1.2519	1.1970	1.3096	4.503	3	0.569	2.560	0.1943	0.8778
*50.6	0.5667	0.6145	0.8524	0.8188	0.8861	3.949	3	5.833	2.560	0.1943	0.5667
101.3	0.0000	0.0000	0.0914	0.0914	0.0914	0.000	3				0.0000

Auxiliary Tests	Statistic	Critical	Skew
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.945487	0.884	-0.45766
Bartlett's Test indicates equal variances (p = 0.74)	4.32944	18.47532	

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob
Dunnett's Test	25.3	50.6	35.7796	3.952569	0.131009	0.141495	0.078641	0.008642	1.4E-04

Linear Interpolation (200 Resamples)

Point	%	SD	95% CL(Exp)		Skew
IC05	5.138	8.709	0.000	50.805	1.3444
IC10	27.559	7.429	0.000	33.613	-1.6753
IC15	31.399	1.720	23.565	37.052	-0.2641
IC20	35.239	1.549	28.262	40.362	-0.2004
IC25	39.079	1.429	32.869	44.165	-0.0865
IC40	50.600	1.465	44.980	56.515	0.2100
IC50	59.050	1.338	52.936	63.979	-0.0645



Oyster Larval Development Test-Proportion Normal

Start Date:	14/06/2007	Test ID:	ECX07-1805	Sample ID:	Brine
End Date:	17/06/2007	Lab ID:	Freo	Sample Type:	SA Desalination Plant
Sample Date:	18/05/2007	Protocol:	ESA SOP 106	Test Species:	CR-Crassostrea gigas
Comments:					

Conc-%	1	2	3	4
Control	0.6532	0.6081	0.7883	0.6757
Diluent Control	0.5856	0.7207	0.7658	0.6081
0.79	0.6532	0.6982	0.7207	0.6532
1.6	0.6081	0.7207	0.6306	0.6757
3.2	0.3604	0.7207	0.6982	0.6757
6.3	0.0100	0.0200	0.0100	0.0200
12.7	0.0000	0.0000	0.0000	0.0000
25.3	0.0000	0.0000	0.0000	0.0000
50.6	0.0000	0.0000	0.0000	0.0000
101.3	0.0000	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root						1-Tailed		Number
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp
Control	0.6813	1.0168	0.9733	0.8944	1.0927	8.716	4				
Diluent Control	0.6701	1.0000	0.9614	0.8714	1.0656	9.725	4				131
0.79	0.6813	1.0168	0.9713	0.9411	1.0140	3.743	4	-0.151	2.360	0.1560	128
1.6	0.6588	0.9832	0.9477	0.8944	1.0140	5.598	4	0.207	2.360	0.1560	136
3.2	0.6138	0.9160	0.9030	0.6439	1.0140	19.256	4	0.883	2.360	0.1560	154
*6.3	0.0150	0.0224	0.1210	0.1002	0.1419	19.906	4	12.713	2.360	0.1560	394
12.7	0.0000	0.0000	0.0500	0.0500	0.0500	0.000	4				400
25.3	0.0000	0.0000	0.0500	0.0500	0.0500	0.000	4				400
50.6	0.0000	0.0000	0.0500	0.0500	0.0500	0.000	4				400
101.3	0.0000	0.0000	0.0500	0.0500	0.0500	0.000	4				400

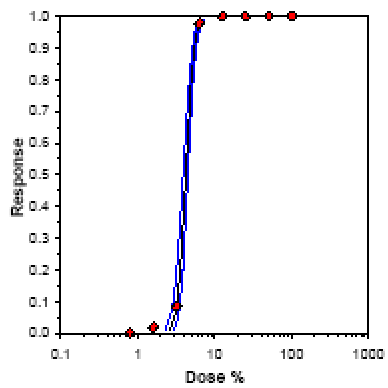
Auxiliary Tests	Statistic	Critical	Skew
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.88445	0.868	-1.50534
Bartlett's Test indicates equal variances (p = 0.02)	11.8899	13.2767	
The control means are not significantly different (p = 0.86)	0.188807	2.446914	

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob
Dunnett's Test	3.2	6.3	4.489989	31.25	0.152393	0.22666	0.546988	0.008739	3.5E-09

Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma
Slope	11.52634	1.062579	9.443683	13.60899	0.3275	0.365603	12.59158	1	0.625194	0.086758
Intercept	-2.2062	0.757022	-3.68996	-0.72243						
TSCR	0.329167	0.013565	0.302579	0.355755						

Point	Probits	%	95% Fiducial Limits	
EC01	2.674	2.650727	2.257201	2.974942
EC05	3.355	3.037316	2.657722	3.347957
EC10	3.718	3.265948	2.897979	3.56747
EC15	3.964	3.429851	3.071312	3.724766
EC20	4.158	3.565962	3.215732	3.855555
EC25	4.326	3.68703	3.344407	3.972156
EC40	4.747	4.010644	3.688301	4.286078
EC50	5.000	4.218848	3.908493	4.490765
EC60	5.253	4.437862	4.13814	4.709424
EC75	5.674	4.827376	4.538589	5.109675
EC80	5.842	4.991271	4.703119	5.283318
EC85	6.036	5.189346	4.898375	5.497744
EC90	6.282	5.449774	5.148941	5.787431
EC95	6.645	5.860003	5.530162	6.260831
EC99	7.326	6.714641	6.282711	7.302368



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				Copepod Reproduction		
Start Date:	10/08/2007	Test ID:	ECX07-1805		Sample ID:	SA Brine
End Date:	8/09/2007	Lab ID:	Freo		Sample Type:	SA Desalination Plant Discharge
Sample Date:	10/08/2007	Protocol:	GEOTECH WIENV-62		Test Species:	Gladioferens imparipes
Comments:						

Conc-%	1	2	3	4
Control	0.9230	0.7340	1.0000	1.0000
0.66	1.0000	1.0000	0.9700	0.5200
1.3	0.4260	1.0000		
2.7	1.0000	0.5000		
5.25	0.1890	1.0000		
10.5	1.0000	0.5000		
21.1	0.0300	0.0200	0.0100	0.0000
42.2	0.0000	0.0000	0.0000	0.0000

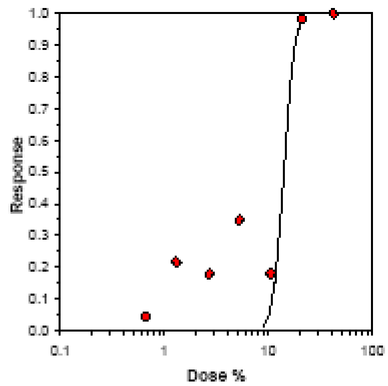
			Transform: Arcsin Square Root						1-Tailed		Number
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp
Control	0.9143	1.0000	1.3400	1.0289	1.5208	17.484	4				35
0.66	0.8725	0.9543	1.3109	0.8054	1.5208	26.092	4	0.106	2.746	0.7552	51
1.3	0.7130	0.7799	1.1160	0.7111	1.5208	51.302	2	0.665	2.746	0.9249	57
2.7	0.7500	0.8203	1.1531	0.7854	1.5208	45.096	2	0.555	2.746	0.9249	50
5.25	0.5945	0.6503	0.9853	0.4498	1.5208	76.866	2	1.053	2.746	0.9249	81
10.5	0.7500	0.8203	1.1531	0.7854	1.5208	45.096	2	0.555	2.746	0.9249	50
*21.1	0.0150	0.0164	0.1165	0.0500	0.1741	46.067	4	4.449	2.746	0.7552	394
42.2	0.0000	0.0000	0.0500	0.0500	0.0500	0.000	4				400

Auxiliary Tests					Statistic		Critical		Skew
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)					0.946041		0.868		-0.22585
Bartlett's Test indicates equal variances (p = 0.14)					9.756529		16.81187		
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob
Bonferroni t Test	10.5	21.1	14.88456	9.52381	0.64289	0.678382	0.681921	0.151262	0.011002

			Maximum Likelihood-Probit							
Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma
Slope	11.80204	4.616473	-0.06496	23.66904	0.0875	81.34926	11.07048	4.4E-16	1.147834	0.084731
Intercept	-8.54678	5.802415	-23.4623	6.368782						
TSCR	0.195714	0.042771	0.085769	0.305659						

Point	Probits	%	95% Fiducial Limits
EC01	2.674	8.927287	
EC05	3.355	10.19679	
EC10	3.718	10.94577	
EC15	3.964	11.48194	
EC20	4.158	11.92675	
EC25	4.326	12.32206	
EC40	4.747	13.37726	
EC50	5.000	14.05509	
EC60	5.253	14.76726	
EC75	5.674	16.03186	
EC80	5.842	16.56323	
EC85	6.036	17.20488	
EC90	6.282	18.04766	
EC95	6.645	19.37331	
EC99	7.326	22.12828	

Significant heterogeneity detected (p = 4.38E-16)



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Juvenile Prawn Growth Test

Start Date:	22/06/2007	Test ID:	ECX07-1805	Sample ID:	Brine
End Date:	13/07/2007	Lab ID:	Freo	Sample Type:	SA Desalination Plant
Sample Date:	18/05/2007	Protocol:	Geotech WI	Test Species:	Western King Prawn
Comments:					

Conc-%	1	2	3
Control	1.0000	1.0000	0.8730
0.79	1.0000	0.9700	1.0000
1.6	1.0000	1.0000	0.8330
3.2	0.9340	1.0000	1.0000
6.3	0.8310	1.0000	0.7810
12.7	1.0000	0.8530	1.0000
25.3	1.0000	1.0000	1.0000
50.6	1.0000	1.0000	0.8100
101.3	0.0200	0.0300	0.0100

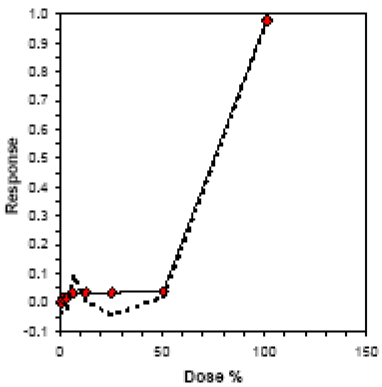
			Transform: Arcsin Square Root						1-Tailed		Isot
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean
Control	0.9577	1.0000	1.4160	1.2064	1.5208	12.818	3				0.9733
0.79	0.9900	1.0338	1.4794	1.3967	1.5208	4.842	3	-0.467	2.580	0.3502	0.9733
1.6	0.9443	0.9861	1.3971	1.1498	1.5208	15.330	3	0.139	2.580	0.3502	0.9600
3.2	0.9780	1.0212	1.4508	1.3110	1.5208	8.349	3	-0.257	2.580	0.3502	0.9600
6.3	0.8707	0.9092	1.2506	1.0838	1.5208	18.882	3	1.219	2.580	0.3502	0.9400
12.7	0.9510	0.9930	1.4063	1.1773	1.5208	14.101	3	0.071	2.580	0.3502	0.9400
25.3	1.0000	1.0442	1.5208	1.5208	1.5208	0.000	3	-0.772	2.580	0.3502	0.9400
50.6	0.9367	0.9781	1.3671	1.1198	1.5208	16.691	3	0.213	2.580	0.3502	0.9367
*101.3	0.0200	0.0209	0.1367	0.1002	0.1741	26.717	3	9.411	2.580	0.3502	0.0200

Auxiliary Tests	Statistic	Critical	Skew
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.933948	0.894	-0.47241
Equality of variance cannot be confirmed			

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob
Dunnett's Test	50.6	101.3	71.59455	1.976285	0.210267	0.215387	0.55851	0.02763	1.7E-07

Linear Interpolation (200 Resamples)

Point	%	SD	95% CL(Exp)		Skew
IC05	51.264	21.474	0.000	55.305	-0.3695
IC10	53.955	6.772	16.520	57.779	-4.0061
IC15	56.647	2.357	44.413	60.254	-2.2269
IC20	59.339	1.899	47.906	62.728	-1.1360
IC25	62.031	1.776	51.308	65.202	-1.1327
IC40	70.106	1.409	61.515	72.625	-1.1170
IC50	75.489	1.166	68.320	77.574	-1.0972



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Adult Prawn Growth

Start Date:	17/07/2007	Test ID:	ECX07-1805	Sample ID:	Brine
End Date:	14/08/2007	Lab ID:	Freo	Sample Type:	SA Desalination Plant
Sample Date:	18/05/2007	Protocol:	Geotech WI	Test Species:	Western King Prawn
Comments:					

Conc-%	1	2	3
Control	0.8630	0.6880	1.0000
0.79	0.8770	0.6620	0.6820
1.6	0.8500	0.7060	0.6870
3.2	0.6030	0.5480	0.3850
12.7	0.6180	0.5440	0.4260
25.3	0.3670	0.0000	0.6030
50.6	0.0000	0.0000	0.0000
101.3	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root						1-Tailed		Number
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp
Control	0.8503	1.0000	1.2302	0.9781	1.5208	22.221	3				45
0.79	0.7403	0.8706	1.0448	0.9504	1.2125	13.931	3	0.987	2.500	0.4692	78
1.6	0.7477	0.8793	1.0493	0.9771	1.1731	10.265	3	0.964	2.500	0.4692	75
3.2	0.5120	0.6021	0.7973	0.6694	0.8891	14.331	3	2.306	2.500	0.4692	146
12.7	0.5293	0.6225	0.8150	0.7111	0.9045	11.963	3	2.212	2.500	0.4692	141
*25.3	0.3233	0.3802	0.5300	0.0500	0.8891	81.589	3	3.731	2.500	0.4692	203
50.6	0.0000	0.0000	0.0500	0.0500	0.0500	0.000	3				300
101.3	0.0000	0.0000	0.0500	0.0500	0.0500	0.000	3				300

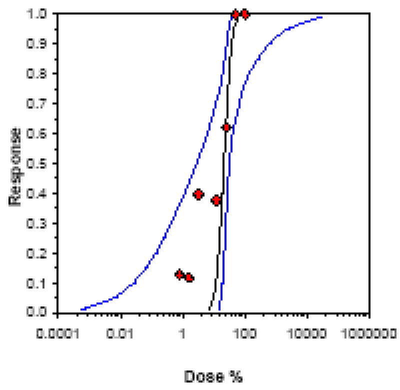
Auxiliary Tests							Statistic		Critical		Skew
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)							0.961414		0.858		-0.46683
Bartlett's Test indicates equal variances (p = 0.25)							6.565877		15.08632		
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU			MSDu	MSDp	MSB	MSE	F-Prob
Dunnett's Test	12.7	25.3	17.92512	7.874016			0.412836	0.464695	0.183735	0.052843	0.035715

Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma
Slope	5.005438	1.71048	0.608516	9.402359	0.15	80.54761	11.07048	6.5E-16	1.329482	0.199783
Intercept	-1.65464	2.445066	-7.93987	4.630592						
TSCR	0.296824	0.052255	0.162499	0.43115						

Point	Probits	%	95% Fiducial Limits	
EC01	2.674	7.323488	0.000552	14.35326
EC05	3.355	10.02002	0.00714	17.29284
EC10	3.718	11.84269	0.027777	19.20997
EC15	3.964	13.25625	0.069182	20.70624
EC20	4.158	14.49909	0.142364	22.0577
EC25	4.326	15.6578	0.263425	23.37398
EC40	4.747	19.00499	1.207748	27.81542
EC50	5.000	21.35414	2.897527	32.1748
EC60	5.253	23.99366	6.433725	40.21262
EC75	5.674	29.12283	16.11561	87.58822
EC80	5.842	31.45021	19.59499	141.2481
EC85	6.036	34.39883	22.90569	264.8789
EC90	6.282	38.50472	26.31351	619.0059
EC95	6.645	45.50881	30.63409	2297.937
EC99	7.326	62.26533	38.23909	28671.25

Significant heterogeneity detected (p = 6.45E-16)



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Larval Fish Growth

Start Date: 13/11/2007	Test ID: ECX07-1805	Sample ID: SA Desalination Plant	
End Date: 21/11/2007	Lab ID: Freo	Sample Type: Brine	
Sample Date: 28/08/2007	Protocol: GEOTECH WIENV62	Test Species: Yellowtail Kingfish	
Comments:			

Conc-%	1	2	3
Control	1.0000	1.0000	0.8610
0.79	0.9480	0.4960	0.7610
1.6	0.6390	0.8000	0.7840
3.2	0.8910	0.9820	0.4230
6.3	0.6010	0.5510	0.7190
12.7	0.6660	0.6320	0.5360
25.3	0.0000	0.0000	0.1800
50.6	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root						1-Tailed		Number
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp
Control	0.9537	1.0000	1.4101	1.1887	1.5208	13.595	3				14
0.79	0.7357	0.7714	1.0614	0.7834	1.3407	26.256	3	1.971	2.530	0.4476	79
1.6	0.7410	0.7770	1.0403	0.9263	1.1071	9.540	3	2.090	2.530	0.4476	78
3.2	0.7653	0.8025	1.1262	0.7081	1.4362	33.379	3	1.604	2.530	0.4476	71
*6.3	0.6237	0.6540	0.9119	0.8365	1.0121	9.912	3	2.816	2.530	0.4476	113
*12.7	0.6113	0.6410	0.8963	0.8214	0.9546	7.675	3	2.892	2.530	0.4476	116
*25.3	0.0600	0.0629	0.1794	0.0500	0.4381	124.911	3	6.956	2.530	0.4476	282
50.6	0.0000	0.0000	0.0500	0.0500	0.0500	0.000	3				300

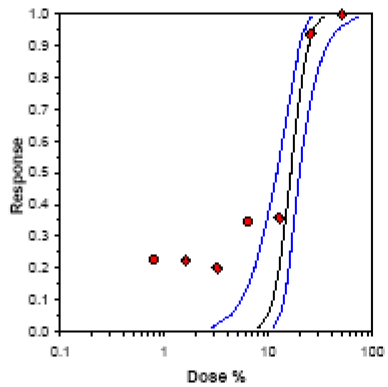
Auxiliary Tests					Statistic		Critical		Skew
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)					0.97188		0.873		-0.34911
Bartlett's Test indicates equal variances (p = 0.32)					7.012969		16.81187		
Hypothesis Test (1-tail, 0.05)	**NOEC**	**LOEC**	**ChV**	**TU**	**MSDu**	**MSDp**	**MSB**	**MSE**	**F-Prob**
Dunnett's Test	3.2	6.3	4.489989	31.25	0.301016	0.308925	0.430588	0.046958	3.4E-04

Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma
Slope	7.498539	1.582755	3.429945	11.56713	0.046667	34.76016	11.07048	1.7E-06	1.215096	0.133359
Intercept	-4.11144	2.017397	-9.29732	1.074431						
TSCR	0.236033	0.029008	0.161465	0.3106						

Point	Probits	%	95% Fiducial Limits	
EC01	2.674	8.032594	2.828396	11.24898
EC05	3.355	9.902376	4.417433	13.03435
EC10	3.718	11.07106	5.584808	14.14425
EC15	3.964	11.93653	6.528522	14.97712
EC20	4.158	12.67237	7.378399	15.70075
EC25	4.326	13.33971	8.18192	16.37583
EC40	4.747	15.18133	10.51089	18.39126
EC50	5.000	16.40952	12.09166	19.93107
EC60	5.253	17.73708	13.74571	21.85824
EC75	5.674	20.18578	16.45921	26.33698
EC80	5.842	21.24878	17.48011	28.68182
EC85	6.036	22.5587	18.62596	31.89126
EC90	6.282	24.32219	20.01655	36.73298
EC95	6.645	27.19271	22.02804	45.79291
EC99	7.326	33.52248	25.86579	70.57559

Significant heterogeneity detected (p = 1.68E-06)



Mulloway Larval Growth

Start Date:	27/10/2007	Test ID:	ECX07-1805	Sample ID:	RO Brine
End Date:	3/11/2007	Lab ID:	Freo	Sample Type:	SA Desalination Plant
Sample Date:	27/10/2007	Protocol:	GEOTECH WIENV -64	Test Species:	Mulloway
Comments:					

Conc-%	1	2	3
Control	0.8790	0.9270	1.0000
1.6	0.8100	0.6740	0.8460
3.2	0.7740	0.6140	0.6500
6.3	0.7670	0.6470	0.7390
12.7	0.7240	0.6860	0.4920
25.3	0.0100	0.0200	0.0300
50.6	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root						1-Tailed		Number
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp
Control	0.9353	1.0000	1.3445	1.2155	1.5208	11.754	3				19
*1.6	0.7767	0.8304	1.0835	0.9631	1.1675	9.869	3	3.011	2.500	0.2167	67
*3.2	0.6793	0.7263	0.9712	0.9004	1.0754	9.489	3	4.306	2.500	0.2167	97
*6.3	0.7177	0.7673	1.0121	0.9346	1.0671	6.821	3	3.835	2.500	0.2167	84
*12.7	0.6347	0.6785	0.9244	0.7774	1.0177	13.937	3	4.846	2.500	0.2167	110
*25.3	0.0200	0.0214	0.1367	0.1002	0.1741	26.717	3	13.909	2.500	0.2167	294
50.6	0.0000	0.0000	0.0500	0.0500	0.0500	0.000	3				300

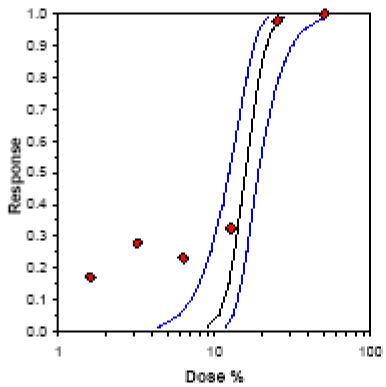
Auxiliary Tests					Statistic		Critical		Skew
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)					0.974794		0.858		0.032906
Bartlett's Test indicates equal variances (p = 0.63)					3.436978		15.08632		
Hypothesis Test (1-tail, 0.05)	**NOEC**	**LOEC**	**ChV**	**TU**	**MSDu**	**MSDp**	**MSB**	**MSE**	**F-Prob**
Dunnett's Test	<1.6	1.6			0.133416	0.140488	0.49686	0.011273	2.6E-07

Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma
Slope	9.491399	1.799764	4.494442	14.48836	0.063333	23.38187	9.487728	1.1E-04	1.19798	0.105359
Intercept	-6.3705	2.203633	-12.4868	-0.25222						
TSCR	0.222456	0.029041	0.141825	0.303087						

Point	Probits	%	95% Fiducial Limits	
EC01	2.674	8.971792	4.28476	11.6552
EC05	3.355	10.58475	5.967017	13.17964
EC10	3.718	11.55999	7.127479	14.12822
EC15	3.964	12.26825	7.99714	14.84379
EC20	4.158	12.86198	8.745895	15.46916
EC25	4.326	13.39419	9.426792	16.05568
EC40	4.747	14.835	11.27121	17.81529
EC50	5.000	15.77538	12.43232	19.14548
EC60	5.253	16.77537	13.59223	20.75789
EC75	5.674	18.57989	15.44566	24.23472
EC80	5.842	19.3487	16.14659	25.93495
EC85	6.036	20.28509	16.94149	28.17125
EC90	6.282	21.52793	17.91639	31.40245
EC95	6.645	23.51144	19.33473	37.13513
EC99	7.326	27.73834	22.01558	51.53008

Significant heterogeneity detected (p = 1.06E-04)



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Larval Fish Growth

Start Date:	31/07/2007	Test ID:	ECX07-1805	Sample ID:	Brine
End Date:	7/08/2007	Lab ID:	Freo	Sample Type:	SA Desalination Plant
Sample Date:	18/05/2007	Protocol:	Geotech WIENV-62	Test Species:	Pink Snapper
Comments:					

Conc-%	1	2	3
Control	1.0000	0.9600	1.0000
0.79	0.9700	0.8900	0.9400
1.6	0.9700	0.9100	1.0000
3.2	0.9300	1.0000	1.0000
6.3	1.0000	0.9900	1.0000
12.7	0.8500	1.0000	0.9500
25.3	0.7500	0.7400	0.7300
50.6	0.0200	0.0100	0.0100
101.3	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root						1-Tailed		Number
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp
Control	0.9867	1.0000	1.4703	1.3694	1.5208	5.943	3				4
0.79	0.9333	0.9459	1.3176	1.2327	1.3967	6.234	3	1.892	2.560	0.2066	20
1.6	0.9600	0.9730	1.3945	1.2661	1.5208	9.132	3	0.939	2.560	0.2066	12
3.2	0.9767	0.9899	1.4462	1.3030	1.5208	8.681	3	0.274	2.560	0.2066	7
6.3	0.9967	1.0101	1.5041	1.4706	1.5208	1.925	3	-0.418	2.560	0.2066	1
12.7	0.9333	0.9459	1.3464	1.1731	1.5208	12.912	3	1.536	2.560	0.2066	20
*25.3	0.7400	0.7500	1.0358	1.0244	1.0472	1.101	3	5.384	2.560	0.2066	78
*50.6	0.0133	0.0135	0.1141	0.1002	0.1419	21.120	3	16.804	2.560	0.2066	296
101.3	0.0000	0.0000	0.0500	0.0500	0.0500	0.000	3				300

Auxiliary Tests	Statistic	Critical	Skew
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.95956	0.884	-0.27065
Bartlett's Test indicates equal variances (p = 0.06)	13.64201	18.47532	

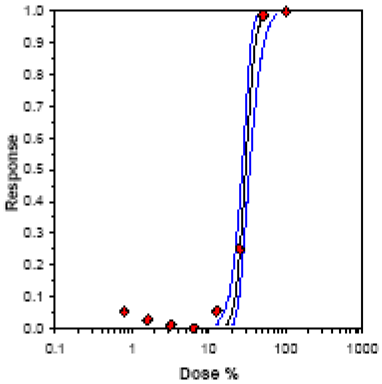
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob
Dunnett's Test	12.7	25.3	17.92512	7.874016	0.081317	0.082143	0.645561	0.009772	1.3E-10

Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma
Slope	9.715673	1.506136	6.030288	13.40106	0.013333	27.45901	12.59158	1.2E-04	1.478022	0.102926
Intercept	-9.35998	2.194345	-14.7293	-3.9906						
TSCR	0.035501	0.009337	0.012653	0.058349						

Point	Probits	%	95% Fiducial Limits	
EC01	2.674	17.32124	12.49667	20.33232
EC05	3.355	20.35742	16.05208	23.08425
EC10	3.718	22.1879	18.27897	24.78857
EC15	3.964	23.51502	19.90343	26.07436
EC20	4.158	24.62616	21.25175	27.20132
EC25	4.326	25.62116	22.4367	28.26263
EC40	4.747	28.31037	25.44873	31.46077
EC50	5.000	30.06227	27.21724	33.84612
EC60	5.253	31.92257	28.9322	36.63439
EC75	5.674	35.27318	31.70557	42.20839
EC80	5.842	36.69837	32.8001	44.75547
EC85	6.036	38.43246	34.08425	47.97447
EC90	6.282	40.73121	35.72452	52.42474
EC95	6.645	44.39363	38.23032	59.90333
EC99	7.326	52.17525	43.2599	77.20388

Significant heterogeneity detected (p = 1.19E-04)



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Embryo Development

Start Date:	7/06/2007	Test ID:	ECX07-1805	Sample ID:	RO Brine
End Date:	29/10/2007	Lab ID:	Freo	Sample Type:	SA Desalination Plant
Sample Date:	7/06/2007	Protocol:	GEOTECH WI	Test Species:	Giant Cuttlefish
Comments:					

Conc-%	1	2	3	4	5
Control	0.6364	0.6364	0.7273	0.3636	0.5455
0.4	0.6364	0.6364	0.6364	0.7273	0.7273
0.79	0.5455	0.5455	0.5455	0.6364	0.7273
1.6	0.8182	0.5455	0.5455	0.5455	0.5455
3.2	0.6364	0.5455	0.6364	0.5455	0.9091
6.3	0.5455	0.5455	0.6364	0.5455	0.5455
12.7	0.0909	0.0000	0.0909	0.0909	0.0909
25.3	0.0000	0.0000	0.0000	0.0000	0.0000
50.6	0.0000	0.0000	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root						1-Tailed		Number
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp
Control	0.5818	1.0000	0.8693	0.6473	1.0213	16.243	5				23
0.4	0.6727	1.1563	0.9626	0.9235	1.0213	5.566	5	-1.327	2.409	0.1694	18
0.79	0.6000	1.0313	0.8675	0.8309	1.0213	9.563	5	-0.259	2.409	0.1694	22
1.6	0.6000	1.0313	0.8908	0.8309	1.1303	15.030	5	-0.305	2.409	0.1694	22
3.2	0.6545	1.1250	0.9547	0.8309	1.2645	18.780	5	-1.214	2.409	0.1694	19
6.3	0.5636	0.9688	0.8494	0.8309	0.9235	4.875	5	0.283	2.409	0.1694	24
*12.7	0.0727	0.1250	0.2753	0.1513	0.3063	25.171	5	8.444	2.409	0.1694	51
25.3	0.0000	0.0000	0.1513	0.1513	0.1513	0.000	5				55
50.6	0.0000	0.0000	0.1513	0.1513	0.1513	0.000	5				55

Auxiliary Tests	Statistic	Critical	Skew
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.918927	0.91	0.980486
Bartlett's Test indicates equal variances (p = 0.08)	11.3993	16.81187	

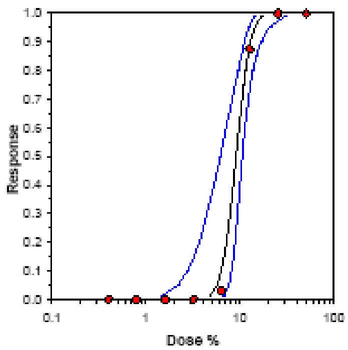
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob
Dunnett's Test	6.3	12.7	8.944831	15.87302	0.168622	0.288974	0.289749	0.012371	1.0E-09

Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits	
Slope	8.280412	2.4054	3.565829	12.995
Intercept	-2.94697	2.553904	-7.95262	2.058681
TSCR	0.378294	0.029225	0.321013	0.435576

Control	Chi-Sq	Critical	P-value	Mu	Sigma
0.418182	1.086491	12.59158	0.98	0.959731	0.120767

Point	Probits	%	95% Fiducial Limits	
EC01	2.674	4.772935	1.446115	6.725312
EC05	3.355	5.768827	2.233245	7.630267
EC10	3.718	6.382082	2.811743	8.172255
EC15	3.964	6.832263	3.281789	8.566634
EC20	4.158	7.212593	3.708314	8.899566
EC25	4.326	7.555712	4.115567	9.201255
EC40	4.747	8.494454	5.330428	10.0468
EC50	5.000	9.11447	6.200497	10.63917
EC60	5.253	9.779742	7.170633	11.33238
EC75	5.674	10.9948	8.91828	12.88578
EC80	5.842	11.51785	9.604606	13.72915
EC85	6.036	12.15901	10.36028	14.94089
EC90	6.282	13.01669	11.22702	16.86889
EC95	6.645	14.40043	12.37636	20.63477
EC99	7.326	17.40513	14.37529	31.12698



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Days to Hatch

Start Date:	7/06/2007	Test ID:	ECX07-1805	Sample ID:	RO Brine
End Date:	29/10/2007	Lab ID:	Freo	Sample Type:	SA Desalination Plant
Sample Date:	7/06/2007	Protocol:	GEOTECH WI	Test Species:	Giant Cuttlefish
Comments:					

Conc-%	1	2	3	4	5
Control	1.0000	0.7730	0.5350	1.0000	1.0000
0.79	0.7140	1.0000	1.0000	0.5760	1.0000
1.6	0.8440	1.0000	0.4520	0.3540	0.8280
3.2	1.0000	0.5010	0.4010	0.8940	0.7260
6.3	0.5910	0.5590	0.9800	1.0000	0.4200
12.7	0.0100	0.0200	0.0300	0.0200	0.0100
25.3	0.0000	0.0000	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root						1-Tailed		Number
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp
Control	0.8616	1.0000	1.2914	0.8204	1.5208	25.296	5				70
0.79	0.8580	0.9958	1.2861	0.8617	1.5208	25.300	5	0.026	2.360	0.4699	71
1.6	0.6956	0.8073	1.0407	0.6372	1.5208	34.352	5	1.259	2.360	0.4699	153
3.2	0.7044	0.8175	1.0504	0.6857	1.5208	32.302	5	1.210	2.360	0.4699	148
6.3	0.7100	0.8240	1.0752	0.7051	1.5208	34.586	5	1.085	2.360	0.4699	145
*12.7	0.0180	0.0209	0.1316	0.1002	0.1741	24.000	5	5.824	2.360	0.4699	491
25.3	0.0000	0.0000	0.0500	0.0500	0.0500	0.000	5				500

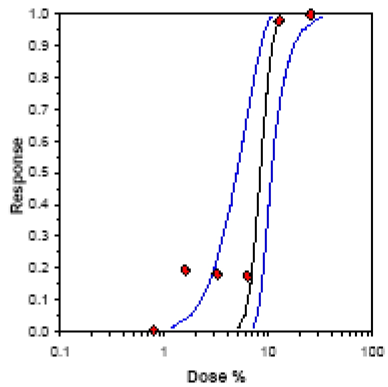
Auxiliary Tests					Statistic		Critical		Skew
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)					0.940128		0.9		-0.01911
Bartlett's Test indicates equal variances (p = 0.02)					13.57006		15.08632		
Hypothesis Test (1-tail, 0.05)	**NOEC**	**LOEC**	**ChV**	**TU**	**MSDu**	**MSDp**	**MSB**	**MSE**	**F-Prob**
Dunnett's Test	6.3	12.7	8.944831	15.87302	0.38793	0.419864	0.928078	0.099132	4.7E-05

Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma
Slope	10.97958	2.726511	3.40956	18.54961	0.14	55.44713	9.487728	2.6E-11	0.922248	0.091078
Intercept	-5.12589	2.688528	-12.5905	2.338673						
TSCR	0.220997	0.034544	0.125088	0.316906						

Point	Probits	%	95% Fiducial Limits	
EC01	2.674	5.132962	1.174615	7.083776
EC05	3.355	5.921576	1.830941	7.836145
EC10	3.718	6.390373	2.311048	8.300557
EC15	3.964	6.72746	2.698082	8.649018
EC20	4.158	7.008002	3.04603	8.952142
EC25	4.326	7.257988	3.37475	9.235236
EC40	4.747	7.928173	4.329926	10.07931
EC50	5.000	8.360794	4.9861	10.71798
EC60	5.253	8.817022	5.687638	11.50548
EC75	5.674	9.631164	6.885461	13.30839
EC80	5.842	9.974722	7.34664	14.25602
EC85	6.036	10.39068	7.86087	15.56882
EC90	6.282	10.93878	8.466002	17.58545
EC95	6.645	11.80477	9.281637	21.44602
EC99	7.326	13.61843	10.63807	32.26449

Significant heterogeneity detected (p = 2.62E-11)



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Length at Hatch

Start Date:	7/06/2007	Test ID:	Ecx07-1805	Sample ID:	RO Brine
End Date:	29/10/2007	Lab ID:	Freo	Sample Type:	SA Desalination Plant
Sample Date:	6/06/2007	Protocol:	GEOTECH WI	Test Species:	Giant Cuttlefish
Comments:					

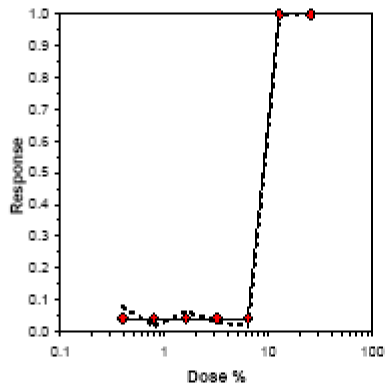
Conc-%	1	2	3	4	5
Control	0.9850	1.0000	1.0000	0.9800	1.0000
0.4	0.9150	0.9150	0.9280	0.8490	0.9540
0.79	0.9150	1.0000	1.0000	1.0000	0.9670
1.6	0.9280	0.9410	0.9020	0.9150	0.9670
3.2	0.9800	0.9670	0.9150	1.0000	0.9540
6.3	0.9930	0.9930	0.9800	0.9280	0.9930
12.7	0.0000	0.0000	0.0000	0.0000	0.0000
25.3	0.0000	0.0000	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root						1-Tailed		Number
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp
Control	0.9930	1.0000	1.4678	1.4289	1.5208	3.064	5				4
*0.4	0.9122	0.9186	1.2751	1.1717	1.3546	5.202	5	4.315	2.360	0.1164	43
0.79	0.9764	0.9833	1.4451	1.2750	1.5208	7.686	5	0.867	2.360	0.1164	11
*1.6	0.9306	0.9372	1.3060	1.2524	1.3881	4.007	5	3.647	2.360	0.1164	35
3.2	0.9632	0.9700	1.3935	1.2750	1.5208	6.523	5	1.914	2.360	0.1164	19
6.3	0.9774	0.9843	1.4378	1.2991	1.4870	5.669	5	1.014	2.360	0.1164	12
12.7	0.0000	0.0000	0.0500	0.0500	0.0500	0.000	5				500
25.3	0.0000	0.0000	0.0500	0.0500	0.0500	0.000	5				500

Auxiliary Tests					Statistic		Critical		Skew
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)					0.956087		0.9		-0.63911
Bartlett's Test indicates equal variances (p = 0.55)					3.962947		15.08632		
Hypothesis Test (1-tail, 0.05)	**NOEC**	**LOEC**	**ChV**	**TU**	**MSDu**	**MSDp**	**MSB**	**MSE**	**F-Prob**
Dunnett's Test	6.3	12.7	8.944631	15.87302	0.032342	0.032565	0.034828	0.006079	0.001289

Trimmed Spearman-Karber

Trim Level	EC50	95% CL	
0.0%			
5.0%	8.8141	8.7552	8.8733
10.0%	8.8141	8.7552	8.8733
20.0%	8.8141	8.7552	8.8733
Auto-4.0%	8.8141	8.7552	8.8733



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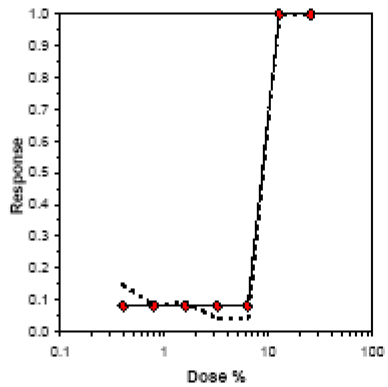
			Weight at Hatch				
Start Date:	7/06/2007	Test ID:	Ecx07-1805		Sample ID:	RO Brine	
End Date:	29/10/2007	Lab ID:	Freo		Sample Type:	SA Desalination Plant	
Sample Date:	6/06/2007	Protocol:	GEOTECH WI		Test Species:	Giant Cuttlefish	
Comments:							

Conc-%	1	2	3	4	5
Control	0.9840	1.0000	0.9690	0.9640	0.9990
0.4	0.8160	0.9030	0.8670	0.7610	0.8370
0.79	0.7400	1.0000	0.9430	1.0000	0.8270
1.6	0.9230	0.8960	0.7460	0.9030	0.9890
3.2	1.0000	0.8570	0.9690	0.9990	0.8820
6.3	1.0000	1.0000	1.0000	0.7210	1.0000
12.7	0.0000	0.0000	0.0000	0.0000	0.0000
25.3	0.0000	0.0000	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root						1-Tailed		Number
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp
Control	0.9832	1.0000	1.4555	1.3799	1.5392	4.968	5				9
*0.4	0.8368	0.8511	1.1588	1.0600	1.2541	6.302	5	2.872	2.360	0.2438	81
0.79	0.9020	0.9174	1.3098	1.0357	1.5208	16.760	5	1.411	2.360	0.2438	49
1.6	0.8918	0.9070	1.2595	1.0426	1.4657	11.955	5	1.897	2.360	0.2438	54
3.2	0.9414	0.9575	1.3714	1.1830	1.5392	12.056	5	0.815	2.360	0.2438	29
6.3	0.9442	0.9603	1.4195	1.0143	1.5208	15.956	5	0.349	2.360	0.2438	28
12.7	0.0000	0.0000	0.0500	0.0500	0.0500	0.000	5				500
25.3	0.0000	0.0000	0.0500	0.0500	0.0500	0.000	5				500

Auxiliary Tests					Statistic		Critical		Skew
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)					0.945973		0.9		-0.81379
Bartlett's Test indicates equal variances (p = 0.17)					7.773679		15.08632		
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob
Dunnett's Test	6.3	12.7	8.944831	15.87302	0.110248	0.111726	0.060137	0.026677	0.081523

	Trimmed Spearman-Karber		
Trim Level	EC50	95% CL	
0.0%			
5.0%			
10.0%	8.6769	8.5902	8.7644
20.0%	8.6769	8.5902	8.7644
Auto-8.0%	8.6769	8.5902	8.7644



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Width at Hatch

Start Date:	7/06/2007	Test ID:	Ecx07-1805	Sample ID:	RO Brine
End Date:	29/10/2007	Lab ID:	Freo	Sample Type:	SA Desalination Plant
Sample Date:	6/06/2007	Protocol:	GEOTECH WI	Test Species:	Giant Cuttlefish
Comments:					

Conc-%	1	2	3	4	5
Control	0.9120	1.0000	0.8940	0.9830	1.0000
0.4	0.8270	0.8340	0.9790	0.8270	1.0000
0.79	0.9280	1.0000	1.0000	1.0000	0.9280
1.6	0.9450	1.0000	0.8760	0.9790	1.0000
3.2	1.0000	1.0000	0.9460	1.0000	1.0000
6.3	1.0000	1.0000	1.0000	0.9120	1.0000
12.7	0.0000	0.0000	0.0000	0.0000	0.0000
25.3	0.0000	0.0000	0.0000	0.0000	0.0000

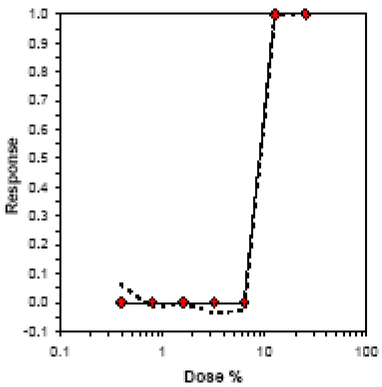
			Transform: Arcsin Square Root						1-Tailed		Number
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp
Control	0.9578	1.0000	1.3961	1.2392	1.5208	9.704	5				22
0.4	0.8934	0.9328	1.2762	1.1418	1.5208	14.332	5	1.469	2.360	0.1958	53
0.79	0.9712	1.0140	1.4321	1.2991	1.5208	8.477	5	-0.410	2.360	0.1958	14
1.6	0.9604	1.0027	1.4030	1.2140	1.5208	9.342	5	-0.059	2.360	0.1958	20
3.2	0.9892	1.0328	1.4839	1.3363	1.5208	5.561	5	-1.034	2.360	0.1958	5
6.3	0.9824	1.0257	1.4705	1.2696	1.5208	7.638	5	-0.874	2.360	0.1958	9
12.7	0.0000	0.0000	0.0500	0.0500	0.0500	0.000	5				500
25.3	0.0000	0.0000	0.0500	0.0500	0.0500	0.000	5				500

Auxiliary Tests	Statistic	Critical	Skew
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.906819	0.9	-0.17952
Bartlett's Test indicates equal variances (p = 0.79)	2.404161	15.08632	

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob
Dunnett's Test	6.3	12.7	8.944831	15.87302	0.100201	0.103251	0.027706	0.0172	0.195317

Graphical Method

Trim Level	EC50
0.0%	8.9448
	8.9448



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			Cuttlefish Post Hatch Length		
Start Date:	7/06/2007	Test ID:	ECX07-1805	Sample ID:	RO Brine 78 ppt
End Date:	29/10/2007	Lab ID:	Freo	Sample Type:	SA Desalination Plant
Sample Date:	6/06/2007	Protocol:	GEOTECH WI	Test Species:	Sepia apama
Comments:					

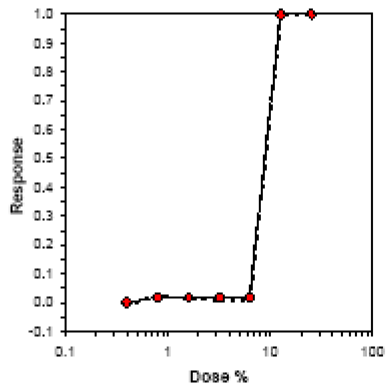
Conc-%	1	2	3	4	5
Control	1.0000	1.0000	1.0000	0.9500	0.9700
0.4	1.0000	0.9760	0.9760	1.0000	1.0000
0.79	0.9110	0.9630	1.0000	0.9370	1.0000
1.6	0.9690	1.0000	0.9630	0.9760	0.9240
3.2	0.9760	1.0000	1.0000	1.0000	0.9040
6.3	0.8850	1.0000	1.0000	1.0000	1.0000
12.7	0.0000	0.0000	0.0000	0.0000	0.0000
25.3	0.0000	0.0000	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root					Rank	1-Tailed	Number
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp
Control	0.9840	1.0000	1.4609	1.3453	1.5208	5.752	5			8
0.4	0.9904	1.0065	1.4786	1.4153	1.5208	3.909	5	29.50	16.00	4
0.79	0.9622	0.9778	1.4007	1.2679	1.5208	8.297	5	23.00	16.00	19
1.6	0.9704	0.9862	1.4141	1.2915	1.5208	6.169	5	23.50	16.00	15
3.2	0.9760	0.9919	1.4467	1.2558	1.5208	8.024	5	27.50	16.00	12
6.3	0.9770	0.9929	1.4616	1.2248	1.5208	9.056	5	29.00	16.00	12
12.7	0.0000	0.0000	0.0500	0.0500	0.0500	0.000	5			500
25.3	0.0000	0.0000	0.0500	0.0500	0.0500	0.000	5			500

Auxiliary Tests					Statistic	Critical	Skew
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)					0.895297	0.9	-0.90829
Bartlett's Test indicates equal variances (p = 0.71)					2.931538	15.08632	
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU			
Steel's Many-One Rank Test	6.3	12.7	8.944831	15.87302			

Trimmed Spearman-Karber

Trim Level	EC50	95% CL	
0.0%	8.5289	8.3928	8.6673
5.0%	8.8901	8.8527	8.9277
10.0%	8.8901	8.8527	8.9277
20.0%	8.8901	8.8527	8.9277
Auto-0.0%	8.5289	8.3928	8.6673



Cuttlefish Post Hatch Weight

Start Date:	7/06/2007	Test ID:	ECX07-1805	Sample ID:	RO Brine 78 ppt
End Date:	29/10/2007	Lab ID:	Freo	Sample Type:	SA Desalination Plant
Sample Date:	6/06/2007	Protocol:	GEOTECH WI	Test Species:	Sepia apama
Comments:					

Conc-%	1	2	3	4	5
Control	0.8670	1.0000	1.0000	0.9670	1.0000
0.4	0.9810	0.9540	1.0000	0.9890	1.0000
0.79	1.0000	0.8600	0.9670	0.9670	0.9030
1.6	0.9230	0.9320	1.0000	0.9140	0.9320
3.2	0.9410	1.0000	1.0000	1.0000	0.8320
6.3	0.8600	0.9680	1.0000	1.0000	1.0000
12.7	0.0000	0.0000	0.0000	0.0000	0.0000
25.3	0.0000	0.0000	0.0000	0.0000	0.0000

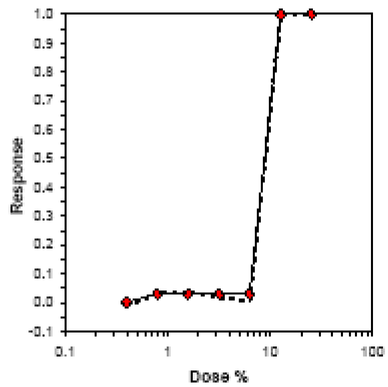
			Transform: Arcsin Square Root						1-Tailed		Number
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp
Control	0.9708	1.0000	1.4357	1.2280	1.5208	9.023	5				14
0.4	0.9848	1.0144	1.4589	1.3546	1.5208	4.758	5	-0.287	2.360	0.1911	8
0.79	0.9394	0.9677	1.3477	1.1873	1.5208	9.655	5	1.087	2.360	0.1911	30
1.6	0.9402	0.9685	1.3395	1.2732	1.5208	7.638	5	1.188	2.360	0.1911	31
3.2	0.9546	0.9833	1.4072	1.1485	1.5208	11.908	5	0.351	2.360	0.1911	23
6.3	0.9656	0.9946	1.4261	1.1873	1.5208	10.215	5	0.093	2.360	0.1911	17
12.7	0.0000	0.0000	0.0500	0.0500	0.0500	0.000	5				500
25.3	0.0000	0.0000	0.0500	0.0500	0.0500	0.000	5				500

Auxiliary Tests	Statistic	Critical	Skew
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.937458	0.9	-0.63523
Bartlett's Test indicates equal variances (p = 0.70)	2.963577	15.08632	

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob
Dunnett's Test	6.3	12.7	8.944831	15.87302	0.084552	0.086114	0.011932	0.016393	0.609369

Trimmed Spearman-Karber

Trim Level	EC50	95% CL	
0.0%	8.2519	8.0819	8.4254
5.0%	8.8512	8.8018	8.9009
10.0%	8.8512	8.8018	8.9009
20.0%	8.8512	8.8018	8.9009
Auto-0.0%	8.2519	8.0819	8.4254



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Cuttlefish Post Hatch Width

Start Date: 7/06/2007	Test ID: ECX07-1805	Sample ID:	RO Brine 78 ppt
End Date: 29/10/2007	Lab ID: Freo	Sample Type:	SA Desalination Plant
Sample Date: 6/06/2007	Protocol: GEOTECH WI	Test Species:	Sepia apama
Comments:			

Conc-%	1	2	3	4	5
Control	0.8670	1.0000	1.0000	0.9670	1.0000
0.4	0.9810	0.9540	1.0000	0.9890	1.0000
0.79	1.0000	0.8600	0.9670	0.9670	0.9030
1.6	0.9230	0.9320	1.0000	0.9140	0.9320
3.2	0.9410	1.0000	1.0000	1.0000	0.8320
6.3	0.8600	0.9680	1.0000	1.0000	1.0000
12.7	0.0000	0.0000	0.0000	0.0000	0.0000
25.3	0.0000	0.0000	0.0000	0.0000	0.0000

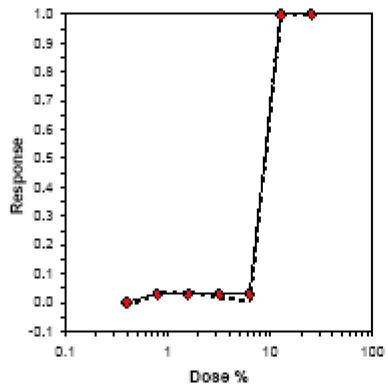
			Transform: Arcsin Square Root						1-Tailed		Number
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp
Control	0.9708	1.0000	1.4357	1.2280	1.5208	9.023	5				14
0.4	0.9848	1.0144	1.4589	1.3546	1.5208	4.758	5	-0.287	2.360	0.1911	8
0.79	0.9394	0.9677	1.3477	1.1873	1.5208	9.655	5	1.087	2.360	0.1911	30
1.6	0.9402	0.9685	1.3395	1.2732	1.5208	7.638	5	1.188	2.360	0.1911	31
3.2	0.9546	0.9833	1.4072	1.1485	1.5208	11.908	5	0.351	2.360	0.1911	23
6.3	0.9656	0.9946	1.4281	1.1873	1.5208	10.215	5	0.093	2.360	0.1911	17
12.7	0.0000	0.0000	0.0500	0.0500	0.0500	0.000	5				500
25.3	0.0000	0.0000	0.0500	0.0500	0.0500	0.000	5				500

Auxiliary Tests	Statistic	Critical	Skew
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.937458	0.9	-0.63523
Bartlett's Test indicates equal variances (p = 0.70)	2.963577	15.08632	

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob
Dunnett's Test	6.3	12.7	8.944831	15.87302	0.084552	0.086114	0.011932	0.016393	0.609369

Trimmed Spearman-Karber

Trim Level	EC50	95% CL	
0.0%	8.2519	8.0819	8.4254
5.0%	8.8512	8.8018	8.9009
10.0%	8.8512	8.8018	8.9009
20.0%	8.8512	8.8018	8.9009
Auto-0.0%	8.2519	8.0819	8.4254



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Cuttlefish Post Hatch Survival							
Start Date:	7/06/2007	Test ID:	ECX07-1805		Sample ID:	RO Brine	
End Date:	29/10/2007	Lab ID:	Freo		Sample Type:	SA Desalination Plant	
Sample Date:	7/06/2007	Protocol:	Geotech WI		Test Species:	Sepia apama	
Comments:							

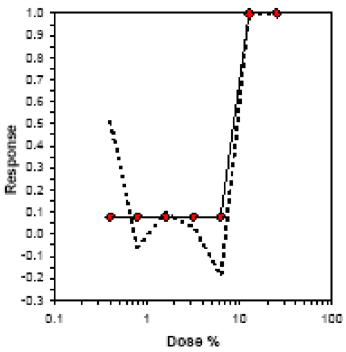
Conc-%	1	2	3	4	5
Control	0.7500	0.7500	1.0000	0.6667	0.3333
0.4	0.2500	0.2500	0.2500	0.4000	0.6000
0.79	1.0000	0.6667	0.6667	0.7500	0.8000
1.6	0.5000	1.0000	0.6667	0.3333	1.0000
3.2	0.5000	1.0000	0.7500	0.3333	0.8571
6.3	1.0000	1.0000	0.7500	1.0000	0.6667
12.7	0.0000	0.0000	0.0000	0.0000	0.0000
25.3	0.0000	0.0000	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root						1-Tailed		Number
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp
Control	0.7000	1.0000	1.0021	0.6155	1.3453	26.094	5				5
*0.4	0.3500	0.5000	0.6283	0.5236	0.8861	25.480	5	2.645	2.360	0.3334	14
0.79	0.7767	1.1095	1.0686	0.9553	1.2780	12.506	5	-0.471	2.360	0.3334	4
1.6	0.7000	1.0000	0.9824	0.6155	1.2780	30.061	5	0.139	2.360	0.3334	6
3.2	0.6881	0.9830	0.9818	0.6155	1.2780	28.138	5	0.143	2.360	0.3334	6
6.3	0.8833	1.2619	1.1673	0.9553	1.2780	13.278	5	-1.169	2.360	0.3334	2
12.7	0.0000	0.0000	0.2527	0.2527	0.2527	0.000	5				20
25.3	0.0000	0.0000	0.2527	0.2527	0.2527	0.000	5				20

Auxiliary Tests					Statistic		Critical		Skew
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)					0.957893		0.9		-0.14128
Bartlett's Test indicates equal variances (p = 0.53)					4.108651		15.08632		
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob
Dunnett's Test	6.3	12.7	8.944831	15.87302	0.325663	0.458695	0.166689	0.049906	0.019779

Trimmed Spearman-Karber

Trim Level	EC50	95% CL	
0.0%			
5.0%			
10.0%	8.6840	8.2174	9.1771
20.0%	8.6840	8.2174	9.1771
Auto-7.8%	8.6840	8.2174	9.1771

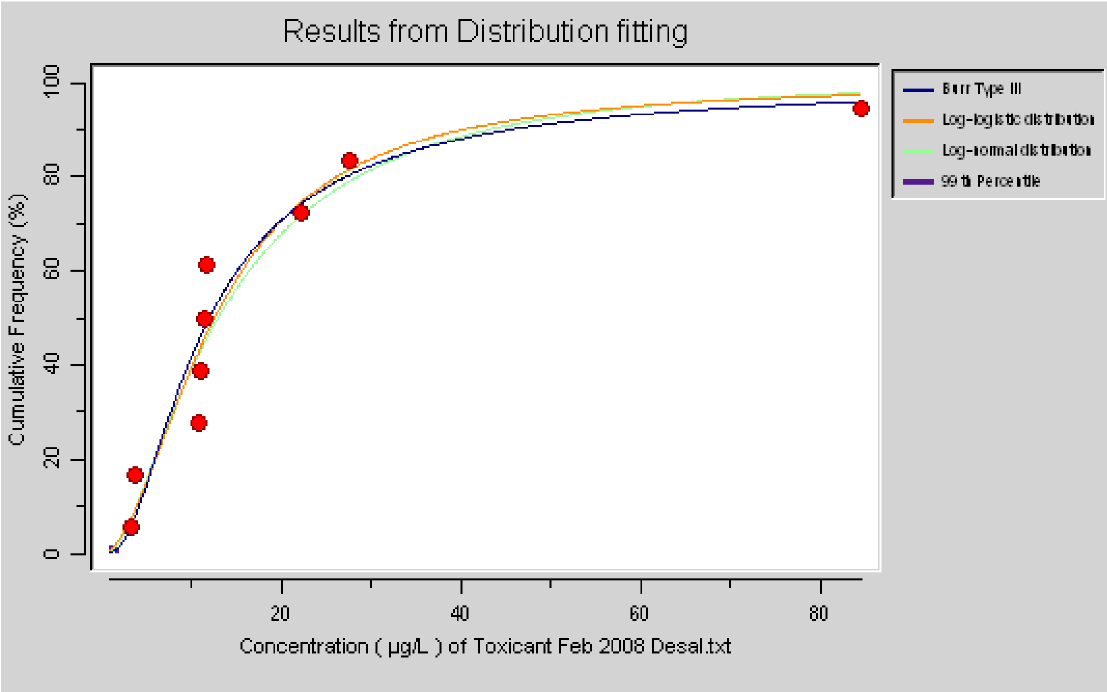


Page 1 ToxCalc v5.0.23 Reviewed by:_____

Appendix 3

BURRLIOZ Data

BurrliOZ Results
ECX07-1805 ARUP RO Brine
Species Protection Trigger Values



PC99 50% = 1.76 (501 Bootstrap Samples)
Burr Type III Distribution fitted to 9 observations

PC95 50% = 3.13 (501 Bootstrap Samples)
Burr Type III Distribution fitted to 9 observations

PC90 50% = 4.20 (501 Bootstrap Samples)
Burr Type III Distribution fitted to 9 observations

PC80 50% = 5.96 (501 Bootstrap Samples)
Burr Type III Distribution fitted to 9 observations

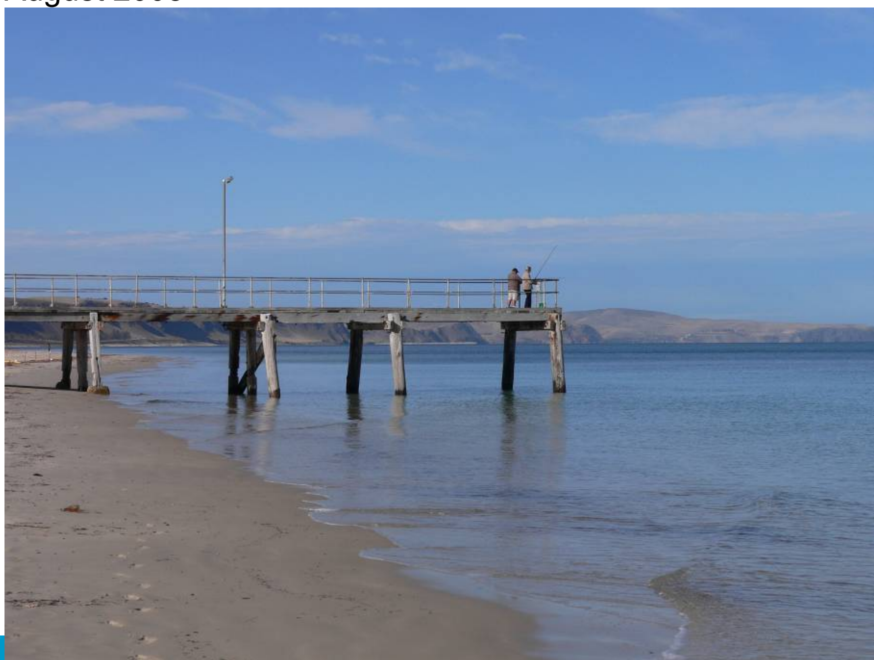
APPENDIX O10.5

Selection of species and other factors that affect dilution factors for saline brine discharge from the proposed desalination plant at Point Lowly, South Australia (report by Dr Michael Warne, CSIRO)

See overleaf for report.

Selection of species and other factors that affect dilution factors for saline brine discharge from the proposed desalination plant at Point Lowly, South Australia.

Michael Warne
CLW Report 09/08
August 2008



CONTENTS

EXECUTIVE SUMMARY

Fifteen organisms were tested and evaluated as part of the Environmental Impact Statement for the proposed desalination plant at Point Lowly for their appropriateness to calculate dilution factors for the saline brine. This report provides an assessment of all the WET results, and the species protection values presented here use the most appropriate dataset available and thus supercede all previous values.

Seven of the tested fifteen species comprise the best dataset; being the unicellular alga *Isochrysis galbana,* the macroalga *Ecklonia radiata,* the Western King Prawn *Melicertus latisulcatus,* the Pacific Oyster *Crassostrea gigas,* the Pink Snapper *Pagrus auratus,* the Mulloway *Argyrosomus japonicus* and the Giant Cuttlefish *Sepia apama.*

A second dataset which retained the previous species but added the macroalga *Hormosira banksii,* the copepod *Gladioferens imparipes* and the fish *Seriola lalandi* was also evaluated as this maximized the number of test species but contained toxicity data from a mixture of exposure durations from acute to chronic tests and included data derived using diluent water with different salinities.

The chosen list of species contains more species belonging to more taxonomic groups than the minimum required by the Australian and New Zealand water quality guidelines and used in the evaluation of the Western Australia desalination plant. Therefore there will be greater confidence in the dilution factors being derived for the proposed desalination plant at Point Lowly than for the WA plant.

Use of the best dataset (i.e. that comprising the first seven species listed above) resulted in a concentration that should protect 99% of species (PC99) of 2.35% saline brine and a dilution factor of 45 at 40 ppt diluent salinity. The corresponding values for the second best dataset (i.e. that comprising the additional three species) are 2.48% saline brine and a dilution factor of 41 respectively. The best dataset is recommended for use, even though it contained fewer species than the second best dataset, because all the toxicity data it contains are based upon sub-chronic or chronic exposure, all tests were conducted at one salinity (i.e. 40 ppt) and it results in a more conservative (larger) dilution factor.

A dilution factor of 45 would theoretically protect 99% of marine species typical of Upper Spencer Gulf from experiencing a sub-chronic toxic effect of greater than 10% in receiving

water with a salinity of 40 ppt. However, this salinity corresponds to the low end of the range of salinities reported at Point Lowly (i.e. 39 – 42 ppt) and it may therefore underestimate the dilution factor required when the receiving water has a salinity of 42 ppt. The one toxicity value available measured at a higher salinity (i.e. 45 ppt) was for the Giant Cuttlefish. To protect this species from experiencing a sub-chronic effect of greater than 10% a dilution factor of 55 is required. It was therefore decided to calculate the concentration of saline brine and the corresponding dilution factor that would protect 100% of species in receiving water with a salinity of 40 ppt.

A concentration of 1.23% saline brine and a dilution of 85 would theoretically protect 100% of marine species typical of Upper Spencer Gulf from experiencing a sub-chronic toxic effect of greater than 10% in seawater with a salinity of 40 ppt. In addition, this dilution factor would result in less than a 3% reduction in post-hatch survival of the Giant Cuttlefish in seawater with a salinity of 45 ppt. As 45 ppt is greater than that experienced at Point Lowly, the reduction in post-hatch survival of the Giant Cuttlefish in seawater with the maximum salinity experienced at Point Lowly (i.e. 42 ppt) would be less than 3%.

ARUP/ENSR informed the author of this report that the minimum dilution factor that will be achieved at the Giant Cuttlefish breeding site closest to the discharge point is 116. Such a dilution factor would protect 100% of species in seawater with a salinity of 40 ppt with a considerable margin of safety. In addition, it would cause less than a 1% reduction in post-hatch survival of the Giant Cuttlefish in seawater with a salinity of 45 ppt at the breeding site closest to the point of discharge and therefore an even lower effect in seawater with a salinity of 42 ppt.

Uncertainty remains over the exact dilution factor needed to protect marine species typical of the Upper Spencer Gulf from sub-chronic effects associated with the discharge of saline brine from the proposed desalination plant into receiving water with a salinity of 42 ppt. This has arisen because the WET tests were generally conducted at 40 ppt rather than at the maximum recorded values at Point Lowly (i.e. 42 ppt) and the test organisms were not acclimatised to test conditions for the typical duration. The magnitude of the effect of these two factors on the species protection values is not known, however the effects that they have on toxicity counteract each other. Electing to protect 100% of species in seawater with a salinity of 40 ppt attempts to overcome this uncertainty. The best way to overcome this would be to conduct additional sub-chronic or chronic WET tests using diluent water with a salinity of 42 ppt or at a salinity of 43 ppt if it is desired to be more conservative.

BACKGROUND

Dr Warne (CSIRO) was approached by ARUP/ENSR to review two years of studies undertaken as part of the Environmental Impact Statement for the proposed desalination plant at Point Lowly, South Australia and to provide his expert opinion on a number of issues related to the toxicity tests. Specifically, it was requested that the following issues be addressed:

1. which species should be used to derive dilution factors;
2. how do the species tested for this project compare with those undertaken for the Western Australia desalination plant;
3. what role if any could a lack of test species acclimation have on the toxicity results;
4. what effect if any could the use of diluent water with different salinities have on the toxicity results;
5. what effect does exposure duration have on toxicity data;
6. whether it is possible to combine EC10 and NOEC type toxicity data to derive dilution factors; and
7. to derive a set of dilution factors to protect 99% of species and provide information on how these were derived.

The following report addresses each of these issues. The reports that present the two years of data reviewed are provided in Appendices O10.2 to O10.4.

TYPES OF WET TESTING

There are two different approaches that can be used to conduct direct toxicity assessment (DTA) which is also called whole effluent toxicity testing (WET).

1. **to use generic species** that occur in that environmental media. For example, a WET test at Point Lowly would use species that occur within Australian marine waters. This is also called the Standard DTA approach (Van Dam and Chapman, 2001).
2. **to use endemic organisms** that actually occur in the ecosystem that is being assessed. For example, a WET test at Point Lowly would use species that are found in the marine waters around Point Lowly or closely related organisms. This is also called the Site-specific DTA approach (Van Dam and Chapman, 2001).

There are strengths and limitations to both approaches.

The key limitations of the generic species approach are that:

- the resulting toxicity data may not be relevant to the particular ecosystem being considered – as the species tested may not be present or closely related species may not be present; and
- usually the dilution water is not from the particular ecosystem and therefore site-specific characteristics of the water can not be taken into account.

The strength of this approach is that toxicity data for many generic species are often available and therefore there is greater confidence in the outcomes as more species can be tested.

The limitations of the endemic species approach are that:

- toxicity tests may be not already be developed for endemic species and developing tests takes considerable time and money;
- generally, toxicity data is generated for the minimum acceptable number of species for the desired purpose.

The effect of the above limitations is decreasing as the number of species that have been used in DTA increases (e.g. Van Dam and Chapman, 2001). An excellent review of the status of DTA within Australia and New Zealand is the work by Van Dam and Chapman (2001).

The strength of the endemic species approach is that the toxicity data are directly relevant to the particular ecosystem being studied.

I believe that it is generally accepted within ecotoxicology that the second approach (i.e. to use endemic organisms) is the preferred approach providing toxicity data are available for a similar number of species and taxonomic groups of organisms. Van Dam and Chapman (2001) state that:

> “For the purposes of Australian water managers, who generally oversee specific geographical regions and are concerned with local water quality, site-specific DTA is likely to be the most appropriate approach.”

This is the certainly the approach recommended for conducting DTA by the Australian and New Zealand guidelines for marine and fresh water quality (ANZECC and ARMCANZ, 2000).

Overall, the early toxicity testing undertaken to assess the toxicity of the saline brine for Point Lowly followed the generic species approach with the exception of the Giant Cuttlefish *Sepia apama*. The species used were (Geotechnical Services, 2006a), *S. apama*, *Penaeus monodon* – crustacean; *Seriola lalandi* – fish; *Nitzschia closterium* – diatom; *Hormosira* banksii – brown macroalga; *Heliocidaris tuberculata* – echinoid; and *Saccostrea commercialis* – bivalve (Hydrobiology, 2006). The use of the above generic organisms caused some problems mainly as they were acclimated to normal salinity marine water (i.e. 33 ppt), while the salinity of the Point Lowly region varies between 39 and 42. At the salinities encountered at Point Lowly, two of the tested species (i.e. the Sydney rock oyster and the sea urchin) died in salinities that occur naturally at Point Lowly – thus highlighting their unsuitability as test organisms. Also, neither of these species was endemic to the Point Lowly region. Given the above, I recommended that it would be desirable to conduct further toxicity tests, preferably using species found in Spencer Gulf, to increase the number of species for which there are toxicity data and to increase the relevance of the resulting dilution factors.

As a result of my previous recommendation subsequent toxicity testing has been undertaken to follow the endemic species approach (see Appendix O10.4). A list of all the species that have been used to determine the toxicity of saline brine and whether they are endemic to the Upper Spencer Gulf (where Point Lowly is located) is presented in Table 1. The information on the distribution of species was provided by ARUP/ENSR.

SELECTION OF SPECIES USED TO DERIVE THE DILUTION FACTOR

A series of criteria were used to determine the most appropriate species to be used in deriving dilution factors. These were:

- are the test species relevant to the region being examined?
- were the species tested in water with similar physicochemical conditions as at Point Lowly?
- did the tests meet appropriate quality assurance and quality control criteria?
- were the test species exposed to the toxicant for the same duration (e.g. chronic, sub-chronic or acute)?

Another consideration is that the method used to derive the dilution factors becomes more reliable and more representative as the number of species for which there is toxicity data increases. The Australian and New Zealand water quality guidelines (ANZECC and ARMCANZ, 2000) recommend using chronic tests for a minimum of five species representing four taxonomic groups in order to derive a high reliability trigger value.

Finally, it is important to adopt a pragmatic approach to WET testing (Chapman et al., 2001; van Dam and Chapman, 2001). For example, it will rarely be possible to generate regionally relevant toxicity data for more than five species due to time and cost considerations. However, the limited number of species is offset against the much greater environmental relevance of the toxicity data to the site being considered.

Table 1. Information on the test organisms used in the whole effluent toxicity testing of saline brine for the Point Lowly desalination plant.

Species	Present in USG	Notes	Phase*
Microalga - *Nitzschia closterium*	Yes	Widely distributed in Australian waters	1
Microalga - *Isochrysis galbana*	Genus yes, species unknown		2
Microalga - *Ecklonia radiata*	No	Widely distributed throughout SA waters	2
Macroalga - *Hormosira banksii*	Yes	Widely distributed throughout SA waters	1
Copepod - *Gladioferens imparipes*	Unknown		2
Tiger Prawn - *Penaeus monodon*	No		1
Western King Prawn - *Melicertus latisulcatus*	Yes		2
Blue Swimmer Crab - *Portunus pelagicus*	Yes		2
Pacific Oyster - *Crassostrea gigas*	Yes	In aquaculture	2
Sydney Rock Oyster - *Saccostrea commercialis*	No		1
Sea urchin - *Heliocidaris tuberculata*	No	Distributed on rocky reefs from Southern Queensland to central New South Wales	1
Yellowtail Kingfish - *Seriola lalandi*	Yes	Also an important aquaculture species	1 & 2
Snapper - *Pagrus auratus*	Yes		2
Mulloway - *Argyrosomus japonicus*	Yes		2
Australian Giant Cuttlefish - *Sepia apama*	Yes	Important breeding habitat at Point Lowly	1 & 2

*Phases 1 and 2 refer to testing conducted in 2006 and 2007 respectively.

Do the test species have regional relevance?

Based on the occurrence of the test organisms within the Upper Spencer Gulf (USG) toxicity data for the following nine species could be used: *Nitzschia closterium*; *Hormosira banksii*;

Melicertus latisulcatus; Portunus pelagicus; Crassostrea gigas; Seriola lalandi; Pagrus auratus; Argyrosomus japonicus; Sepia apama. Based on unidentified members of the same genus of algae being present in the Upper Spencer Gulf *Isochrysis galbana* could also be used. *Ecklonia radiata,* as far as it is known, does not occur in the Upper Spencer Gulf, but it is widely distributed throughout South Australian waters so it could also be used.

In terms of the regional relevance, the copepod *Gladioferens imparipes* could also be considered for use in determining dilution factors. While it is not clear that this particular specis is present in the Upper Spencer Gulf it still has regional relevance. The reasons for this are that:

1. it is an herbivorous calanoid copepod (Rippingale and Hodgkin, 1974) found in south-western Australian marine waters and copepods in general play important roles in coastal marine ecosystems (e.g. Willis, 1999) as they take in energy through the consumption of phytoplankton and algae transfer energy to higher trophic levels by being consumed by birds, fishes and mammals; and
2. copepods are planktonic crustaceans. Thus while they are motile they generally move with the surrounding water. All crustacea spend at least the early part of their life as plankton and move with the movement of the water – however for most macrocrustaceans (e.g. barnacles, crabs, lobsters) only the early lifestages (which are generally the more sensitive lifestages) are planktonic. Therefore, it is argued that copepods are appropriate indicators of the early life stages of crustaceans. There definitely are crustaceans present in Upper Spencer Gulf.

Therefore 12 species were suitable for use as endemic organisms.

Were the toxicity tests conducted in water similar to that of Point Lowly?

According to information provided by ARUP/ENSR the salinity of the water at Point Lowly ranges from 39 to 42 ppt. The salinity of the diluent used for the recommended test species from the previous section are presented in Table 2.

As salinity can act as a toxicant it is likely that the toxicity data for at least some of the recommended test species will underestimate and some overestimate the toxicity measured using 42 ppt diluent water.

Table 2. The salinity of the diluent water used in the toxicity tests for the species that have been recommended for use in deriving the dilution factors for the saline brine.

Recommended test species	Salinity of diluent water (ppt)
Isochrysis galbana	39.9
Ecklonia radiata	39.9
Hormosira banksii	37
Gladioferens imparipes	39.9
Melicertus latisulcatus	39.9
Crassostrea gigas	39.9
Seriola lalandi (phase I and II)	40 and 35
Pagrus auratus	39.9
Argyrosomus japonicus	39.9
Sepia apama (phase I and II)	45 and 40

The toxicity tests conducted by Geotechnical Services (Appendix O10.3) showed that the salinity of the diluent water affected the toxicity to *S. apama*. They recalculated the toxicity of the saline brine at 42 ppt and found that it was 2 - 3.2 fold higher (i.e. the EC50 values were 2-3.2 times smaller) at 45 ppt (i.e. the salinity of the diluent water) than at 42 ppt (i.e. the upper end of the range of salinities found at Point Lowly). However, there is evidence in the WET toxicity data from both the Perth and proposed Point Lowly desalination plants that the toxicity is not caused exclusively by the salinity of the brine. This being the case, it is not possible to correctly adjust the toxicity of the saline brine solely in terms of salinity. The *S. apama* toxicity tests conducted in phase II were conducted using diluent water with a salinity of 39.9 ppt (Geotechnical Services; see Appendix O10.4) which is essentially identical to the lower end of the range of salinities at Point Lowly (i.e. 40 ppt). Therefore the toxicity results for *S. apama* from phase II are the more appropriate for deriving dilution factors when assessed in terms of the water being similar to that at Point Lowly.

Hydrobiology (see Appendix O10.2) conducted WET testing but did not adjust their toxicity values to salinities other than 36 ppt (as Geotechnical Services did). However, for five of the six species salinity controls were conducted (the exception was Kingfish). The effect of increasing salinity was not consistent for all species. For some species (i.e. *Heliocidaris tuberculata, Nitzschia closterium, Peneaus monodon,* and *Saccostrea commercialis),* increased salinity increased toxicity, while for others (i.e. *Hormosira banksii, Seriola lalandi*), increased salinity had no statistically significant effect ($p \leq 0.05$) within the range of salinities reported as occurring in the Spencer Gulf (Geotechnical Services; see Appendix O10.3), but above this range toxicity increased with increased salinity. There is, therefore, the potential that the toxicity values for *H. tuberculata, N. closterium, P. monodon* and *S. commercialis* from phase I underestimate the toxicity of the saline brine at Point Lowly. Therefore, all four of these species should not be included in the derivation of the dilution factors as they underestimate the toxicity of the saline brine at 40 – 42 ppt.

For the Yellowtail kingfish *(S. lalandi)* toxicity results were generated in both phase I and II and resulted in very similar values. From phase I the no observed effect concentration (NOEC) was 12.5% saline brine. The phase II test yielded a concentration that causes a 10% effect (EC10) of 11.1% saline brine. However neither of these tests was ideal. The phase I test was conducted at 40 ppt but the exposure was acute (96 hour exposure of larvae) while for phase II the exposure was sub-chronic but it was conducted at 35 ppt (see Appendices O10.2 and O10.4). Neither is ideal, but *S. lalandi* data from both phase I and II could both be used to derive dilution factors.

What is the effect of a lack of test species acclimation on toxicity?

Countering the potential underestimation discussed in the previous section, is the fact that the test organisms used in the WET testing were either not acclimated to the test conditions or were not acclimatised for the usual duration (i.e. 2 to 7 days). Acclimation is routinely conducted when organisms are collected from the wild and subsequently used in toxicity tests or there are marked changes in experimental conditions. The test organisms that were included in the best dataset to calculate the dilution factors were all conducted in water with a salinity of 40 ppt. These organisms, with the exception of the cuttlefish and the adult prawns, therefore were all transferred from normal marine water with a salinity of approximately 35-36 ppt to water with a salinity of 40 ppt. Not acclimatising the test organisms would correspond to organisms moving instantaneously from regions where the background salinity occurs into the desalination plant discharge zone and then remaining there for the duration of the toxicity test. This exposure scenario is not likely to occur and the resulting toxicity data are most likely to overestimate the actual toxicity. As such not acclimatising the organisms is a conservative approach. It is not possible to estimate the extent of this overestimation of the toxicity given the data currently available.

Did the tests meet appropriate quality assurance and quality control criteria?

Portunus pelagicus (Blue Swimmer Crab) can not be used as the test failed due to excessive mortality in the controls.

The percentage hatch of *S. apama* in the phase II toxicity tests was not optimal (i.e. values for the control was 61.8% while values for the 0.4 to 6.3% saline brine treatments ranged from 56.3 to 67.2%) (Appendix O10.4) and was much less than that reported for the phase I toxicity tests (i.e. 100% hatch in the control and 75.5 to 89.1% for the 0.4 to 6.3% saline brine treatments). Generally, standardised toxicity tests have a set of validation criteria on which it is determined whether the test is of suitable quality or not and should therefore be accepted or rejected. A key validation criterion is always a stated level of toxic effect for the control - generally permitting a maximum effect of 10 – 20%. The permitted variation in the per cent effect reflects the innate variability of the test species. The toxicity test for *S. apama* is not standardised and therefore I am not aware that it has such a validation criteria, however, it is unlikely that any validation criterion would permit a 40% effect. We therefore have the situation where the *S. apama* results from phase I with their greater per cent hatch in the control are more reliable, but they were measured at 45 ppt and thus may overestimate the toxicity at 40 – 42 ppt. The results from phase II are less reliable but were measured in diluent water with a salinity of 40 ppt and thus within the range of measured salinities at Point Lowly. Thus neither dataset is ideal but both provide useful data.

Were the test species exposed to the toxicant for the same duration?

Acute and chronic toxicity data were not combined to derive the Australian and New Zealand water quality guidelines as they would have different statistical distributions (ANZECC and ARMCANZ, 2000; Warne, 2001). The toxicity tests for the species recommended so far to derive dilution factors are all classed as either chronic (i.e. *Nitzschia closterium*) or sub-chronic toxicity tests with the exception of the *G. imparipes* and phase I *S. lalandi* tests which are acute. Sub-chronic tests are not strictly chronic tests, which require a prolonged exposure of the test organisms to the toxicant. But generally, sub-chronic tests are markedly more sensitive (i.e. they can detect toxicity at considerably lower concentrations) than acute toxicity tests because they expose sensitive early life-stages of the test organisms. For the purposes of deriving water quality guidelines and dilution factors, sub-chronic data can be treated as chronic estimates of toxicity.

If the *G. imparipes* and/or *S. lalandi* acute toxicity data are to be used to derive dilution factors, then it would mean that both acute and chronic data were being combined. This is not appropriate as stated in the Australian and New Zealand water quality guidelines (ANZECC and ARMCANZ, 2000). It might be possible to use a default assessment factor to convert the acute values to chronic values but the magnitude of these is arbitrary and there is little scientific basis for this (Warne, 1998). It is the author's opinion that it would be preferable to only use sub-chronic or chronic toxicity data rather than use estimates of chronic toxicity.

RECOMMENDED SPECIES FOR THE CALCULATION OF DILUTION FACTORS AND THE RATIONALE

There are a number of limitations associated with some of the WET data which have been discussed in the preceding text. These revolve around the fact that some of the WET tests were conducted using diluent water with salinity outside the range found at Point Lowly and that some of the WET tests only use acute exposure. It is the author's strong opinion that the most internally consistent dataset which permits the largest number of species should be used to derive the dilution factors. By internally consistent it is meant that:

- toxicity data for only one type of exposure (i.e. chronic or acute) and
- data determined using diluent water with salinity within the range of Point Lowly (i.e. 40 – 42 ppt)

should be used to derive the dilution factors.

Based on this, the best dataset was that using chronic toxicity data measured in diluent water with a salinity of 40 ppt (Table 3). A discussion on the whether EC10 and NOEC data are equivalent and can be combined to derive dilution factors is provided in Appendix 1 of this report. The toxicity data for *S. apama* from phase II was included despite being of questionable quality, as it was conducted in diluent water with a salinity of 40 ppt.

The second best dataset was considered to be that which permitted the most species to be used to derive the dilution factors even if some acute, chronic, and values measured in different salinity diluent water were combined (Table 3). In addition to the chronic toxicity values measured at 40 ppt the best toxicity values for *H. banksii*, *G. imparipes* and *S. lalandi* were included in the second best dataset. In the case of *S. lalandi* both toxicity values from phase I and II are not ideal (see previous explanation) and therefore the most conservative value (i.e. 11.1) was used which also happens to be the chronic toxicity value for *S. lalandi*.

H. banksii was included as it has regional relevance and the toxicity data from salinity controls shows that there was no difference in the toxicity measured within the range 37 to 45 ppt. Therefore the toxicity of the saline brine measured in dilutent water with a salinity of 37 ppt could be used to estimate the toxicity when tested in diluent water with a salinity of 42 ppt. The acute EC10 value for *G. imparipes* was included due to regional relevance. The organisms and toxicity values presented in Table 3 are those recommended for the derivation of concentrations that should protect 99% of species (PC99) and dilution factors.

Table 3. The species and the toxicity values for the two preferred datasets to be used for deriving the dilution factors*.*

Test species	Taxonomic group	EC10 and NOEC values (% brine)	
		Best dataset	2nd best dataset
H. banksii	Macroalga		16[a]
I. galbana	Diatom	84.4	84.4
E. radiata	Macroalga	27.6	27.6
C. gigas	Bivalve	3.3	3.3
G. imparipes	Crustacean		10.9[b]
P. auratus	Fish	22.2	22.2
S. lalandi	Fish		10.6[c]
A. japonicus	Fish	11.6	11.6
M. latisulcatus	Crustacean	11.8	7.5[d]
S. apama	Cephalopod	6.3	6.3

[a] the NOEC for *H. banksii* was measured in diluent water with a salinity of 37 ppt.

[b] the EC10 for *G. imparipes* is an acute toxicity value.

[c] the EC10 value for *S. lalandi* was measured in diluent water with a salinity of 35 ppt and calculated by the author using data generated by Geotechnical Services (Appendix O10.4).

[d] the EC10 value for *M. latisulcatus* was calculated by the author using data generated by Geotechnical Services (Appendix O10.4).

The best dataset contains toxicity data for seven species that belong to six taxonomic groups of organisms. The second best dataset contains toxicity data for ten species that belong to six taxonomic groups of organisms. Thus both datasets exceed the minimum data requirements of the BurrliOZ method (Campbell et al., 2000) and the Australian and New Zealand water quality guidelines (ANZECC and ARMCANZ, 2000) (i.e. at least five species that belong to at least four taxonomic groups of organisms).

DERIVATION OF DILUTION FACTORS

It is appropriate that the level of protection at Point Lowly be a PC99 (i.e. theoretically protecting 99% of species) given the close proximity of the breeding ground of the Giant Cuttlefish (*S. apama*). Therefore only the PC99 and the corresponding dilution factors are presented in the following text. The PC99 and dilution factor for the best dataset are 2.35% saline brine and 45 (rounded up from 42.6) respectively. The corresponding values for the second best dataset are 2.48% saline brine and 41 (rounded up from 40.3) respectively. It is worth noting that the PC99 and dilution factors derived using the best dataset (even though they are based on toxicity data for fewer species) are more conservative (i.e. requiring a greater dilution of brine) than those derived using the second best dataset. Therefore, in order to be conservative the PC99 and dilution factor for the best dataset are preferred.

If the PC99 and dilution factor for the best dataset are achieved then theoretically 99% of marine organisms typical of Upper Spencer Gulf will be protected from experiencing sub-chronic toxic effects of greater than 10% caused by the discharge of saline brine into water with a salinity of 40 ppt. It is important to note however, that the salinity that this PC99 value and dilution factor is based on is at the lower end of the range of salinites experienced at Point Lowly. Therefore, it is possible that the PC99 and dilution factor are underestimated compared to those that would be derived using toxicity data generated using diluent water with a salinity of 42 ppt (the upper range of salinities reached at Point Lowly).

There is toxicity data for the Giant Cuttlefish conducted in diluent water with a salinity of 45 ppt (a higher salinity than that experienced at Point Lowly). The most sensitive endpoint measured at 45 ppt was post-hatch survival which resulted in a chronic EC10 of 1.86% saline brine. The saline brine would need to be diluted by a factor of 55 (rounded up from 53.8) in order to ensure that the Giant Cuttlefish would not experience more than a 10% reduction in post-hatch survival in seawater with a salinity of 45 ppt. As this salinity is greater than that experienced at Point Lowly, the dilution factor of 55 is likely to exceed that needed to provide the same level of protection to the Giant Cuttlefish in seawater with a salinity of 42 ppt.

It was decided to protect all species (i.e. 100% of marine species typical of Upper Spencer Gulf). Using the best dataset recommended previously, the first concentration of saline brine that BurrliOZ states would protect 100% of species is 1.23%. This corresponds to a dilution factor of 85 (rounded up from 81.3). This dilution factor should theoretically protect 100% of marine species typical of Upper Spencer Gulf from experiencing sub-chronic toxic effects of greater than 10% caused by the discharge of saline brine into water with a salinity of 40 ppt.

Due to the close proximity of the Giant Cuttlefish's breeding ground to the proposed discharge site, it was decided to ascertain what level of protection a dilution factor of 85 would provide based on the lowest toxicity value for that species. The concentration of 1.23% saline brine lay between the EC1 and EC5 values (Appendix O10.3). To determine the per cent effect that 1.23% saline brine will have to the most sensitive endpoint of the Giant Cuttlefish, the concentrations of saline brine were plotted against the per cent reduction in post-hatch survival values (Appendix 09.3) and regressed (Figure 1).

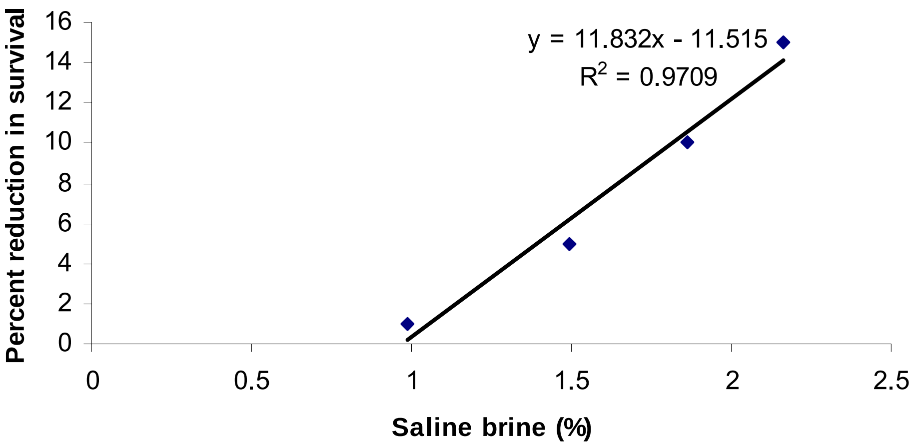


Figure 1. Plot of percent saline brine in seawater at 40ppt against the percent reduction in post-hatch survival of the Giant Cuttlefish (*Sepia apama*) and the regression line and equation for this data.

The resulting regression equation could predict approximately 97% of the variation in toxicity (i.e. R^2 = 0.971) and therefore accurately fits the data. By substituting the value of 1.23% into the regression equation (Fig. 1) it was determined that this would cause a 2.89% reduction in post-hatch survival of the Giant Cuttlefish. Therefore if a dilution factor of 85 is achieved then:

> theoretically 100% of marine species typical of Upper Spencer Gulf would be protected from experiencing sub-chronic toxic effects of greater than 10% in sea water with a salinity of 40 ppt; and

there would be less than a 3% reduction in post-hatch survival of the Giant Cuttlefish in seawater with a salinity of 45 ppt.

As 45 ppt is greater than that experienced at Point Lowly, the reduction in post-hatch survival of the Giant Cuttlefish in seawater with the maximum salinity experienced at Point Lowly (i.e. 42 ppt) would be less than 3%. With the toxicity data available it is not possible to provide a more accurate estimate of the per cent reduction in post-hatch survival that would be experienced by the Giant Cuttlefish at 42 ppt.

The level of protection that is provided by adopting a dilution factor of 85 is higher than the highest level of protection offered in the Australian and New Zealand water quality guidelines (ANZECC and ARMCANZ, 2000) (i.e. 100% of species compared to 99% of species). This increased level of protection increases the required dilution factor from 45 to 85.

The last step in the derivation of the Australian and New Zealand water quality guidelines was to ground-truth the trigger values (Warne, 2001) and if necessary to adjust the trigger values downwards by manipulating the calculations or the data in various ways (e.g. by increasing the level of protection from 95% to 99% or using a larger assessment factor (AF)). This was done by comparing the trigger values to all the raw toxicity data paying particular attention to field-based, mesocosm or microcosm toxicity data.

This ground-truthing step was conducted for the present report. The PC100 value for the best dataset was compared to all the toxicity data that had been generated by the WET testing (i.e. Appendices O10.2 to O10.4). The lowest toxicity value derived by the WET testing was an EC10 value of 1.86% saline brine for *S. apama*. The endpoint measured by this toxicity value was survival of young post hatching – which is a very relevant endpoint given the close proximity of the Giant Cuttlefish breeding ground to the proposed site of the desalination plant. However, this toxicity value was determined using diluent water with a salinity of 45 ppt, which is greater than the salinity range at Point Lowly and therefore the value most probably overestimates the toxicity that would occur at Point Lowly. Nonetheless the preferred PC100 value for the best dataset (i.e. 1.18% saline brine) is lower than the lowest toxicity value measured (i.e. 1.86% saline brine).

The author has been informed by ARUP/ENSR that the lowest dilution factor of the saline brine that would be achieved at the Giant Cuttlefish breeding site closest to the discharge point will be 116. This corresponds to a saline brine concentration of 0.86%. Using the regression equation presented earlier, 0.86% saline brine would have no effect on post-hatch survival. However, the relationship below the 1% reduction in post-hatch survival may not conform to the relationship observed above this level (Figure 1). A reasonably conservative approach would be to assume there is a linear relationship between the control and the 1% effect level. By making this assumption the following equation is obtained

% reduction in survival = 0.989 x % saline brine ($R^2 = 1$)

Using this regression equation 0.86% saline brine would cause a 0.85% reduction in post-hatch survival in seawater with a salinity of 45 ppt.

Thus, if a 116 fold dilution of the saline brine is achieved at the closest Giant Cuttlefish breeding site to the discharge point then the largest effect on the endpdoints that were measured for the Giant Cuttlefish would be a less than 1% reduction in post-hatch survival.

COMPARISON WITH THE SPECIES USED FOR THE WA DESALINATION PLANT

The species that were used to assess the saline brine from the Perth Seawater Desalination Plant into Cockburn sound were: the marine bacteria *Vibrio fischeri*; the macroalga *Ecklonia radiata*; the blue mussel *Mytilis edulis*; the unicellular algae *Nitzschia closterium* and *Isochrysis sp*; the copepod *Gladioferens imparipes;* and the Pink Snapper *Pagrus auratus* (Geotechnical Services, 2006, 2007a, 2007b). The *V. fischeri* was only used to determine the range of concentrations to be used for the other species and was not used in the calculations of the dilution factors (Geotechnical Services, 2006). Thus only five species that belonged to five different taxonomic groups were used to derive the dilution factors. This meets the minimum data requirements to use the BurrliOZ species sensitivity distribution method and to derive a trigger value in accordance with the Australian and New Zealand water quality guidelines (ANZECC and ARMCANZ, 2000).

In comparison, it is recommended that toxicity data for ten species that belong to seven different taxonomic groups be used to derive the dilution factors for the proposed Point Lowly desalination plant study. These also meet the minimum data requirements of the Australian and New Zealand water quality guidelines (ANZECC and ARMCANZ, 2000). There should be greater confidence in the dilution factors calculated for Point Lowly than for the WA desalination plant as toxicity data for more species and more taxonomic groups is being used. The inclusion of toxicity data for *S. apama* in the derivation is very important and appropriate as there is a breeding ground located close to the proposed desalination plant site.

CONCLUSIONS

A series of whole effluent toxicity tests have been conducted over two years by two organisations. Different subsets of these species have been combined in various reports to produce a range of species protection values and dilution factors (refer Appendices O10.2 to O10.4). This report provides an assessment of all the WET results, and the species protection values presented here use the most appropriate dataset available and thus supercede all previous values.

The suite of organisms tested as part of the Environmental Impact Statement for the proposed desalination plant at Point Lowly were evaluated for their appropriateness to calculate dilution factors for the saline brine effluent. The best possible dataset is based solely on sub-chronic and chronic toxicity data measured in diluent water with a salinity of 40 ppt. Based on this the recommended species are *Isochrysis galbana, Ecklonia radiata, Melicertus latisulcatus, Crassostrea gigas, Pagrus auratus, Argyrosomus japonicus* and *Sepia apama.* However, a second dataset which retained the previous species but added *Hormosira banksii, Gladioferens imparipes* and *S. lalandi* was also evaluated as this maximized the number of test species. Both datasets contain more species belonging to more taxonomic groups than that used in the evaluation of the Western Australia desalination plant and exceed the minimum data requirements of the Australian and New Zealand water quality guidelines. Therefore there will be greater confidence in the dilution factors being derived for the proposed desalination plant being examined in this report than for the WA plant.

Use of the best dataset in BurrliOZ yielded a concentration that should protect 99% of species (PC99) of 2.35% saline brine and a dilution factor of 45. The corresponding values for the second best dataset are 2.48% and 41 respectively. The best dataset yielded larger dilution factors then the second dataset, and it is therefore recommended for deriving dilution factors. If the PC99 and dilution factor for the best dataset are achieved then theoretically 99% of marine organisms typical of Upper Spencer Gulf will be protected from experiencing sub-chronic toxic effects of greater than 10% caused by the discharge of saline brine into water with a salinity of 40 ppt.

The salinity of seawater at Point Lowly ranges from 39 to 42 ppt. Therefore the dilution factor derived for seawater with a salinity of 40 ppt may underestimate that required at 42 ppt. A toxicity value for the Giant Cuttlefish tested in seawater with a salinity of 45 ppt was available. To protect the Giant Cuttlefish at this salinity from sub-chronic effects of greater than 10% would require a dilution factor of 55. Therefore it was decided to protect 100% of marine species typical of Upper Spencer Gulf in seawater with a salinity of 40 ppt from sub-chronic toxic effects larger than 10%. This is achieved by a dilution factor of 85. In addition, this dilution would lead to the Giant Cuttlefish experiencing less than a 3% reduction in post-hatch survival of in seawater with a salinity of 45 ppt.

As 45 ppt is greater than that experienced at Point Lowly, the reduction in post-hatch survival of the Giant Cuttlefish in seawater with the maximum salinity experienced at Point Lowly (i.e. 42 ppt) would be less than 3%.

ARUP/ENSR informed the author of this report that the minimum dilution factor that will be achieved at the Giant Cuttlefish breeding site closest to the discharge point is 116. Such a dilution factor would protect 100% of species in seawater with a salinity of 40 ppt with a considerable margin of safety. In addition, it would cause less than a 1% reduction in post-hatch survival of the Giant Cuttlefish in seawater with a salinity of 45 ppt at the breeding site closest to the discharge point and therefore an even lower effect in seawater with a salinity of 42 ppt.

Uncertainty remains over the exact dilution factor needed to protect marine species typical of the Upper Spencer Gulf from sub-chronic effects associated with the discharge of saline brine from the proposed desalination plant into receiving water with a salinity of 42 ppt. This has arisen because the WET testing data were generally conducted at 40 ppt. Electing to protect 100% of species in seawater with a salinity of 40 ppt addresses some of this uncertainty. The best way to overcome this would be to conduct additional chronic WET tests using diluent water with a salinity of 42 ppt or at a salinity of 43 ppt if it is desired to be more conservative.

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APPENDIX 1 - USE OF EC10 AND/OR NOEC TOXICITY DATA

The current Australian and New Zealand Water Quality Guidelines use no observed effect concentration (NOEC) data to derive high reliability Trigger Values (TVs) but EC/LC50 toxicity data to derive moderate and both classes of low reliability TVs (ANZECC & ARMCANZ, 2000; Warne, 2001). The relative merits of NOEC and lowest observed effect concentration (LOEC) toxicity data (which are collectively called hypothesis-based toxicity values) have been discussed in the literature. Critics of NOEC data such as Hoekstra and Van Ewijk (1993), Noppert et al. (1994) and Chapman et al. (1996) feel that such data should not be used for regulatory purposes. They prefer point estimates of toxicity such as the concentration that is lethal to 5% of a population (i.e. LC5) or the concentration that causes a 10% effect (i.e. EC10). The problems with the use of NOEC and LOEC data are that:

- only tested concentrations can be NOEC or LOEC values (therefore such values are somewhat predetermined by the concentrations used in the toxicity test);
- the term NOEC is misleading. A NOEC is the highest concentration used in a toxicity test that causes an effect not significantly different to the control(s). It therefore does not correspond to ‘no effect’. Typically, the NOEC corresponds to a 10 to 30% effect (Moore and Caux, 1997; USEPA, 1991 and Hoekstra and Van Ewijk, 1993);
- this measure of toxicity can easily be manipulated and does not encourage high quality work. For instance, less rigorous procedures would increase the variability between replicates. This in turn, would increase the size of the difference needed between the treatment and control means in order for a statistically significant difference to be found (i.e. the NOEC value is likely to increase).
- a problem related to the third dot point is that TVs derived using this data do not have as clear a definition as those derived using EC10 data. The TVs based on NOECs would theoretically protect X% of species from experiencing statistically significant inhibitory impacts. The TVs based on EC10 data would theoretically protect X% of species from experiencing inhibitory impacts greater than 10%.

An example of the problems that can arise with using hypothesis-based toxicity data compared to point estimates is provided by the toxicity data for saline brine to the Mulloway. For that species the NOEC is < 1.6% saline brine while the EC10 is 11.56% brine. The hypothesis based method compared the values for each treatment to the control and found that the first treatment (i.e. 1.6% brine) was significantly different to the control – hence the NOEC became < 1.6% brine. However, the concentration response curve is unusual – in that there is a marked difference between the control and the lowest treatment but then with subsequent increases in the brine content there was very little increase in toxic effect until above 12.7% brine at which point all growth essentially stopped. This tends to indicate that there was possibly another toxicant present in the diluent water which caused this initial low level effect. So the point estimates of toxicity were calculated using the growth rate of the first treatment as the starting point from which the toxicity values were determined.

Despite the above problems NOEC data were recommended in preference to toxicity data such as EC10 values in the Australian and New Zealand guidelines (ANZECC and ARMCANZ, 2000) for the following reasons:

- there was a general lack of EC10 type data in the scientific literature; and
- there are large amounts of NOEC data available in the literature.

However, the Australian and New Zealand WQGs (ANZECC & ARMCANZ, 2000) point out that the methods used to derive the trigger values are not data specific. Thus, TVs could be derived using EC10 values if there was sufficient data. In fact, these same documents suggested that the use of NOEC data "be phased out" as EC10 type data become available (ANZECC & ARMCANZ, 2000; Warne, 1998).

Just this year NOEC and LOEC type data and the hypothesis-based statistical methods used to derive them have come under further attack. Newman (2008) has written a scathing article which reveals that the methods used to derive the NOEC and LOEC are statistically flawed and that these methods should be replaced 'whenever possible' by confidence interval-based methods. Warne (in prep) also argues strongly that NOEC and LOEC data should not be generated from now on and that any that is generated should be rejected by regulators and journals.

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