RPS

WHOLE EFFLUENT TOXICITY TESTING OF SIMULATED REVERSE OSMOSIS BRINE EFFLUENTS

Gorgon Development, Barrow Island

VOLUME 1 OF 2

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EXECUTIVE SUMMARY

RPS Environment and Planning Pty Ltd was commissioned by Chevron Australia Pty Ltd (Chevron) to design and coordinate a programme of ecotoxicological testing of whole effluents that might be released by a reverse osmosis (RO) desalination plant on Barrow Island. Testing protocols and Draft B of this report were reviewed by CSIRO. Whole Effluent Toxicity (WET) testing was done by Ecotox Services Australasia on simulated whole effluents that mimic what may be released by the proposed RO plant. The selection of chemicals included in the simulated effluent was based on estimation of what might be in whole effluents under normal running conditions, and included a range of potential vendor's systems and different waste streams, such as from backwashing and cleaning in place procedures.

Six marine species relevant to Barrow Island were used in seven bioassays to assess the toxicity of twelve simulated whole effluents. Despite testing across a wide range of simulated whole effluents, there was no evidence that different types or combinations of waste streams made any difference to the toxicity of simulated whole effluents. Overall, the toxicity of the simulated whole effluents tested was not detectable as different from whole effluents that were composed of just brine (61 ppt) or sodium hypochlorite (0.53 mg L^{-1}) in seawater. The twelve repeats of each bioassay were then treated as replicate runs for a general RO desalination whole effluent and averaged, which allowed for more precise estimates of endpoints such as LC10 and EC10 (concentrations where 10% of test organisms died or were affected, respectively). Bioassays which measured toxicity effects on urchin fertilisation and larval development, oyster larval development and fish imbalance were more precise than bioassays which measured juvenile prawn and juvenile polychaete mortality and algal growth rates.

Estimates of LC10 or EC10 from each bioassay were then used in a BurrliOZ species sensitivity distribution analysis to determine the (safe) concentrations of whole effluent which should have only small (10%) effects on 99% of species (i.e. PC99). Choice of correction factor used to combine data from acute bioassays with chronic data and how these were applied made a difference to the estimate of PC99. Results of explicit hypotheses about correction factors, however, did not support the application of acute to chronic (ACR) correction factors larger than two. When an ACR correction factor of two was applied to acute LC10 data the lower 95% confidence interval estimate of PC99 was 10.3% of stock whole effluent, suggesting that a one in ten dilution should provide enough dilutions that less than 1% of species will be affected. When an ACR correction factor of two was applied to acute LC50 data the lower 95% confidence interval estimate of PC99 was 14.2% of stock whole effluent, corresponding to a one in seven dilution. An alternative, more conservative method of determining a safe concentration, using the minimum concentration of whole effluent where no biological effect was detected (NOEC) and applying a safety factor of ten, suggested that 2.5% of stock whole effluent solution should be dilute enough to protect 99% of species. Thus, ensuring dilution of whole effluent to a one in forty dilution would almost certainly mean no effects would be detectable past the near-field mixing zone of a relatively small outfall such as that proposed for Barrow Island.

This study is a prior assessment of the potential toxic effects of RO desalination waste discharges on the biota of Barrow Island because actual RO waste from Barrow Island is not as yet available to WET test directly. Notwithstanding the limitations associated with testing simulated rather than actual whole effluents, this work suggests that the types of whole effluents that are expected to be released at Barrow Island will be of low toxicity. Once a plant is operating on Barrow Island it would be prudent to further WET test and monitor the effluents it produces.

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1.0 INTRODUCTION

1.1 Background

The continuous provision of locally produced freshwater is critical for all stages of the proposed Gorgon Gas Development on Barrow Island, Western Australia. Chevron Australia Pty Ltd (Chevron), on behalf of the Gorgon Joint Venture (GJV), is seeking ministerial approval to discharge effluent from a proposed desalination plant on Barrow Island, via ocean outfall. The proposed desalination plant would process sea water using reverse osmosis (RO) technology to provide freshwater for the construction phase and ongoing operations of the Gorgon Development. A by-product of producing freshwater via desalination, however, is waste effluent which will contain concentrated brine (total dissolved solids = 61 ppt) and various chemicals used in processing sea water and maintaining the plant. The brine effluent from the RO plant would be released via an ocean outfall near the proposed Barrow Island Materials Offloading Facility (MOF).

During consultation, the Department of Environment and Conservation (DEC) recommended that assessment of the toxicity of waste effluents from the proposed RO plant at Barrow Island be based on toxicity testing of *whole effluents* directly, rather than assessments of the toxicity of individual chemical components within effluents. This is because the impacts of brine and chemical waste in combination in whole effluents may cause unpredictable synergistic effects, greater than the predicted effects of individual chemicals or brine separately.

RPS Environment and Planning (RPS) was commissioned by Chevron to coordinate a program of ecotoxicological testing of whole effluents that could be released via ocean outfall from the proposed RO desalination plant on Barrow Island. Using a range of organisms relevant to Barrow Island, Whole Effluent Toxicity (WET) testing was done with a range of simulated whole effluents that mimic what may be released by the proposed RO plant. WET testing was undertaken by Ecotox Services Australasia on behalf of RPS.

The chemicals to be included in the simulated effluents were selected to represent the range of likely effluents as estimated by potential RO plant suppliers. Four potential vendors under consideration to supply the RO plant provided information about the likely chemical treatments used in their RO systems. The four systems differ in the range of specific treatment chemicals used and the mixes of chemicals that may be released at different times. Consequently, there are a large number of potential effluents that could be released (and WET tested), depending on which specific plant is commissioned. The approach taken to rationalise this very large number of potential whole effluents was to WET test a subset of whole effluents and test directly for any evidence of differences in toxicity among these. If any evidence of synergistic toxicity was found then further WET testing would be done on all possible combinations.

This report outlines:

- How simulated RO whole effluents for WET testing were selected and the logical basis for testing whether different types of simulated whole effluents were more toxic than others.
- How simulated RO whole effluents were formulated.
- The range of bioassays conducted in the WET testing.
- Which test species were selected.
- Detailed analyses and interpretation of the results.

A review of the proposed protocol for WET testing and the final report was conducted by Dr Jenny Stauber of the Centre for Environmental Contaminants, CSIRO Division of Land and Water. Her review of Draft B of this report and a summary of the changes made in response to her comments/suggestions are given in Appendix 1.

1.2 Designing Simulated Whole Effluents

Given that the composition of whole effluents produced by the eventual RO plant at Barrow Island will not be known until a vendor is selected and the plant design is finalised, one option for WET testing is to test whole effluents collected from operational RO desalination plants in other locations. This was decided against because differences in the composition of the processing chemicals used and the sea water sources at the various plants might render this irrelevant to understanding potential toxicological effects at Barrow Island. Instead, WET testing of simulated whole effluents created in the laboratory was undertaken. These simulated whole effluents were formulated based on advice provided to RPS by Kellog Joint Venture (KJV), who are responsible for designing and commissioning of the RO plant for Barrow Island. Estimates of potential chemical use and running conditions provided by KJV are based on what is known about the sea water quality at Barrow Island and information provided to KJV by the potential suppliers of RO plants; exact details of running conditions and likely chemical usage, however, can only be known once an actual plant is operating at Barrow Island. Sea water from near the proposed MOF at Barrow Island was concentrated into brine by evaporation and chemicals were added at the concentrations at which they are likely to be used under a normal range of operating conditions.

RO plants also produce different waste streams at different times, according to various maintenance protocols. Generally, there are three processes involved in running an RO plant, each of which affects the composition of the waste stream:

- Normal desalination operations - during which pre-treatment chemicals such as biocides, flocculants and anti-scalants are added to the inlet stream, most of which are concentrated and released into the main brine effluent; post-treatment chemicals are also added to the outlet stream to neutralize the effects of some added pre-treatment chemicals.

- Backwashing - when additional biocides (e.g. Sodium hypochlorite) are added to the flushing water when solid waste is removed from intake filters.

- Cleaning in place (CIP) procedures - periodic cleaning of ultra-filtration and RO membranes including the addition of biocides, cleaning chemicals and pH adjusters.

Brine effluent at Barrow Island will include waste from both backwashing and CIP procedures. Backwashing waste will be produced every six hours and added to the main brine effluent from a constant flow storage tank. As a result it will be a constant component of the final effluent. CIP is expected to occur once every thirty days. Thus, the RO plant at Barrow Island will likely release two different waste streams over a month: brine + process chemicals + backwash waste for twenty-nine days, and brine + process chemicals + backwash waste + CIP for one day.

The four potential vendors of the RO plant at Barrow Island (Veolia, ITT, Osmoflo, GE) each provided estimates of their likely chemical uses under the range of normal operating conditions. RPS provided water quality data from the east coast of Barrow Island for the vendors to base their chemical use scenarios on. Each vendor's RO process nominally uses a different set of proprietary and non-proprietary chemicals. For these chemicals (and their main by-products) KJV has produced a summary of the concentrations likely to be present in the main effluent stream under normal operating conditions. All discharges will mainly comprise brine with a concentration of total dissolved solids of 61 ppt. Sodium hypochlorite is a standard additive for all backwashing procedures. The only significant difference in the composition of whole effluents amongst the potential vendors was in pre-treatment chemicals.

A standard suite of chemicals are added to the effluent during CIP procedures. Five additional chemicals, however, may also be added during CIP (NaOH, EDTA, Na-DSS, HCl or Na_2SO_4). Thus, in total there are four potential (vendor-specific) effluents that will include brine, process chemicals and backwash waste, plus five alternative CIP waste variants that each could be added to these four main brine producing effluents once a month. In total, twenty-four potential whole effluents were identified for WET testing.

This study chose to measure the toxicity of ten of the twenty-four potential whole effluent combinations that might be released at Barrow Island (plus two other control treatments), ensuring these ten effluent combinations were representative of the range of effluent types that might be released at Barrow Island. This approach allowed for more generalised testing of hypotheses in relation to differences in toxicity to biota from different types of whole effluents and a test of whether there was any evidence of synergistic toxic effects.

1.3 Experimental Design

Alternative Models for the Toxicity of Whole Effluent Components

At least eight alternative models can be constructed about the toxicity of different whole effluents from an RO plant at Barrow Island, and how synergistic effects may or may not develop when different components of effluents are combined with brine and/or each other. Models A, B, D, E and F (below) are non-interacting in that the simple presence or absence of a particular component determines the toxicity of whole effluents. In contrast, in models C, G and H, the toxicity of an effluent depends on combinations of components together. By WET testing the twelve effluent combinations listed below (treatments 1–12), it is possible to test among these models because each makes unique predictions about differences in toxicity among treatments. In the hypotheses below '1 > 2, 3' indicates that the toxicity of whole effluent 1) will be greater than for whole effluent 2) and whole effluent 3). '1 = 2' suggests there will be no detectable difference in toxicity between whole effluents 1) and 2). Note in bioassays the parameter chosen to measure 'toxicity' (e.g. [EC50] or [EC10]) is measured as less when toxicity is greater.

Simulated Whole Effluent Treatments

See Section 2.4 for detailed descriptions of the chemical composition of each treatment.

1. Brine only (no other additives).
2. Veolia Brine Effluent + Backwash.
3. Veolia Brine Effluent + Backwash + CIP (variant i).
4. Veolia Brine Effluent + Backwash + CIP (variant iv).
5. ITT Brine Effluent + Backwash.
6. ITT Brine Effluent + Backwash + CIP (variant ii).
7. Osmoflo Brine Effluent + Backwash.
8. Osmoflo Brine Effluent + Backwash + CIP (variant iii).
9. Osmoflo Brine Effluent + Backwash + CIP (variant iv).
10. GE Brine Effluent + Backwash.
11. GE Brine Effluent + Backwash + CIP (variant v).
12. Backwash only (NaClO 0.53 mg L^{-1}).

Models and Hypotheses

A. Brine *per se* is the most toxic component of the effluent and the presence or absence of flocculants, biocides and/or other backwashing or CIP components does not affect toxicity.

Hypothesis: 1 = 2 = 3 = 4 = 5 = 6 = 7 = 8 = 9 = 10 = 11 > 12

B. The backwash chemical (NaClO) is the most toxic component of effluent and the presence or absence of other components and/or brine does not affect toxicity.

Hypothesis: 2 = 3 = 4 = 5 = 6 = 7 = 8 = 9 = 10 = 11 = 12 > 1

C. Backwash and brine is more toxic in combination than either alone and other components do not affect toxicity.

Hypothesis: 2 = 3 = 4 = 5 = 6 = 7 = 8 = 9 = 10 = 11 > 1, 12

D. Certain vendor's effluents are more toxic than others, regardless of CIP chemicals used.

Hypothesis: 2 = 3 = 4
and: 5 = 6
and: 7 = 8 = 9
and: 10 = 11
and: (2 > 1, 12 or 5 > 1, 12 or 7 > 1,12 or 10 > 1, 12)
and not: 2 = 3 = 4 = 5 = 6 = 7 = 8 = 9 = 10 = 11

E. CIP chemicals are the most toxic component, or make whole effluents more toxic, regardless of which CIP variant is used or vendor.

Hypothesis: 3 = 4 = 6 = 8 = 9 = 11 > 1, 2, 5, 7, 10, 12

F. Some specific CIP variants are the most toxic component or make a whole effluent more toxic, regardless of which vendor's treatment they are used with.

Hypothesis: 3 > 1, 2, 4, 5, 6, 7, 8, 9, 10, 11, 12
or: 4 = 9 > 1, 2, 3, 5, 6, 7, 8, 10, 11, 12
or: 6 > 1, 2, 3, 4, 5, 7, 8, 9, 10, 11, 12
or: 8 > 1, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12
or: 11 > 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12

G. CIP chemicals are the most toxic component or make whole effluents more toxic, regardless of which CIP variant is used, but depending on which vendor's treatment they are used with.

Hypothesis: 3 = 4 > 1, 2, 12
and: 6 > 1, 5, 12
and: 8 = 9 > 1, 7, 12
and: 11 > 1, 10, 12
and not: 3 = 4 = 5 = 8 = 9 = 11

H. Some CIP variants make a whole effluent more toxic, depending on which vendor's treatment they are combined with.

Hypothesis:	3 > 1, 2, 4, 5, 6, 7, 8, 9, 10, 11, 12
or:	4 > 1, 2, 3, 5, 6, 7, 8, 9, 10, 11, 12
or:	6 > 1, 2, 3, 4, 5, 7, 8, 9, 10, 11, 12
or:	8 > 1, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12
or:	10 > 1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 12
or:	11 > 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11

Of key interest was whether any of the whole effluents that contained CIP chemical variants were more toxic than whole effluents tested that did not contain these chemicals; i.e. whether there was any support for models E–H. Although each of the above models could have been supported uniquely by the outcomes of the WET testing, in reality it would be difficult to distinguish among models (F–H) because only some combinations of vendors' treatments x CIP variants were tested. For example, if effluent 6) was found to be the most toxic effluent it would not be possible to distinguish if that was because:

- Any CIP in conjunction with ITT's running chemicals is more toxic (model F).
- CIP variant ii is the most toxic component alone regardless of vendor's running chemicals (model G).
- The combination of ITT's running chemicals and CIP variant ii creates a uniquely toxic effluent (model H).

Alternatively, if either 4) or 10) was the most toxic effluent then only model H would be supported. Consequently, if there was any evidence that any of the whole effluents with CIP were more toxic than those without the CIP additions (i.e. support for any of models E–H), a conservative approach would be for this to have triggered the assessment of further combinations of vendors x CIP variants in a second round of WET testing.

1.4 Choice of Test Species

One of the goals for this WET testing protocol was to ensure that that the results are relevant to marine taxa that live near the proposed MOF at Barrow Island. ANZECC/ARMCANZ (2000) guidelines suggest WET testing local species, randomly chosen from as wide a range of groups as possible to ensure this. At a minimum, the guidelines suggest using at least five (local) species from at least four taxonomic groups in WET tests. The test organisms should also encompass a range of functional groupings, representing different parts of the ecosystem.

Six marine species were chosen for WET testing in this study on the basis of satisfying the ANZECC/ARMCANZ (2000) guidelines and the availability of sufficient individuals. These species represent six major taxonomic groups:

- Echinoderms: *Heliocidaris tuberculata*.
- Molluscs: *Saccostrea glomerata*.
- Crustaceans: *Penaeus monodon*.
- Polychaetes: *Diopatra dentata*.
- Fish: *Lates calcarifer*.
- Algae: *Isochrysis galbana* (Tahitian isolate).

These species thus also covered a range of trophic levels:

- Primary producers (*I. galbana*).
- Grazers (*H. tuberculata*).
- Filter feeders (*S. glomerata*).
- Predators (*P. monodon, D. dentata, L. calcarifer*).

For the sea urchin (*H. tuberculata*), two different bioassays were done (fertilisation success; larval development), testing the effects of whole effluents on different physiological processes/life history stages. For the comparisons of toxicity among simulated whole effluents each of the urchin bioassays were treated as independent. For the calculation of species protection trigger levels, however, the results from the two urchin bioassays were combined to provide just one estimate for urchins. For the other five species, a different bioassay was done for each; details of each bioassay are given in Section 3.0 and in the full laboratory report in Appendix 3.

Barrow Island lies within the distributional range of most of the test species (*P. monodon*, *I. galbana*, *L. calcarifer* and *D. dentata*) and two (*H. tuberculata*, *S. glomerata*) are very close relatives of species found there. Although it has an exclusively eastern Australian distribution (Laedsgaard et al. 1991), *H. tuberculata* was chosen because it is a close relative of a number of common echinometrid urchins with tropical Indo-Pacific distributions that are known to include Barrow Island (e.g. *Echinometra mathaei*). Urchins are also potentially sensitive to changes in salinity given their unique water vascular system (e.g. Havenhand et al. 2008). Genetically, *S. glomerata* (was *S. commercialis*) is very closely related to both *S. cucullata* and *S. mordex,* which are both found at Barrow Island (Lam and Morton 2006). The taxa chosen for these tests were also selected on the basis of availability at the testing location in Sydney, sourced from the wild or aquaculture, as well as the long experience Ecotox Services Australasia has working with these species in terms of husbandry and quality assurance for the tests. While it would be ideal if test organisms were sourced from local populations at Barrow Island, collecting and transporting organisms from there to an ecotoxicology laboratory could lead to quality control issues in terms of the physiological condition of test organisms.

2.0 BIOASSAY PREPARATION

2.1 Sample and Test Containers

All sample containers and vessels used for the preparation of samples were cleaned using the following procedure (based on USEPA 2002). New sample bottles and equipment only required a rinse in 10% acid (see below) followed by rinsing with deionised water and dilution water, and did not require the acetone rinsing step:

- Soaked fifteen minutes in tap water and scrubbed with laboratory grade detergent.
- Rinsed twice with deionised or milli-q water.
- Carefully rinsed once with fresh, dilute (10% vol/vol) nitric acid to remove scale, metals and bases.
- Rinsed three times with deionised or milli-Q water, and allowed to dry.

2.2 Dilution Water

The dilution water used for the preparation of test treatments (i.e. dilutions of simulated whole effluents) was filtered sea water (FSW) of 35 ± 1 ppt collected from the Sydney Region. Ongoing monitoring of sea water at several sites around Barrow Island by RPS has measured salinity consistently between 34 and 36 ppt. The sea water was filtered through a 0.45 µm acid washed (10% HNO_3) cellulose acetate membrane capsule filter with 0.65 µm pre-filter (Sartorius) using a Masterflex peristaltic pump. Once filtered, the FSW was stored in a clean container(s) in the dark until required. The sea water was kept at the test temperature if used that day or at 4 °C if kept longer.

2.3 Creating Simulated Brine and Whole Effluents

Sea water for creating simulated brine was collected from near the proposed MOF on Barrow Island, in clean, 5 L, high-density, polyethylene carboys. Collection containers were rinsed on site with sea water three times immediately prior to filling with sea water. The filled carboys were shipped to the Ecotox Services Australasia laboratory inside insulated containers by express air freight. A completed chain of custody form accompanied the samples. The chain of custody form documented the sample name, date and time of sample collection, identified the person that collected the sample as well as details of time of sample delivery and custody. Upon arrival in Sydney, the Barrow Island sea water was filtered and stored, as above.

Brine was simulated at Ecotox Services Australasia by concentrating Barrow Island sea water (lab ID 2817) in 80 L, high-density, polyethylene carboys. The Barrow Island sea water was filtered to 0.45 µm using a peristaltic pump and Sartorius Sartobran filter capsule prior to being poured into the carboys. Evaporation was assisted by heating the sea water to 30 °C with the aid of aquarium heaters, and with vigorous aeration using aquarium aerators. The salinity of the sea water was monitored daily and upon reaching 60 ppt, the solution was poured off into 2.5 L amber glass Winchesters for holding at 4 °C until required for testing. The process took approximately four weeks.

A final brine stock solution (total dissolved solids = 61 ppt) was used in bioassays – corresponding to an upper limit expectation of wastewater concentration possible during normal operations of the RO plant. Stock brine and mixed effluents were measured for salinity, pH and dissolved oxygen (DO) using a hand-held WTW LF330 salinity/conductivity meter, pH330 pH meter, and Oxi330 DO meter, respectively.

Whole effluent test solutions consisted of Barrow Island brine stock and RO process chemicals at concentrations outlined in the tables in Section 2.4. The 'brine only' treatment did not have any chemicals added and the 'backwash only' treatment was NaClO added to dilution sea water (not brine). Each whole effluent was prepared the night before the each bioassay (i.e. ~ fourteen hours earlier). Salinity, DO and pH of the effluents were measured immediately before use in the bioassays.

2.4 Composition of Simulated Whole Effluents

2.4.1 Treatment 1 – Brine Only

This effluent consisted of the simulated brine only, without the addition of any process chemicals. It acted as a control treatment, with which to compare the effect of the process chemicals.

2.4.2 Treatment 2 – Veolia Brine Effluent + Backwash

Chemical Name	Chemical	CAS Number	[Effluent] (mg L^{-1})
Hydrex 4110	Polycarboxylates	†	5
Ferric hydroxide	$Fe(OH)_3$	20344-49-4	14
Sodium metabisulphite SMBS-c12 Neutral	$Na_2S_2O_4$	7681-57-4	3
Sodium hypochlorite	NaClO	7681-52-9	0.53

2.4.3 Treatment 3 – Veolia Brine Effluent + Backwash + CIP (Variant i)

Chemical Name	Chemical	CAS Number	[Effluent] (mg L^{-1})
Hydrex 4110	Polycarboxylates	†	5
Ferric hydroxide	$Fe(OH)_3$	20344-49-4	14
Sodium metabisulphite SMBS-c12 Neutral	$Na_2S_2O_4$	7681-57-4	3
Sodium hypochlorite	NaClO	7681-52-9	0.7
Sodium hydroxide (pH 12, T 35 °C max)	NaOH	1310-73-2	13.8
Sodium metabisulphite – SMBS - Shock	$Na_2S_2O_4$	7681-57-4	3.4
Citric acid	Citric acid	77-92-9	66.7
Sodium hydroxide (pH 12, T 35 °C max)	NaOH	1310-73-2	6.8*

* NaOH added additional to 13.8mgL^{-1} above

2.4.4 Treatment 4 – Veolia Brine Effluent + Backwash + CIP (Variant iv)

Chemical Name	Chemical	CAS Number	[Effluent] (mg L^{-1})
Hydrex 4110	Polycarboxylates	†	5
Ferric hydroxide	$Fe(OH)_3$	20344-49-4	14
Sodium metabisulphite SMBS-c12 Neutral	$Na_2S_2O_4$	7681-57-4	3
Sodium hypochlorite	NaClO	7681-52-9	0.7
Sodium hydroxide (pH 12, T 35 °C max)	NaOH	1310-73-2	13.8
Sodium metabisulphite – SMBS – Shock	$Na_2S_2O_4$	7681-57-4	3.4
Citric acid	Citric acid	77-92-9	66.7
iv) HCl (pH 1-2, T 25 °C max)	HCl	7647-01-0	13.6

2.4.5 Treatment 5 – ITT Brine Effluent + Backwash

Chemical Name	Chemical	CAS Number	[Effluent] (mg L^{-1})
PermaTreat PC-1020T	Organophosphate	†	5
Ferric hydroxide	$Fe(OH)_3$	20344-49-4	14
Sodium metabisulphite SMBS-c12 Neutral	$Na_2S_2O_4$	7681-57-4	3
Sodium hypochlorite	NaClO	7681-52-9	0.53

2.4.6 Treatment 6 – ITT Brine Effluent + Backwash +CIP (Variant ii)

Chemical Name	Chemical	CAS Number	[Effluent] (mg L^{-1})
PermaTreat PC-1020T	Organophosphate	†	5
Ferric hydroxide	$Fe(OH)_3$	20344-49-4	14
Sodium metabisulphite SMBS-c12 Neutral	$Na_2S_2O_4$	7681-57-4	3
Sodium hypochlorite	NaClO	7681-52-9	0.7
Sodium hydroxide (pH 12, T 35 °C max)	NaOH	1310-73-2	13.8
Sodium metabisulphite – SMBS – Shock	$Na_2S_2O_4$	7681-57-4	3.4
Citric acid	Citric acid	77-92-9	66.7
ii) Ethylene dyamine tetraacetic (pH 12, T 35 °C max	EDTA	60-00-4	67.8

2.4.7 Treatment 7 – Osmoflo Brine Effluent + Backwash

Chemical Name	Chemical	CAS Number	[Effluent] (mg L^{-1})
Osmotreat-Antiscalant	Phosphonate	†	12
Ferric hydroxide	$Fe(OH)_3$	20344-49-4	14
Sodium metabisulphite SMBS-c12 Neutral	$Na_2S_2O_4$	7681-57-4	3
Sodium hypochlorite	NaClO	7681-52-9	0.53

2.4.8 Treatment 8 – Osmoflo Brine Effluent + Backwash + CIP (Variant iii)

Chemical Name	Chemical	CAS Number	[Effluent] (mg L^{-1})
Osmotreat-Antiscalant	Phosphonate	†	12
Ferric hydroxide	$Fe(OH)_3$	20344-49-4	14
Sodium metabisulphite SMBS-c12 Neutral	$Na_2S_2O_4$	7681-57-4	3
Sodium hypochlorite	NaClO	7681-52-9	0.7
Sodium hydroxide (pH 12, T 35 °C max)	NaOH	1310-73-2	13.8
Sodium metabisulphite – SMBS – Shock	$Na_2S_2O_4$	7681-57-4	3.4
Citric acid	Citric acid	77-92-9	66.7
iii) Na-DSS (pH 12, T 35 °C max)	Detergent	151-21-3	1.69

2.4.9 Treatment 9 – Osmoflo Brine Effluent + Backwash + CIP (Variant iv)

Chemical Name	Chemical	CAS Number	[Effluent] (mg L^{-1})
Osmotreat-Antiscalant	Phosphonate	†	12
Ferric hydroxide	$Fe(OH)_3$	20344-49-4	14
Sodium metabisulphite SMBS-c12 Neutral	$Na_2S_2O_4$	7681-57-4	3
Sodium hypochlorite	NaClO	7681-52-9	0.7
Sodium hydroxide (pH 12, T 35 °C max)	NaOH	1310-73-2	13.8
Sodium metabisulphite – SMBS - Shock	$Na_2S_2O_4$	7681-57-4	3.4
Citric acid	Citric acid	77-92-9	66.7
iv) HCl (pH 1-2, T 25 °C max)	HCl	7647-01-0	13.6

2.4.10 Treatment 10 – GE Brine Effluent + Backwash

Chemical Name	Chemical	CAS Number	[Effluent] (mg L^{-1})
MDC 150	Phosphonate	†	6
Betzdearborn DCL30	SBS	7631-90-5	6
Ferric hydroxide	$Fe(OH)_3$	20344-49-4	14
Sodium metabisulphite SMBS-c12 Neutral	$Na_2S_2O_4$	7681-57-4	3
Sodium hypochlorite	NaClO	7681-52-9	0.53

2.4.11 Treatment 11 – GE Brine Effluent + Backwash + CIP (Variant v)

Chemical Name	Chemical	CAS Number	[Effluent] (mgL^{-1})
MDC 150	Phosphonate	†	6
Betzdearborn DCL30	SBS	7631-90-5	6
Ferric hydroxide	$Fe(OH)_3$	20344-49-4	14
Sodium metabisulphite SMBS-c12 Neutral	$Na_2S_2O_4$	7681-57-4	3
Sodium hypochlorite	NaClO	7681-52-9	0.7
Sodium hydroxide (pH 12, T 35 °C max)	NaOH	1310-73-2	13.8
Sodium metabisulphite – SMBS - Shock	$Na_2S_2O_4$	7681-57-4	3.4
Citric acid	Citric acid	77-92-9	66.7
v) SMBS (pH 5, T 25 °C max)	$Na_2S_2O_4$	7681-57-4	67.8

† CAS Registry numbers unavailable = proprietary chemicals sourced from manufacturers by KJV

2.4.12 Treatment 12 – Backwash Chemicals Only

NaClO (0.53 mg L^{-1}) in filtered 35 ppt dilution sea water (no brine in this treatment).

3.0 TOXICITY TESTING

3.1 Test Regime

The following bioassays were performed with each of the twelve simulated whole effluents (treatments):

- 72 hr (chronic) microalgal growth inhibition test using *Isochrysis galbana* (Tahitian isolate).
- 1 hr (chronic) fertilisation success using the sea urchin, *Heliocidaris tuberculata.*
- 72 hr (chronic) larval development test using the sea urchin, *Heliocidaris tuberculata.*
- 48 hr (chronic) larval development using the rock oyster, *Saccostrea glomerata.*
- 96 hr (acute) larval mortality using juvenile prawns, *Penaeus monodon.*
- 96 hr (acute) mortality using the juvenile polychaetes, *Diopatra dentata.*
- 96 hr (acute) larval fish imbalance test using barramundi, *Lates calcarifer*.

Five concentrations of each whole effluent treatment, in addition to the filtered (dilution) sea water blank, were used in each bioassay (Table 1). Bioassays for each of the test species are summarized below in Tables 2 to 8.

Table 1: Test Dilutions used in the WET Tests

Dilution Factor	1 X	2 X	4 X	8 X	16 X	Filtered sea water
% of Whole Effluent	100%	50%	25%	12.5%	6.3%	0%
Salinity (Based on 61 ppt Brine Diluted into 35 ppt Control Sea Water)	61 ppt	48 ppt	42 ppt	38 ppt	37 ppt	35 ppt

Table 2: Summary of Test Conditions for the Microalgal Growth Inhibition Test – Based on USEPA Method 1003.0 and Stauber et al. (1996)

Test Species	Unicellular flagellated golden-brown *Isochrysis galbana* (Tahitian isolate)
Test Type	Static, non-renewal
Test Duration	72 hr
Test End-point	Growth (cell yield)
Test Temperature	25 or 28 $\pm$ 1 ^{o}C
Test Chamber Size/Volume	6 mL in 20 mL scintillation vials
Source of Test Organisms	Laboratory culture, 5 to 6 days in age
Test Concentrations	100, 50, 25, 12.5 and 6.3%, plus FSW Controls
Test Acceptability Criteria	Control cell yield > 2×10^5 cells per mL after 72 hr. Variability in the controls less than 20%

Table 3: Summary of Test Conditions for the Sea Urchin Fertilisation Success Test – Based on APHA Method 8810c, Modified for *H. tuberculata* by Simon and Laginestra (1997)

Test Species	Sea urchin, *Heliocidaris tuberculata*
Test Type	Static, non-renewal
Test Duration	1 hr spermatozoa exposure plus 20 minutes fertilisation time
Test End-point	Fertilisation success
Test Temperature	20 $\pm$ 1 ^{o}C
Test Chamber Size/Volume	5 mL in 9 mL tissue culture tube
Source of Test Organisms	Field collection, Sydney coastal region
Test Concentrations	100, 50, 25, 12.5 and 6.3%, plus FSW Controls
Test Acceptability Criteria	>70% fertilisation in FSW controls, reference toxicant results within prescribed range

Table 4: Summary of Test Conditions for the Sea Urchin Larval Development Test – Based on APHA Method 8810D and Simon and Laginestra (1997)

Test Species	Sea urchin, *Heliocidaris tuberculata*
Test Type	Static, non-renewal
Test Duration	72 hr
Test End-point	Normal pluteus larvae
Test Temperature	20 $\pm$ 1 ^{o}C
Test Chamber Size/Volume	5 mL in 9 mL tissue culture tube
Source of Test Organisms	Field collection, Sydney coastal region
Test Concentrations	100, 50, 25, 12.5 and 6.3%, plus FSW Controls
Test Acceptability Criteria	$\geq$ 70% normal larvae in FSW controls, reference toxicant results within prescribed range

Table 5: Summary of Test Conditions for the Rock Oyster Larval Development Test – Based on Krassoi et al. (1997)

Test Species	Rock oyster, *Saccostrea glomerata (commercialis)*
Test Type	Static, non-renewal
Test Duration	48 hr
Test End-point	Larval development to D-veliger stage
Test Temperature	25 ± 1 ^{o}C
Test Chamber Size/Volume	5 mL in 9 mL tissue culture tube
Source of Test Organisms	Oyster farms in NSW or Queensland.
Test Concentrations	100, 50, 25, 12.5 and 6.3%, plus FSW Controls.
Test Acceptability Criteria	≥ 70% normal larvae in FSW controls, reference toxicant results within prescribed range.

Table 6: Summary of Test Conditions for the Larval Fish Imbalance Test – Based on USEPA 1993 and OECD Method 203

Test Species	Barramundi, *Lates calcarifer*
Test Type	Static, non-renewal
Test Duration	96 hr
Test End-point	Imbalance, including survival
Test Temperature	28 ^{o}C
Test Chamber Size/Volume	200 mL in 250 mL borosilicate glass beakers
Source of Test Organisms	Hatchery reared, South Australia. 9 days post-hatch, ~ 4mm
Test Concentrations	100, 50, 25, 12.5 and 6.3%, plus FSW Controls
Test Acceptability Criteria	≥ 80% survival in FSW controls, reference toxicant results within prescribed range

Table 7: Summary of Test Conditions for the Acute Toxicity Test with the Juvenile Tiger Prawn – Based on USEPA OPPTS 850.1045

Test Species	Tiger Prawn, *Penaeus monodon*
Test Type	Static, non-renewal
Test Duration	96 hr
Test End-point	Survival of larvae
Test Temperature	28 ^{o}C
Test Chamber Size/Volume	200 mL in 250 mL borosilicate glass beakers
Source of Test Organisms	Hatchery reared, Queensland. 15 day post-larvae
Test Concentrations	100, 50, 25, 12.5 and 6.3%, plus FSW Controls
Test Acceptability Criteria	≥ 80% survival in FSW controls, reference toxicant results within prescribed range

Table 8: Summary of Test Conditions for the Acute Toxicity Test with the Juvenile Beach Worm – Based on Generic Methods given in USEPA 2002

Test Species	Beach worm, *Diopatra dentata*
Test Type	Static, non-renewal
Test Duration	96 hr
Test End-point	Survival of juvenile worms
Test Temperature	28 ^{o}C
Test Chamber Size/Volume	200 mL in 250 mL borosilicate glass beakers
Source of Test Organisms	Hatchery reared, NSW. ~ 6 months, 15 to 30 mm
Test Concentrations	100, 50, 25, 12.5 and 6.3%, plus FSW Controls
Test Acceptability Criteria	≥ 80% survival in FSW controls, reference toxicant results within prescribed range

4.0 QUALITY ASSURANCE

Specific quality assurance (QA) procedures for undertaking toxicity testing activities, procurement and culturing of test organisms, maintenance and calibration of instruments, cleaning, chain of custody and sample handling procedures were in accordance with Ecotox Services Australasia standard laboratory procedures. Ecotox Services Australasia is the only NATA accredited laboratory doing WET testing in Australia. The urchin fertilisation and larval development tests, oyster larval development and fish larval imbalance tests are currently NATA accredited. The prawn mortality, polychaete mortality and microalgal growth inhibition tests are not specifically NATA accredited.

The following quality assurance procedures were undertaken for all toxicity tests. Tables 9 to 15 give specific QA controls for each of the laboratory toxicity tests. The acceptance criteria for each of these measures had to be met in order for tests to be considered valid. Tests that were invalid were repeated with fresh effluents and test organisms.

Table 9: Specific QA Control Criteria for the Microalgal Growth Inhibition Test

QA Measure	Acceptance Criteria
Control treatment cell yield	> 20,000 cells mL^{-1} at 72 hr
Control variability	CV < 20%
Artificial sea water control cell yield	Not significantly different to control treatment
Positive (copper) control	Within Cusum chart limits

Table 10: Specific QA Control Criteria for the Sea Urchin Fertilisation Success Test

QA Measure	Acceptance Criteria
Control treatment minimum % fertilised	≥ 70%
Artificial sea water control minimum % fertilised	≥ 70%
Reference toxicant test	Within Cusum chart limits

Table 11: Specific QA Control Criteria for the Sea Urchin Larval Development Test

QA Measure	Acceptance Criteria
Control treatment minimum % normal pluteus larvae	≥ 70%
Artificial sea water control minimum % normal	≥ 70%
Reference toxicant test	Within Cusum chart limits

Table 12: Specific QA Control Criteria for the Rock Oyster Larval Development Test

QA Measure	Acceptance Criteria
Control treatment minimum % normal D-veliger larvae	≥ 70%
Artificial sea water control	≥ 70%
Reference toxicant test	Within Cusum chart limits

Table 13: Specific QA Control Criteria for the Larval Fish Imbalance Test

QA Measure	Acceptance Criteria
Control treatment minimum % unaffected	≥ 80%
Artificial sea water control	≥ 80%
Reference toxicant test	Within Cusum chart limits

Table 14: Specific QA Control Criteria for the Acute Juvenile Tiger Prawn Test

QA Measure	Acceptance Criteria
Control treatment minimum % survival	≥ 80%
Artificial sea water control	≥ 80%
Reference toxicant test	Within Cusum chart limits

Table 15: Specific QA Control Criteria for the Acute Beach Worm Test

QA Measure	Acceptance Criteria
Control treatment minimum % survival	≥ 90%
Artificial sea water control	≥ 90%
Reference toxicant test	Within Cusum chart limits

4.1 Sample Tracking

Each simulated effluent was given a Laboratory ID Number for tracking whilst in the custody of Ecotox Services Australasia. ID numbers were documented on the following:

- Sample receipt logbooks and chain of custody forms.
- Toxicity test datasheets.
- Sample characteristics datasheets and other relevant laboratory datasheets.
- Printouts of statistical analyses of the test data.
- Test reports.

When sub-samples were required they had the identical ID number as the parent sample, unless annotated with alternate numbers in the Sample Receipt Log Books.

5.0 STATISTICAL ANALYSES

5.1 Estimates of LC/EC50 and LC/EC10

The concentration of each simulated whole effluent resulting in 50% mortality or effect in each bioassay (LC50 or EC50 respectively) was determined by the probit or trimmed Spearman-Karber method in TOXCALC V5.0 software. Similarly, the LC10 or EC10 estimates (10% mortality or effect) were determined using the probit analysis in the TOXCALC V5.0 software. Where data where unsuitable for probit analysis, the non-linear interpolation method in TOXCALC V5.0 was used to calculate IC10 equivalents instead; for most tests the interpolation method was actually used. Thus, IC10 estimates were calculated in most cases, but will be referred to hereafter as LC10 or EC10 estimates.

The highest concentration where toxicity was not detected statistically (No Observed Effect Concentration = NOEC) and the lowest concentration observed causing toxicity (Lowest Observed Effect Concentration = LOEC) were also determined using TOXCALC V5.0 software.

5.2 Comparison of Toxicity Among Whole Effluent Treatments and Subsequent Averaging Across Whole Effluent Treatments

An ANOVA was used to test for differences in overall toxicity of whole effluents, comparing the mean ranking of estimates of LC50 and EC50 from the seven bioassays among the twelve whole effluent treatments. LC50 and EC50 were used for this comparison because they are more precise measures of the toxicity of whole effluent treatments than LC10 or EC10. The distributions of EC50 and LC50 estimates within whole effluent treatments were quite obviously skewed and most transformations were unable to correct this. Consequently, the ANOVA was run on the ranks of the toxicity within bioassays (i.e. each whole effluent treatment was ranked 1–12 with increasing EC50 or LC50 for each bioassay and the averages of these compared among whole effluent treatments). Following a significant result in the main ANOVA, specific hypotheses regarding the comparative toxicity of the twelve simulated whole effluents were then to be tested using a Student-Newman-Keuls (SNK) multiple comparison test procedure (Winer et al. 1991). All ANOVA and subsequent hypothesis tests were done using SYSTAT V12.0 (SYSTAT Software Inc, USA).

Having failed to detect significant differences among the whole effluent treatments, each whole effluent treatment was then treated as one of twelve replicate tests for each bioassay that could be used to estimate LC10 or EC10 for a general simulated whole effluent. LC10 or EC10 estimates were then averaged within each bioassay to generate an estimate of the toxicity of the general whole effluent in each of the bioassays. These averages were used in the BurrliOZ Species Sensitivity Distribution (SSD) analysis, following the application of acute to chronic correction factors (see below). Following a

suggestion from the CSIRO review of the proposed protocols here, the two estimates of average EC10 for the urchin bioassays were also arithmetically averaged to ensure only one clearly independent datum was used for each species in the SSD analysis.

5.3 Tests of Appropriate Acute to Chronic Ratio (ACR) Correction Factors

Combining acute and chronic data in a SSD analysis such as BurrliOZ (below) requires that data measure toxicity effects in an equivalent way. Common practice is therefore to scale acute data by some correction factor (an Acute to Chronic Ratio, or ACR) to normalise the acute data to match chronic data. What ACR correction factor should be applied, however, is unclear in most cases. Review comments on the proposed protocol from the CSIRO suggested that a low ACR (1–2.5) is probably appropriate, but that a range of correction factors should be assessed. DEC had suggested previously that an ACR as large as 10 might be appropriate; however, ACR larger than two were found to be inappropriate for these data on a number of grounds.

Implicitly, the goal of an appropriate ACR correction factor is that the distribution of adjusted LC50s should be the same as the distribution of EC50s. Thus, this can be tested explicitly by testing the hypothesis that the mean of acute data scaled by the ACR was equal to the mean of chronic data. This was done by first running an ANOVA comparing the three (averaged) LC50s from acute bioassays with the three (averaged) EC50s from the chronic bioassays, which tested the appropriateness of an ACR = 1. Subsequently, hypotheses based on the same ANOVA tested whether the mean of acute bioassay LC50 divided by an ACR correction factor of 10, 3, 2.5 or 2 were equal to the mean of the chronic bioassays.

5.4 BurrliOZ Species Sensitivity Distribution (SSD) Analysis

The BurrliOZ computer program (Campbell et al. 2000) was used to determine median 99, 95 and 90% species protection concentrations (estimates of PC99, PC95 and PC90), based on fits of the cumulative distributions of species sensitivity with varying concentration of simulated whole effluent to a Burr type III distribution (Shao 2000).

Arithmetically averaged estimates of EC10 were used for the model fitting, after correcting the data from the three acute bioassays using ACR correction factors of 1 or 2 (the latter applied in two ways, see below). The two averaged EC10 estimates from the urchin bioassays were also averaged so that only one datum was used for each species when fitting models.

Two methods of combining data from the acute and chronic tests were applied. Initially, acute and chronic data were combined by adjusting LC10 data from acute tests by the ACR correction factor to treat them as equivalent to the EC10 from chronic tests. Thus for ACR = 2, LC10 data were divided by 2. For an ACR = 1, LC10 and EC10 data

were simply combined. This approach is logical given that the basis of a SSD analysis is to identify the location of cumulative sensitivity curves along a toxicant concentration axis; the adjustment by the ACR factor then simply shifts the location of the distribution of acute data to the left by an amount not rejected in tests described in Section 5.3. As pointed out in the review by CSIRO, however, ANZECC/ARMCANZ (2000) guidelines actually suggest equivalent EC10 estimates be derived from acute data by dividing LC50 estimates by the ACR correction factor. While LC/EC50 are more precise measures of the location of a distribution, the logic behind combining different endpoints in this second method is less clear. However, because this second method is accepted practice and was recommended by the CSIRO reviewer, this method of correction was applied in a second round of SSD model fitting for the largest correction factor found applicable with these data (ACR = 2).

In all model fitting 95% confidence interval estimates for the estimates of median PC99, PC95 and PC90 were generated by a bootstrapping process. Pseudo-variances for estimates of PC99, PC95 and PC90 were estimated by manually rerunning the BurrliOZ program, each time with a different one of the six bioassay EC10 data removed from dataset. Variance among the six repeated estimates thus generated were used in calculating a lower 95% confidence interval estimate for PC99, PC95 and PC90, based on a two-tailed t-distribution (Manly 1997).

5.5 Alternative Method of Determining Species Protection Concentrations

A clear feature in many of the bioassays was dilution series in which there were essentially 'all or nothing' responses to simulated whole effluents. That is, in many bioassays a large response would be observed at one concentration, with no response observed the next dilution down the series, such that the LC/EC50 and LC/EC10 would both be between the LOEC and NOEC. As suggested in the CSIRO review, even though the data here were planned and collected using standard methods and dilution series, and also averaged across twelve bioassays, in effect, the data were not really continuous. As such, the data were not ideal for use in SSD analyses like the BurrliOZ program. Therefore, an alternative, conservative, method of determining a species protection concentration suggested in the review by CSIRO was also adopted. That method simply was to determine the minimum NOEC for any bioassay, and then apply a safety factor of ten to that concentration.

6.0 RESULTS

6.1 Toxicity Test Results

Summary reports from ESA for each of the seven bioassays are presented in Appendix 2 for all twelve simulated whole effluents. Full reports from ESA of each of bioassays for each whole effluent are given in Appendix 3. Salinities of stock whole effluents ranged from 60.9 to 61.6 ppt. The measured pH in all tests ranged between 7.8 and 8.3. All tests met QA control criteria.

In nearly all cases the bioassay data were not appropriate for generating an EC10 or LC10 using probit analysis (see Appendix 3 for details of each whole effluent/bioassay), so IC10 equivalent estimates were calculated instead using the non-linear interpolation method. Estimates of NOEC, LOEC, LC/EC50 and LC/EC10 are shown here for each whole effluent for the microalgal growth inhibition bioassay (Table 16), the urchin fertilisation bioassay (Table 17), the urchin larval development bioassay (Table 18), the oyster larval development bioassay (Table 19), the fish imbalance bioassay (Table 20), the prawn mortality bioassay (Table 21) and the polychaete mortality bioassay (Table 22).

Table 16: NOEC, LOEC, EC50 and EC10 Estimates for the Microalgal Growth Inhibition Bioassay

Simulated Whole Effluent	NOEC	LOEC	EC50	EC10
1) Brine only (no other additives)	25	50	72.4	36.7
2) Veolia Brine Effluent + Backwash	25	50	68.3	34.7
3) Veolia Brine Effluent + Backwash + CIP (variant a)	25	50	73.1	40.6
4) Veolia Brine Effluent + Backwash + CIP (variant d)	25	50	70.4	35.1
5) ITT Brine Effluent + Backwash	25	50	69.1	32.9
6) ITT Brine Effluent + Backwash + CIP (variant b)	25	50	72.4	39.0
7) Osmoflo Brine Effluent + Backwash	25	50	65.2	32.4
8) Osmoflo Brine Effluent + Backwash + CIP (variant c)	25	50	74.5	35.5
9) Osmoflo Brine Effluent + Backwash + CIP (variant d)	25	50	68.1	34.1
10) GE Brine Effluent + Backwash	25	50	64.7	31.7
11) GE Brine Effluent + Backwash + CIP (variant e)	25	50	71.0	34.5
12) Backwash only (NaClO 0.53 mg L^{-1})	25	50	70.9	35.4

Units are percentage of stock whole effluent diluted in sea water

Table 17: NOEC, LOEC, EC50 and EC10 Estimates for the Urchin Fertilisation Bioassay

Simulated Whole Effluent		NOEC	LOEC	EC50	EC10
1)	Brine only (no other additives)	25	50	35.3	26.2
2)	Veolia Brine Effluent + Backwash	25	50	34.8	26.2
3)	Veolia Brine Effluent + Backwash + CIP (variant a)	25	50	35.4	26.3
4)	Veolia Brine Effluent + Backwash + CIP (variant d)	25	50	35.4	26.3
5)	ITT Brine Effluent + Backwash	25	50	35.2	26.2
6)	ITT Brine Effluent + Backwash + CIP (variant b)	25	50	35.4	26.3
7)	Osmoflo Brine Effluent + Backwash	25	50	35.4	26.3
8)	Osmoflo Brine Effluent + Backwash + CIP (variant c)	25	50	35.4	26.3
9)	Osmoflo Brine Effluent + Backwash + CIP (variant d)	25	50	35.2	26.2
10)	GE Brine Effluent + Backwash	25	50	35.6	27.2
11)	GE Brine Effluent + Backwash + CIP (variant e)	25	50	36.2	27.9
12)	Backwash only (NaClO 0.53 mg L^{-1})	25	50	35.5	27.1

Units are percentage of stock whole effluent diluted in sea water.

Table 18: NOEC, LOEC, EC50 and EC10 Estimates for the Urchin Larval Development Bioassay

Simulated Whole Effluent		NOEC	LOEC	EC50	EC10
1)	Brine only (no other additives)	25	50	35.4	26.5
2)	Veolia Brine Effluent + Backwash	25	50	35.4	26.5
3)	Veolia Brine Effluent + Backwash + CIP (variant a)	25	50	35.4	26.5
4)	Veolia Brine Effluent + Backwash + CIP (variant d)	25	50	35.2	26.4
5)	ITT Brine Effluent + Backwash	25	50	35.0	26.2
6)	ITT Brine Effluent + Backwash + CIP (variant b)	25	50	35.4	26.5
7)	Osmoflo Brine Effluent + Backwash	25	50	35.0	26.4
8)	Osmoflo Brine Effluent + Backwash + CIP (variant c)	25	50	34.8	26.2
9)	Osmoflo Brine Effluent + Backwash + CIP (variant d)	25	50	35.1	26.2
10)	GE Brine Effluent + Backwash	25	50	35.1	26.3
11)	GE Brine Effluent + Backwash + CIP (variant e)	25	50	35.1	26.2
12)	Backwash only (NaClO 0.53 mg L^{-1})	25	50	35.2	26.4

Units are percentage of stock whole effluent diluted in sea water.

Table 19: NOEC, LOEC, EC50 and EC10 Estimates for the Oyster Larval Development Bioassay

Simulated Whole Effluent	NOEC	LOEC	EC50	EC10
1) Brine only (no other additives)	25	50	34.9	25.5
2) Veolia Brine Effluent + Backwash	25	50	35.4	25.6
3) Veolia Brine Effluent + Backwash + CIP (variant a)	25	50	34.7	25.4
4) Veolia Brine Effluent + Backwash + CIP (variant d)	25	50	33.5	25.3
5) ITT Brine Effluent + Backwash	25	50	34.2	25.5
6) ITT Brine Effluent + Backwash + CIP (variant b)	25	50	35.4	25.6
7) Osmoflo Brine Effluent + Backwash	25	50	34.0	25.4
8) Osmoflo Brine Effluent + Backwash + CIP (variant c)	25	50	35.2	25.5
9) Osmoflo Brine Effluent + Backwash + CIP (variant d)	25	50	35.4	25.6
10) GE Brine Effluent + Backwash	25	50	35.0	25.6
11) GE Brine Effluent + Backwash + CIP (variant e)	25	50	35.4	25.6
12) Backwash only (NaClO 0.53 mg L^{-1})	25	50	34.2	25.5

Units are percentage of stock whole effluent diluted in sea water.

Table 20: NOEC, LOEC, LC50 and LC10 Estimates for the Fish Imbalance Bioassay

Simulated Whole Effluent	NOEC	LOEC	LC50	LC10
1) Brine only (no other additives)	25	50	35.4	32.6
2) Veolia Brine Effluent + Backwash	25	50	35.4	32.6
3) Veolia Brine Effluent + Backwash + CIP (variant a)	25	50	35.4	32.6
4) Veolia Brine Effluent + Backwash + CIP (variant d)	25	50	50.0	42.4
5) ITT Brine Effluent + Backwash	25	50	35.4	32.6
6) ITT Brine Effluent + Backwash + CIP (variant b)	50	100	42.1	40.1
7) Osmoflo Brine Effluent + Backwash	25	50	29.7	22.8
8) Osmoflo Brine Effluent + Backwash + CIP (variant c)	25	50	34.2	26.1
9) Osmoflo Brine Effluent + Backwash + CIP (variant d)	25	50	35.4	32.6
10) GE Brine Effluent + Backwash	25	50	35.4	32.6
11) GE Brine Effluent + Backwash + CIP (variant e)	25	50	35.4	32.6
12) Backwash only (NaClO 0.53 mg L^{-1})	25	50	35.4	32.6

Units are percentage of stock whole effluent diluted in sea water.

Table 21: NOEC, LOEC, LC50 and LC10 Estimates for the Prawn Mortality Bioassay

Simulated Whole Effluent	NOEC	LOEC	LC50	LC10
1) Brine only (no other additives)	50	100	56.6	27.6
2) Veolia Brine Effluent + Backwash	50	100	50.5	11.5
3) Veolia Brine Effluent + Backwash + CIP (variant a)	50	100	63.7	38.4
4) Veolia Brine Effluent + Backwash + CIP (variant d)	50	100	57.8	24.1
5) ITT Brine Effluent + Backwash	25	50	49.3	5.8
6) ITT Brine Effluent + Backwash + CIP (variant b)	25	50	39.4	29.0
7) Osmoflo Brine Effluent + Backwash	50	100	58.9	35.0
8) Osmoflo Brine Effluent + Backwash + CIP (variant c)	25	50	40.9	26.1
9) Osmoflo Brine Effluent + Backwash + CIP (variant d)	25	50	53.4	31.5
10) GE Brine Effluent + Backwash	50	100	70.7	52.9
11) GE Brine Effluent + Backwash + CIP (variant e)	50	100	57.1	33.3
12) Backwash only (NaClO 0.53 mg L^{-1})	50	100	59.7	36.6

Units are percentage of stock whole effluent diluted in sea water.

Table 22: NOEC, LOEC, LC50 and LC10 Estimates for the Polychaete Mortality Bioassay

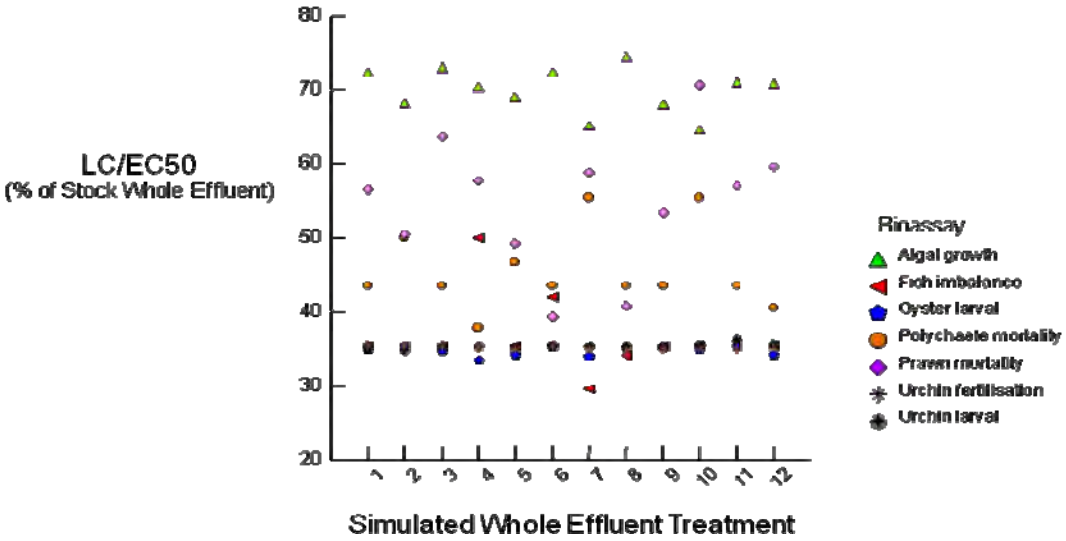
Simulated Whole Effluent	NOEC	LOEC	LC50	LC10
1) Brine only (no other additives)	25	50	43.5	40.6
2) Veolia Brine Effluent + Backwash	25	50	50.0	42.4
3) Veolia Brine Effluent + Backwash + CIP (variant a)	25	50	43.5	40.6
4) Veolia Brine Effluent + Backwash + CIP (variant d)	25	50	37.9	28.0
5) ITT Brine Effluent + Backwash	25	50	46.7	41.5
6) ITT Brine Effluent + Backwash + CIP (variant b)	25	50	43.5	40.6
7) Osmoflo Brine Effluent + Backwash	25	50	55.5	29.5
8) Osmoflo Brine Effluent + Backwash + CIP (variant c)	25	50	43.5	41.0
9) Osmoflo Brine Effluent + Backwash + CIP (variant d)	25	50	43.5	44.1
10) GE Brine Effluent + Backwash	25	50	55.5	44.1
11) GE Brine Effluent + Backwash + CIP (variant e)	25	50	43.5	40.6
12) Backwash only (NaClO 0.53 mg L^{-1})	25	50	40.6	39.6

Units are percentage of stock whole effluent diluted in sea water.

6.2 Differences among Bioassays and Whole Effluents

There were clear differences in the variation in estimates of LC50 or EC50 among simulated whole effluents for the different bioassays (Figure 1). EC50 estimates from the urchin fertilisation, urchin larval and oyster larval bioassays were all consistently low across the twelve whole effluents. The LC50 values from the fish imbalance bioassays

were mostly low for whole effluents, however, there was slightly more variation in the response of the fish to the different effluents than exhibited by the urchins and oysters. In contrast, the EC50 estimates from the algal growth bioassay were high but moderately variable across the whole effluents. The LC50 for the prawn and polychaete mortality bioassays were generally medium to high, but both varied greatly among the whole effluents. For the prawn mortality bioassay, for example, estimates of LC50 ranged from 71% to 39% of stock whole effluent. Similarly, estimates of LC50 for the polychaete mortality bioassay ranged from 56% to 38% of stock whole effluent. Ranges for each bioassay are illustrated in Tables 16 to 22.



Whole Effluent Treatment Codes (1-12) refer to the Treatments detailed In Section 3.4: 1) Brine Only; 2) Veolia Brine Effluent + Backwash; 3) Veolia Brine Effluent + Backwash + CIP (Variant I); 4) Veolia Brine Effluent + Backwash + CIP (Variant Iv); 5) ITT Brine Effluent + Backwash; 6) ITT Brine Effluent + Backwash + CIP (Variant Ii); 7) Osmoflo Brine Effluent + Backwash; 8) Osmoflo Brine Effluent + Backwash + CIP (Variant Iii); 9) Osmoflo Brine Effluent + Backwash + CIP (Variant Iv); 10) GE Brine Effluent + Backwash; 11) GE Brine Effluent + Backwash + CIP (Variant V); 12) Backwash Only (NaClo 0.53mgL^{-1}).

Figure 1: Scatterplot of Estimates of LC/EC50 for Twelve Whole Effluents across Seven Bioassays

There was no indication that any whole effluent was consistently more toxic across bioassays (Figure 1). Prior to testing this statistically, the toxicities of whole effluents for each bioassay were rank-transformed (ranked 1–12) to comply with the ANOVA assumption of normality. ANOVA then comparing average ranked toxicity among whole effluents did not detect any significant differences ($F_{11, 71} = 0.93$, $MS_{error} = 11.15$, $p = 0.52$).

6.3 Tests of Different ACR Correction Factors

The mean of estimates of LC/EC50 for the acute bioassays was not detected as different from the mean of the chronic bioassays for ACR = 1 and ACR = 2, however significant differences ($p < 0.05$) were found for ACR = 2.5, ACR = 3 and ACR = 10 (Table 23). Thus, ACR = 2.5, 3 and 10 were rejected as appropriate correction factors.

Table 23: Specific Tests of Different Acute to Chronic Ratio (ACR) Correction Factors applied to LC/EC50 Bioassay Data

ACR	Hypothesis Tested	F-ratio	Mean Square Error	p-value	Rejected
1	Mean acute bioassays = Mean chronic bioassays	0.03	215.9	0.88	No
2	0.5 x mean acute bioassays = mean chronic bioassays	6.13	215.9	0.06	No
2.5	0.4 x mean acute bioassays = mean chronic bioassays	9.98	215.9	0.03	Yes
3	0.33 x mean acute bioassays = mean chronic bioassays	13.21	215.9	0.02	Yes
10	0.1 x mean acute bioassays = mean chronic bioassays	28.20	215.9	0.003	Yes

6.4 BurrliOZ Model Fitting and Species Protection Concentrations

Mean estimates of LC/EC10 and LC/EC50 for each bioassay are shown in Table 24.

Table 24: Estimates of the Mean LC/EC10 and their Standard Errors (SE) for each of the Bioassays Tested in the WET Testing

Bioassay	Type of Test	Mean LC/EC10 ± SE (% of stock whole effluent)	Mean LC/EC50 ± SE (% of stock whole effluent)
Urchin Larval	Chronic	26.4 ± 0.1	35.2 ± 0.1
Urchin Fertilisation	Chronic	26.6 ± 0.2	35.4 ± 0.1
Oyster Larval	Chronic	25.5 ± 0.1	34.8 ± 0.2
Algal Growth	Chronic	35.2 ± 0.7	70.0 ± 0.9
Fish Imbalance	Acute	32.7 ± 1.5	36.6 ± 1.4
Prawn Mortality	Acute	29.3 ± 3.5	54.8 ± 2.6
Polychaete Mortality	Acute	39.4 ± 1.5	45.6 ± 1.6

For each bioassay estimates of LC/EC10 were arithmetically averaged across the twelve whole effluent treatments.

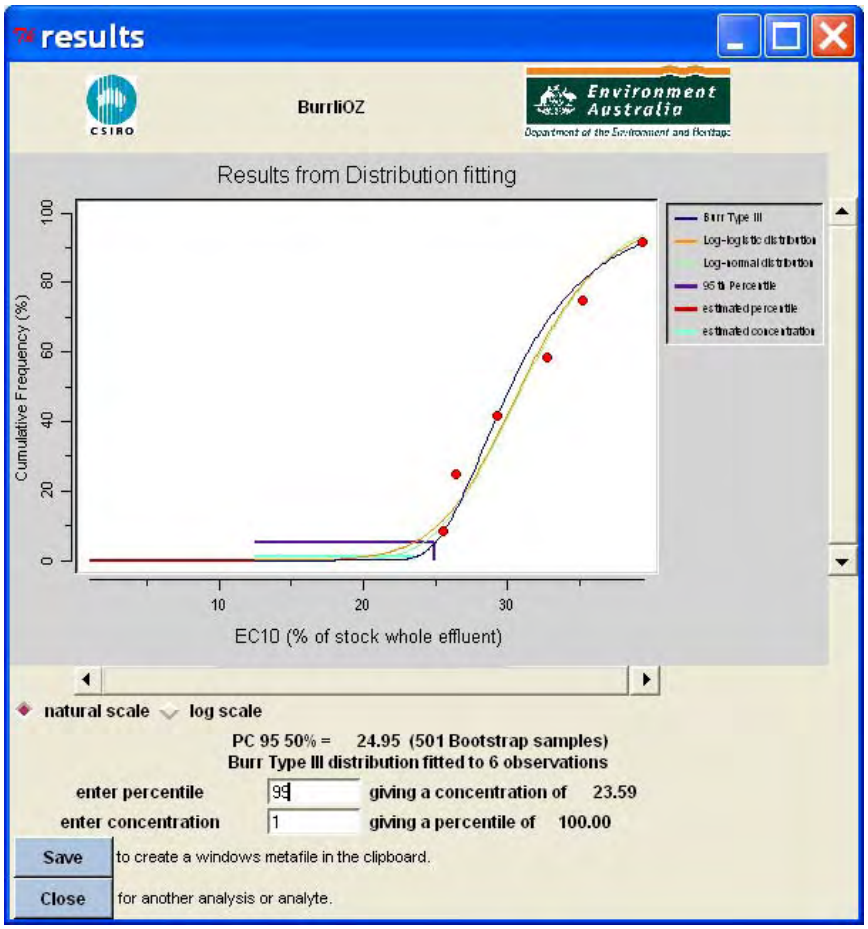
Mean ACR-corrected estimates of EC10, as used in the BurrliOZ SSD analysis, are shown in Table 25.

Table 25: Corrected Estimates of EC10 (% of Stock Whole Effluent) used in the BurrliOZ Model Fitting, after applying a Range of ACR Correction Factors to either Acute LC10 or LC50 Data

Bioassay	Type of Bioassay	ACR = 1	ACR = 2 (adjusting LC10 method)	ACR = 2 (adjusting LC50 method)
Urchin (averaged)	Chronic	26.5	26.5	26.5
Oyster Larval	Chronic	25.5	25.5	25.5
Algal Growth	Chronic	35.2	35.2	35.2
Fish Imbalance	Acute	32.7	16.3	18.3
Prawn Mortality	Acute	29.3	14.7	27.4
Polychaete Mortality	Acute	39.4	19.7	22.8

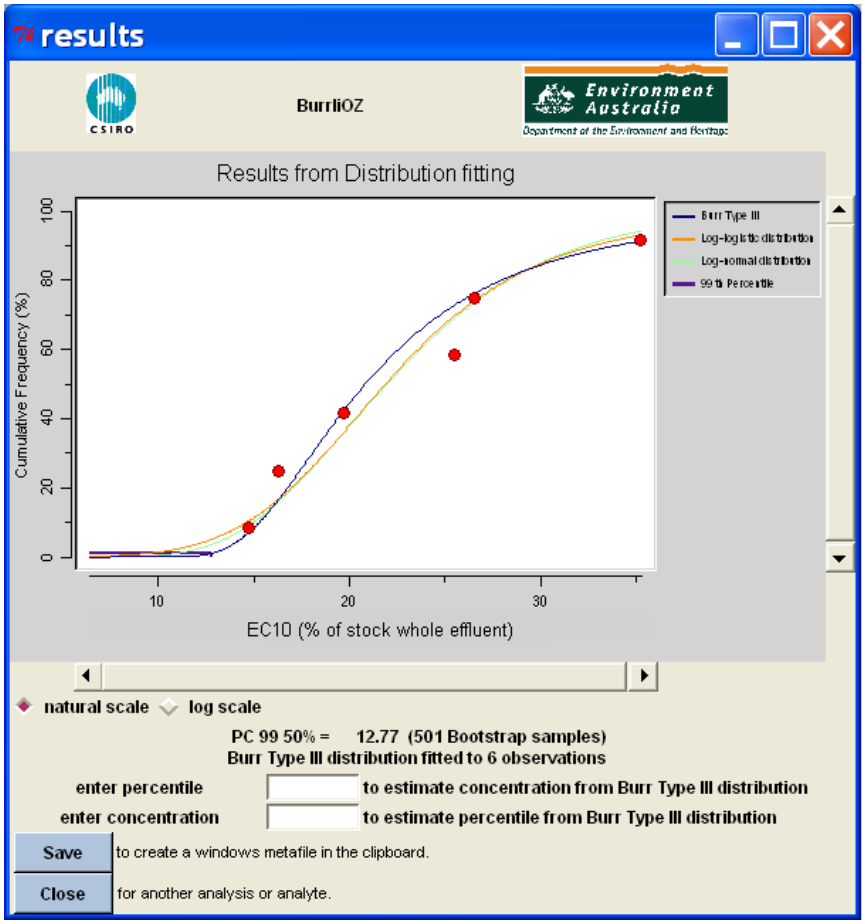
Mean estimates for the urchin egg fertilisation and larval development bioassays were arithmetically averaged to provide one estimate for urchins.

BurrliOZ models fitted using data corrected with different ACR correction factors produced (median) fitted distributions as shown in Figure 2 (ACR = 1, combining LC10 and EC10 data without adjustment), Figure 3 (adjusting LC10s by ACR = 2) and Figure 4 (adjusting LC50s by ACR = 2).



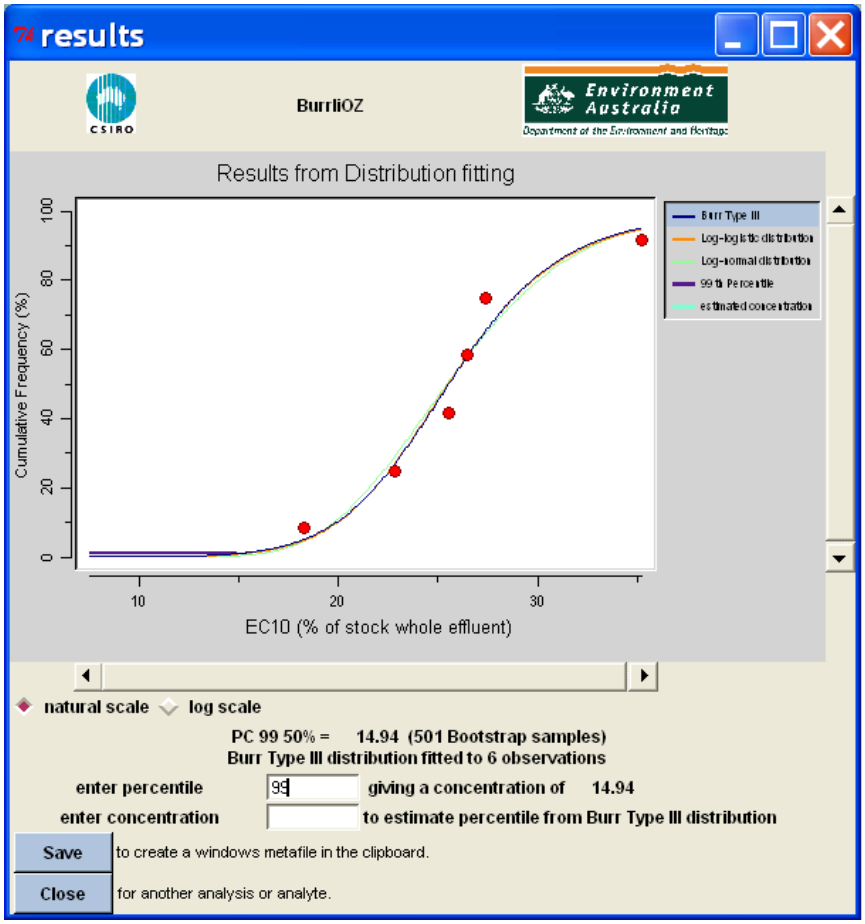
Species sensitivity distribution shown is the average EC10 across whole effluent treatments, calculated for each test. EC10 for the two urchins tests were averaged and represent only one datum in this distribution.

Figure 2: Output from the BurrliOZ Model Fitting Program (Campbell et al. 2000), simply Combining Acute and Chronic Data (i.e. ACR = 1)



Species sensitivity distribution shown is the average EC10 across whole effluent treatments, calculated for each bioassay. EC10 for the two urchins tests were averaged and represent only one datum in this distribution.

Figure 3: Output from the BurrliOZ Model Fitting Program (Campbell et al. 2000), applying an ACR Correction Factor of 2 to the LC10 data from the Fish, Prawn and Polychaete Bioassays



Species sensitivity distribution shown is the average EC10 across whole effluent treatments, calculated for each test. EC10 for the two urchins bioassays were averaged and represent only one datum in this distribution.

Figure 4: Output from the BurrliOZ Model Fitting Program (Campbell et al. 2000), applying an ACR Correction Factor of 2 to the LC50 data from the Fish, Prawn and Polychaete Bioassays

Bootstrap runs, each time removing a different datum from the dataset, were done for all six possible data combinations for the ACR = 2 (LC10-adjusted and LC50-adjusted) datasets. For the ACR = 1 dataset, however, the BurrliOZ program would not run for three data combinations because there was almost no variation in the data. For the ACR = 1 dataset then, only three bootstrap runs could be done and consequently the estimates of the standard error for that correction factor are based on only n = 3 runs.

Fits of the model appeared good for both the ACR = 1 and ACR = 2 corrected data sets. The median, upper 95% confidence interval and lower 95% confidence interval estimates of PC99, PC95 and PC90 are shown in Table 26. Conversion of the lower 95% confidence interval estimates of species protection concentrations into dilutions of stock whole effluent are given in Table 27.

Table 26: Median and 95% Confidence Limit Estimates of Species Protection Concentrations, Expressed as a Percentage of Stock (61 ppt) Whole Effluent

Species Protection Level	ACR = 1	ACR = 2*	ACR = 2†
PC99	23.6 (**16.2, 31.0**)	12.8 (**10.3, 15.2**)	14.9 (**14.2, 15.7**)
PC95	24.9 (**23.6, 26.3**)	14.3 (**12.9, 15.6**)	18.2 (**18.0,18.4**)
PC90	25.8 (**24.5, 27.1**)	15.3 (**14.1, 16.4**)	19.4 (**19.4, 20.4**)

For each species protection level and ACR correction factor, median estimates are shown above, and lower, upper 95% confidence estimates shown in brackets below. * LC10 data adjusted by ACR = 2 correction factor; † LC50 data adjusted by ACR = 2 correction factor.

Table 27: Lower 95% Confidence Interval Estimates of Species Protection Concentrations and Number of Dilutions Required of Stock Whole Effluent (61 ppt) to Achieve that Concentration

Species Protection Level	Lower 95% CI Estimate (% of Stock Whole Effluent)			Dilutions Required		
	ACR = 1	ACR = 2*	ACR = 2†	ACR = 1	ACR = 2*	ACR = 2†
PC99	16.2	10.3	14.2	6.2	9.7	7.0
PC95	23.6	12.9	18.0	4.2	7.8	5.6
PC90	24.5	14.1	19.4	4.1	7.1	5.2

* LC10 data adjusted by ACR = 2 correction factor; † LC50 data adjusted by ACR = 2 correction factor.

6.5 Lowest Observed NOEC

The NOEC was not greater than 25% of stock whole effluent in any of the eighty-four bioassays; 25% of stock effluent corresponds to a salinity of 42 ppt. For each species the minimum NOEC was 25% (Tables 16 to 22).

7.0 DISCUSSION

7.1 Lack of Detectable Differences in Toxicity among Simulated Whole Effluents

A clear pattern in the WET testing results was that for prawn mortality, polychaete mortality and algal growth bioassays, some estimates of LC/EC50 for some whole effluents appeared much lower than for others. Yet none of the estimates of LC/EC50 were consistently less for any whole effluent across the different bioassays. In contrast, estimates of LC/EC50 were much less variable among whole effluents for the urchin fertilisation and larval, oyster larval and fish imbalance bioassays. There are two possible interpretations of these patterns. First is that some whole effluents were differentially toxic to some species, but for other species there were no differences in the toxicity of whole effluents. Second, the differences among whole effluent treatments in LC/EC50 within bioassays simply reflected sampling error associated with making independent estimates of LC/EC50 in repeat tests, and that different bioassays had different levels of error associated with them. The first explanation cannot be ruled out without very extensive testing using replicate trials for each of the whole effluents for each toxicity bioassay, but seems unlikely. The second, simpler explanation appears much more likely. Some sampling error is always incurred whenever an estimate of a parameter like LC/EC50 is measured – such error needs to be accounted for by reporting measures of the precision of estimates like standard errors or 95% confidence intervals (but these must also be based on estimates of variance among independent replicated bioassays rather than error within single tests).

In any case, there was no consistent effect of a whole effluent treatment across a range of species, based on the comparison of the mean rankings of toxicity within the seven bioassays. Thus, this WET testing study failed to detect evidence that any of the twelve simulated RO whole effluents were more toxic than the others. Combining brine and sodium hypochlorite, or adding other vendor's processing or CIP chemicals, did not make a detectable difference to the toxicity of the simulated whole effluents. Consequently, none of the eight proposed hypotheses relating differences in toxicity to different types of whole effluent was supported. Instead, these data support a simple model that RO whole effluents were not any more toxic than either just concentrated brine (61 ppt) or sodium hypochlorite (0.53 mg L^{-1}) in sea water, regardless of what other pre-treatment, post-treatment or CIP chemicals were added.

Given the array of different pre-treatment, post-treatment and CIP chemicals that could be used in different combinations and concentrations in the final RO plant on Barrow Island, only a subset of the large number of potential whole effluents has been tested. There is always a possibility that one of those other untested mixtures might be a unique mix of brine and chemicals that for some reason is more toxic than the tested simulated whole effluents. The simulated whole effluents which were tested, however, were chosen to be representative of a range of different processes and potential

suppliers of the final system that will be installed at Barrow Island. Four different potential vendors' effluents were covered and for each a range of CIP treatments were assessed through WET testing. There was also no reason to expect that any of the tested whole effluents were especially benign (in fact, the opposite was true for some of the CIP variants chosen). Yet none of those whole effluents tested were found to be any more toxic than brine or sodium hypochlorite in sea water. Consequently, the results here do not provide any reason to suggest that unique synergistic toxicity effects in other untested whole effluents are very likely.

7.2 Choice of Appropriate Acute to Chronic Ratio (ACR) and Effect of this on Estimates of PC99

Estimates of PC99 depended on how the chronic and acute WET test data were combined in the BurrliOZ SSD analysis. The choice of ACR correction factor and how this was applied to the acute data made a difference to the estimate of the median PC99, with larger correction factors leading to smaller concentrations.

DEC suggested that an ACR correction factor of 10 might need to be applied to combine the acute with the chronic data in the BurrliOZ analysis. However, applying such a large ACR correction factor is not recent practice in other ongoing desalination WET testing studies; more commonly an ACR of one or two is applied, though often without explicit justification (Rick Krassoi, Ecotox Services Australasia, pers. comm.). The CSIRO reviewer similarly suggested that an ACR of 1–2.5 would be appropriate, but should be assessed on the basis of the WET test results. Statistical testing suggested that low ACR corrections should be applied to the data in this study.

There was direct evidence from the comparison of LC50 and EC50 estimates that an ACR = 2 (or 1) was an appropriate correction factor to apply to the acute data in the current study. Explicit tests of hypotheses that the means of acute and chronic bioassays would be equal when an ACR correction factor was applied were rejected for ACR = 10, 3 and 2.5, but not for ACR = 2 (or 1). Given the goal of applying an ACR correction factor is to normalise the distribution of the acute data to match the distribution of chronic data, these results suggest strongly that a ACR correction factor of no more than two should be applied to these data.

7.3 Alternative Estimate of PC99

As pointed out in the CSIRO review, there are some issues associated with using these data for a SSD analysis to generate a PC99. Although WET testing was carried out using standard methods and usual dilution series, in many cases the resolution of LC/EC10 or LC/EC50 from the bioassays was not great; effectively, the data were not continuous. In many bioassays there was an 'all or nothing' effect in the dilution series; i.e. large effects would be observed in one dilution, but no effects would be observed once the concentration of whole effluent was further diluted by a factor of two. Possibly, this

pattern was observed because most effects detected were simply impacts from osmotic changes, occurring once the salinity of test solutions reached a critical salinity of ~42 ppt. As such, the resolution of the data is constrained by the specific dilution series used in the bioassays, and are not really as expected for use in a SSD.

An alternative suggested by the CSIRO reviewer then, was to use the minimum NOEC for any species tested as an estimate of a likely safe concentration of whole effluent. Additionally, applying a safety factor of 10 to this minimum observed NOEC was suggested to account for the fact that only six species have been tested and that these almost certainly would not include the most sensitive species that could have been tested; noting however, that urchins were specifically included because of their known intolerance to changes in osmotic concentrations. For all six species, the minimum NOEC observed was 25% of stock whole effluent, corresponding to a solution with a salinity of ~ 42 ppt. Applying a safety factor of 10 to this estimate suggests a species protection concentration of 2.5% of stock whole effluent, at which most species should not be affected by the brine effluent. This equates to a 40-fold dilution of the effluent.

7.4 Implications for Future RO Desalination WET Testing Studies

As outlined in the literature review that accompanies this WET testing report, there is little published or freely available information available on the direct toxicity of desalination waste brine on marine species. WET testing currently is being done in association with the construction of a number of desalination plants across Australia, but as yet few WET test data are available in the public domain. However, results of one study for the Perth desalination plant are available and provide a good example of how the work presented here can also be used to inform better design and interpretation of future WET testing studies.

WET testing of brine collected from the Perth desalination plant found apparently very different levels of toxicity of waste brine between the two years of testing (Woodworth 2008). The median PC99 determined in 2006 (0.03% of stock brine = 3,333 dilutions) was much lower than when the same tests were repeated in 2007 with waste effluent collected from the same plant (PC99 = 6.6% = 15 dilutions), and was also lower than in earlier WET tests with simulated brine and pilot plant-produced waste brine (Woodworth 2008). Principally, there was a great disparity between the data collected 2006 and 2007 for bioassays involving copepods and macroalgal fertilisation. Differences in the EC10 estimates between years for the copepod test (2006: 0.12% brine; 2007: 16.8% brine) was attributed to differences in the length of exposure to brine in the test during 2006 and 2007, with the 2006 exposures (and thus data) dismissed as inappropriately long. No explanation was given, however, for the similarly large relative difference in EC10 estimates for macroalgal fertilisation (2006: 92.9% brine; 2007: 20.9% brine).

The estimate of PC99 from the Perth desalination study in 2007 is roughly similar to that estimated here for Barrow Island. However, the apparently large differences between years in that study made interpreting and justifying adjustments made to the 2007 results quite complex (Woodworth 2008). Results of the WET testing presented here for Barrow Island illustrate a possibly simple explanation for the large annual differences in the Perth desalination results. Some toxicity tests may be relatively variable and so if tests are repeated in different years, then estimates of PC99 would be expected to differ as a normal consequence of sampling error. Thus, the copepod (and macroalgal fertilisation) bioassays may be relatively imprecise and the differences between years perhaps simply reflect how different repeat runs are with these tests, rather than some important difference between years. Importantly, in neither year of the Perth desalination were estimates of the precision of the PC99 estimates calculated. It is therefore impossible to determine whether the difference between years was a real difference, or simply the result of sampling error. For this reason, estimates of the precision of estimates of PC99 should always be reported. One of the other outcomes from this work would be a recommendation that where few species are available for WET testing, such as in the Perth desalination study or perhaps future studies at Barrow Island, consideration should be given to repeating toxicity tests to increase the precision of estimates of PC99.

8.0 CONCLUSIONS

Ecotoxicological testing of the effects of a wide range of simulated whole effluents, on a wide array of trophic groups, indicated that the different types or combinations of waste streams make little difference to the toxicity of whole effluents likely to be discharged from the RO desalination plant on Barrow Island. Overall, the toxicity of the tested simulated whole effluents was low and chemical additives did not increase the toxicity above that of raw brine (61 ppt) or sodium hypochlorite (0.53 mg L^{-1}) in sea water. The results suggest that, as long as the concentrations of the chemicals remain the same as those assumed in the current study and no new classes of chemical are added, dilution of the brine discharge stream to 2.5% (a 40-fold dilution) will be sufficient to protect over 99% of the marine species in the waters off the east coast of Barrow Island.

Although the number of data used here in the BurrliOZ SSD analysis was relatively low (six), precise 95% lower confidence interval estimates were calculated for the median estimate of PC99 using an ACR correction factor of 1 or 2. When an ACR = 2 was applied to the acute LC50 data in accordance with ANZECC/ARMCANZ (2000) guidelines, the lower 95% CI estimate of PC99 was 14.2% of stock whole effluent solution, meaning a one in seven dilution in sea water would provide enough dilutions that less than 1% of species would be expected to suffer no more than a 10% impact. When an ACR = 2 correction factor was applied to the acute LC10 data, the lower 95% CI estimate of PC99 was 10.3% of stock whole effluent solution, suggesting that a one in ten dilution should provide enough dilutions that less than 1% of species would be expected to be affected. Note that these are lower confidence interval estimates and so are inherently conservative estimates of PC99. Taking into account some of the limitations in the underlying data, an alternative, more conservative approach for determining a PC99 based on applying a safety factor of 10 to the minimum observed NOEC suggested that 2.5% of stock whole effluent should be safe for 99% of species; this corresponds to a one in forty dilution of waste RO effluent in sea water.

This study is a prior assessment of the potential toxicity effects of RO desalination waste on the biota of Barrow Island. Because actual RO waste from Barrow Island is not available to WET test directly yet, a number of approximations had to be made, not least of which was the use of simulated whole effluents. Thus, one caveat to the conclusions here is that they are based on WET testing of brine created through laboratory-based distillation process of gentle heating and stirring over several weeks, which is a different process to producing brine rapidly through reverse osmosis in an industrial plant. Whether this would make a difference to the toxicity of whole effluents is unclear, but at least some differences are known in brine that gets produced between the desalination processes; for example, reverse osmosis is known to affect concentrations of some trace elements like Boron. Similarly, other components of whole effluent which might be released from a real RO plant may not be well mimicked in the laboratory simulated effluents – for example, residual organic and solid material which might be released as part of backwashing. The whole effluents simulated here also contained chemicals at concentrations predicted in the waste stream, as estimated

will be produced in normal running conditions. Those concentrations can only be estimated when a real plant is running at Barrow Island and specific RO processes and water quality can be measured directly, or at least the supplier and design of this plant is finalised and effluents collected from similar plants.

Notwithstanding the limitations noted above, as a prior assessment of the potential toxicity effects of RO desalination waste on the biota of Barrow Island this work suggests relatively low toxicity for the types of whole effluents that are expected will be released at Barrow Island. Once a plant is operating on Barrow Island it would be prudent to further WET test the whole effluents it produces.

9.0 REFERENCES

ANZECC/ARMCANZ (2000) National water quality management strategy, Australian and New Zealand guidelines for fresh and marine water quality. Australian and New Zealand Environment and Conservation Council, and Agriculture and Resource Management Council of Australia and New Zealand, Canberra, Australia.

Campbell E, Palmer MJ, Shao Q, Warne M StJ, and Wilson M (2000) BurrliOZ: a computer program for calculating toxicant trigger values for ANZECC and ARMCANZ.

Havenhand JN, Butler F, Thorndyke MC, and Williamson JE (2008) Near-future levels of Acidification Reduces Fertilization Success in a Sea Urchin. *Current Biology*, in press.

Krassoi R, Everett D, and Anderson I (1996) Protocol for using doughboy scallop *Chlamys asperrima* (Mollusca: Pectinidae) L. to test the sublethal toxicity of single compounds and effluents. National Pulp Mills Research Program Technical Report No. 17. CSIRO, Canberra.

Laedsgaard P, Byrne, M, and Anderson DT (1991) Reproduction of sympatric populations of *Heliocidaris erythrogramma* and *H. tuberculata* (Echinoidea) in New South Wales. Marine Biology 110, 359-374.

Lam K, and Morton B (2006) Morphological and mitochondrial-DNA analysis of the Indo-west Pacific rock oysters (Ostreidae: *Saccostrea* species). Journal of Molluscan Studies (2006) 72, 235–245.

Manly BF (1997) *Randomization, Bootstrap and Monte Carlo Methods in Biology. 2nd Edition.* Chapman and Hall/CRC Press, Boca Raton.

Shao Q (2000) Estimation for hazardous concentration based on NOEC toxicity data: an alternative approach. Environmetrics 11, 583-59.

Simon J, and Laginestra E (1997) Test for testing sublethal toxicity in effluents, using gametes of sea urchin *Heliocidaris tuberculata*. National Pulp Mills Research Program Technical Report No. 20, CSIRO, Canberra.

Stauber JL, Gunthorpe L, Deavin JG, Munday BL, Krassoi R, and Simon J (1996) Comparative toxicity of effluents from ECF and TCF bleaching of eucalypt kraft pulps. Appita 49, 184–188.

Winer BJ, Brown DR, and Michels KM (1991) *Statistical Principles in Experimental Design*. 3rd Edition. McGraw-Hill, New York, USA.

APPENDIX I

Review of Draft B Report by CSIRO and Subsequent Changes and Responses made by RPS

APPENDIX 1: Review of RPS Report: Whole Effluent Toxicity Testing of Simulated Reverse Osmosis Brine Effluents by Jenny Stauber

Background

CSIRO Centre for Environmental Contaminants Research was engaged by RPS Environment and Planning Pty Ltd to review a draft final report on whole effluent toxicity testing of simulated reverse osmosis brine effluents for the proposed Gorgon Development desalination plant, Barrow Island, WA. The report was prepared by RPS for Chevron Australia Pty Ltd. The following is a review of RPS Report No. M08504:2, Draft B, dated October, 2008.

CSIRO previously reviewed the proposed scope of works and toxicity testing protocols and found these to be appropriate for the aims of the project. Some of the suggested changes were subsequently incorporated into the test program, while other comments e.g. choice of test species and more clearly defining how the data would be analysed, could not be addressed retrospectively.

General Comments

Overall, the experimental design and toxicity testing was appropriate, well planned and clearly described in the report. The toxicity tests used are all well documented standard tests, incorporating good quality assurance procedures. The report is clear, concise and well written. However, there are a number of deficiencies in the toxicity test data analysis and interpretation, which are described in more detail below. Although these deficiencies are unlikely to affect the overall outcome and conclusions, they do need to be addressed if the regulator is to have confidence in the results.

Some important issues that require further analysis and comment include:

1. Problems with the raw data, especially the 'all or nothing' responses which make deriving appropriate LC/EC50 and LC/EC10 values very difficult. This suggests that the statistical analyses (and SSDs) used may not be appropriate to this data set and that the PC99(95) values may be less precise than implied in the report. An alternative safety factor approach (e.g. 0.1 x NOEC of the most sensitive species) may be more useful for this data than SSDs.

2. Non-standard approaches for application of the ACR have been used. The ACR is defined as 'the ratio of the acute EC50 to chronic NOEC.' (ANZECC/ARMCANZ, 2000). So the ACR should have been applied to the acute LC50 or EC50, to get a chronic NOEC value or equivalent. These data should then be combined with chronic NOECs (or EC10s) in the SSD (if SSDs are to be used at all). The authors have instead applied the ACR to acute LC10 data, which is a different, more conservative approach, the justification for which is not explicitly stated.

3. The larval fish imbalance test is an **acute, sub-lethal** test, not a chronic or chronic surrogate test. Because it is only a 96-h exposure, following ANZECC/ARMCANZ(2000) guidelines, fish tests that are <7 days (even larval ones) are not considered to be chronic tests. An ACR needs to also be applied to the fish data before use in the SSD (if used).

These and other issues are discussed in more detail below, section by section.

Methods

Rationale for the WET Testing Program

Given that there was no existing effluent for WET testing, simulated laboratory-made effluents, to cover a range of discharge compositions and scenarios, were used. The authors are well aware of the limitations of this approach, which includes an inability to mimic the reverse osmosis process, and lack of inclusion of other constituents such as organics and particulate material. In particular, if solids and supernatant (and CIP) from the pre-treatment plant are to be disposed of together with the saline concentrate, then the composition and toxicity of the final discharge may be different to that assessed here. An alternative approach is to use an existing effluent from another RO desalination plant e.g. Perth desalination plant effluent has recently been used successfully to assess the potential toxicity of the proposed Wonthaggi desalination plant in Victoria. However, this too has limitations, as outlined in the report. The simulated effluents represent a good compromise and are probably the best that was possible, given that an actual desalination plant discharge was not yet available. **It will be important to actually test the toxicity of the Gorgon development desalination plant discharge during commissioning and operational phases, as part of an ongoing monitoring program, to confirm the PC99(95) dilution estimates.**

Choice of Test Species

Most of toxicity test species used are relevant to the tropical waters of WA, except the sea urchin *Heliocidaris tuberculata*, which is a temperate species with distribution in SE Australia. While I supported its use as a surrogate for local sea urchins, my initial comments suggesting that it was unnecessary to include both the fertilisation endpoint and larval development endpoint, have been confirmed. Both tests gave the same response, with the same EC50, EC10 and NOECs, due to the nature of the effluent i.e. both have the same salinity tolerance. The authors correctly used only one endpoint in the SSDs. Normally this would be the most sensitive endpoint of the two (according to ANZECC/ARMCANZ, 2000), but given their similarity, the authors have combined all the data and taken the mean. It is not clear whether the arithmetic or geometric mean was used. Usually geometric means are used when combining ecotoxicology data, but either in this case would be appropriate. I still believe it would have been better to invest the effort into adding another test species altogether, e.g. tropical zooplankton, to increase the number of usable data in the SSDs from 6 to 7 species, especially given that 8 different species are recommended in ANZECC/ARMCANZ (2000) (although 8 is not always practical).

Experimental Design and Bioassays

The experimental design was well planned and executed. Minor issues include:

1. It wasn't clear whether any pre-adjustment of the simulated effluents was required, e.g. pH and filtering. I had to search through the detailed appendices to find the final pH and salinity of each of the simulated effluents at each dilution. It would be good to include these basic water quality parameters in the body of the report.

2. It should have been made clear in Section 3.1 which tests were acute and chronic. This would have avoided the later error in the data manipulations. The larval fish imbalance test is an **acute, sub-lethal** test, not a chronic or chronic surrogate test. It measures imbalance which is a euphemism for survival in some respects to overcome animal ethics issues. Because it is only a 96-h exposure, following ANZECC/ARMCANZ(2000) guidelines, fish tests that are <7 days are not considered chronic. I have never seen fish 96-h tests, even larval ones, used as chronic tests in SSDs. International and national practice is that they should be treated as acute, sub-lethal tests only and have an ACR applied to their EC50s to derive a chronic value for use in risk assessment, SSDs, etc.

3. Table 6 – size and age of barramundi (larvae) should be stated.

4. Quality Assurance: State which tests are NATA accredited and which are not, otherwise it gives a false impression that all these toxicity tests were NATA accredited.

5. Table 9: What was the initial algal cell density used? This has a major impact on the sensitivity of algal growth tests. Also note that the algal test validity criteria here is >20,000 cells/ml at 72 h, however in Table 2, $>2x10^6$ cells/mL (100-fold higher is used) and in the actual test reports in the appendix, >60,000 cells/ml is the validity criteria. Which one is correct? The actual algal toxicity tests did not meet the upper limit of >2 x 10^6 cell/ml.

Statistical Analyses and Use of the Toxicity Test Data

There are many ways that the data could be analysed and some alternatives are discussed below.

One can argue whether NOECs or EC10s should be used in SSDs – there are advantages and disadvantages in using either estimate. NOECs may be inaccurate by a factor of 2 or 3 (depending on the dilution series used) and rely on good experimental design, with dilution intervals not exceeding 2 or 3 being standard practice. EC10s tend to have large confidence limits, as they are at the tail end of the concentration-response curve. They also correspond to a 10% adverse effect and therefore are not concentrations at which there is no effect. Their use then to derive discharge dilutions where there should be no effect (within a degree of uncertainty) is clearly flawed. I note that the authors, however, have been careful to allow for this by stating that the PC99s derived from this study correspond to no more than a 10% effect, rather than to say 'safe dilution', so this is commendable.

More problematic is the Acute to Chronic ratio (ACR). Following the ANZECC/ARMCANZ(2000) approach (page 8.3-30) and international practice, the ACR is defined as

'the ratio of the acute EC50 to chronic NOEC.'

So the ACR should have been applied to the acute LC50 or EC50, to get a chronic NOEC value or equivalent. These data should then be combined with chronic NOECs (or EC10s in this case) in the SSD. The authors have instead applied the ACR to acute LC10 data, which is a more conservative approach (and sometimes used) but is not strictly according to ANZECC/ARMCANZ (2000). This approach is creeping into use in Australia, without proper justification. This has been a particular problem in WET programs for the Perth, Sydney and Victorian desalination plants. It is not dissimilar to actually using an ACR of 10. According to ANZECC/ARMCANZ(2000) acute NOECs can be estimated from acute LC50s by dividing by 5. So applying an ACR of 2 to the acute LC10 is not dissimilar to applying an ACR of 10 to an acute

LC50 as per ANZECC/ARMCANZ(2000). However, in this case the raw data are problematic as EC10s and EC50s are similar.

The other problem is that the acute fish test was wrongly classified as a chronic test and no ACR was applied. While this probably won't affect the final outcome, **if SSDs are going to be used, then I recommend that all the SSDs be rerun using the three acute data (LC/EC50s) divided by an appropriate ACR, then combined with the three chronic EC10 or NOEC data as per international and national best practice.**

Choice of ACR: The justification for use of low ACRs (1 or 2) is that, assuming the toxicity is mainly due to osmotic shock, there is little difference in the acute and chronic effects of salt. However, all previous work on ACRs has been based on freshwater species exposed to mildly saline waters, not marine species exposed to hypersalinity. I had previously recommended that a range of ACRs be applied to acute EC50 data. The authors have done this (but applied it to the LC/EC10 instead), using a novel approach to determine an appropriate ACR. My only query is whether the objective should be to compare acute LC/EC50s with chronic EC50s, when the ACR is usually related to the chronic NOEC or EC10, not EC50. I understand that the author's approach makes sense intuitively, even though it doesn't strictly follow the ACR definition. **Is it possible to redo this comparison comparing the averaged LC50/EC50s from the 3 acute bioassays, with the averaged EC10s (or geometric mean of NOECs) from the 3 chronic bioassays and reach the same conclusion that an ACR of 10 is excessive?**

The authors have included estimates of variances for PC99, etc enabling better calculation of the 95% confidence limits around these estimates. This is to be commended as a statistically rigorous and appropriately conservative approach.

Results

While much emphasis has been placed on rigorous statistical analysis of the overall data, the same rigour has not been applied to the raw data used to generate the EC50 and EC10 values. There is little discussion or presentation of the actual ecotoxicity results in the body of the report. This is critical, as the raw data are problematic, not through any fault of the program design and execution, but as a consequence of the test organisms' 'all or nothing' responses to hypersalinity as a stressor.

Major issues

1. When you look at the Appendices in detail, it is obvious that for most tests and effluents (particularly the two sea urchin tests, the oyster tests and fish tests) there was an 'all or nothing response', i.e. either no effect was observed at 25% effluent and below, or complete mortality/inhibition was observed at 50% and 100% effluent. This is due to the fact that these species have a similar salinity tolerance – salinities greater than 43‰ completely inhibited these test endpoints. This means that the concentration response curves have no intermediate response, meaning that the usual statistics programs are not particularly suitable for calculating EC50s and EC10s. Compare, for example, the fish tests in effluent 11 and 12. In effluent 11 there was no adverse effect at 50% effluent and for effluent 12 there was 100% imbalance/mortality at the same 50% effluent dilution, yet the EC50 values were identical! Clearly drawing a line through 0 and 100 and trying to interpolate is not biologically meaningful. This means that the EC50s were all very similar and the confidence limits were narrow. Only the algal data (and some of the worm and prawn tests) gave an intermediate response. This is partly the reason the CLs appeared wider around the EC50/EC10 estimates in the algae and prawn tests.

This is a major issue for this data and should be discussed. Conclusions regarding the variability between the tests in Section 6.2 are therefore incorrect and this section needs to be re-written. In hindsight, additional dilutions around 75% effluent may have provided intermediate toxicity response data, but this could not have been forseen by the researchers, who used appropriate and standard dilution series.

2. Another problem with the lack of intermediate values in the concentration-response curves is that the EC10s and EC50s were very similar in many tests/effluents (fish, prawn and worm acute tests in particular), or in some cases the EC10 was higher than the EC50 which is ridiculous and shows up the errors in trying to use these standard statistics protocols. This was the case for the worm test (effluents 8 and 9) and the prawn test (effluent 8). More importantly, this also calls into question whether these EC10 data should be used at all in the SSDs. The NOEC data were usually 25 or 50% (i.e. all species were of similar sensitivity/salinity tolerance) and may have been better to use in the SSDs in this case. However, the problem then becomes that you have stacked NOEC data in the SSDs, with lots of results the same, so this causes bad curve fits and unreliable PC99 estimates. An alternative approach might be to not use SSDs at all, but apply a safety factor of 10 to the NOEC from the most sensitive species, to derive a 'safe' dilution.

3. This also means that it is difficult to compare the toxicity of one effluent with another, and one test endpoint to another, although I suggest that it appears salinity was the major issue (not additives) and that all species appear to have similar salinity tolerance to all effluents.

   All this taken together suggests that these ecotox data are not really suitable for standard statistical analyses or SSDs or at least their limitations should have been considered and discussed in the report. I recommend that instead of SSDs, the application of a safety factor (perhaps 10) to the lowest NOEC from the most sensitive species, to derive a 'safe' dilution. This 'safe' dilution could then be compared with the PC99(95) estimates from the corrected SSDs.

   None of this is likely to change the overall outcome of the study or the magnitude of the PC99s, but it is important these limitations of the data are considered in the broader context.

Discussion

Section 7.1: I don't think you can rule out either explanation in paragraph 1. It is well known that some species are more sensitive to certain toxicants than others e.g. algae and herbicides, chlorine. Usually looking at the reference toxicant data for each test can give some idea of which species are more sensitive to a particular class of compound, e.g. metals. However in this case, toxicity was probably due to osmotic effects and may be a real but small difference between species or just within-test variability. As the authors suggest, at least three definitive tests with each effluent and each species would be needed to begin to get a feel for between- test variability to properly interpret differences between effluents and tests.

Section 7.2: This gives the reader a false sense of security about the precision of the EC10 estimates. The problems with the lack of intermediate responses and the way the raw data are used to calculate the EC50 and EC10s from 'all or nothing' responses negates the implied precision.

I agree with the other conclusions in this section and 7.3.

Section 7.4: While it is tempting to make comparisons with the Perth desalination data, there were also some errors in the way these data were used to generate 'safe' dilutions, e.g from memory, EC10s were sometimes estimated by dividing EC50s by 5, which is incorrect as the concentration-response is rarely linear. Again, looking at how variable the response of each test is to a reference toxicant might help suggest whether the differences between years was due to real differences in the effluent or in the test itself.
The 99% species protection level is appropriate.

Conclusions

In general, this is a well designed and conducted test program which attempts to overcome the limitations of predicting effects before an actual effluent is available. There appears to be little difference in the toxicity of each effluent, and all species/endpoints showed similar sensitivity to salinity stress. The authors and researchers should be commended for their overall approach.

While much emphasis has been placed on rigorous statistical analysis of the overall data, the same rigour has not been applied to the raw data used to generate the EC50 and EC10 values. The problem of the concentration-response curves 'all or nothing' effects and how to best determine toxicity values from such curves has not been addressed in the report. Likewise, non-standard approaches for application of the ACR have been used. This suggests that there might be less precision and confidence in the final derived PC99(95) values than implied in the report.

To check this, I ran a few SSDs with various other combinations of the data (NOECs instead of EC10s, applying an ACR of 2 to the fish test, etc) and came out with similar PC99(95) values, with 11 to 34 fold dilutions required, so I suspect that all of the above comments won't make a large difference to the overall outcomes. I still question though whether these methods are appropriate to this data set, given the nature of the concentration-response data, which is currently buried in the appendices. Perhaps reverting back to the older safety factor approach (0.1 x most sensitive species NOEC) may be useful as a comparison, given the problematic data set.

I believe the authors will easily be able to address these issues and that this will both improve the final report and give the regulator further confidence in a well designed and executed study.

Table 1-1: Responses to CSIRO Review Comments on Whole Effluent Toxicity Testing of Simulated Reverse Osmosis Brine Effluents Draft B – October 2008

Changes Made	CSIRO Comments	RPS Response
	General Comments	
Section 5.5	1. Problems with the raw data, especially the 'all or nothing' responses which make deriving appropriate LC/EC50 and LC/EC10 values very difficult. This suggests that the statistical analyses (and SSDs) used may not be appropriate to this data set and that the PC99(95) values may be less precise than implied in the report. An alternative safety factor approach (e.g. 0.1 x NOEC of the most sensitive species) may be more useful for this data than SSDs.	Agreed. The suggested NOEC approach was also adopted in this revision, given the inherent limitations with some of these data.
Section 5.4	2. Non-standard approaches for application of the ACR have been used. The ACR is defined as 'the ratio of the acute EC50 to chronic NOEC.' (ANZECC/ARMCANZ, 2000). So the ACR should have been applied to the acute LC50 or EC50, to get a chronic NOEC value or equivalent. These data should then be combined with chronic NOECs (or EC10s) in the SSD (if SSDs are to be used at all). The authors have instead applied the ACR to acute LC10 data, which is a different, more conservative approach, the justification for which is not explicitly stated.	Logically, ACR corrections are about transforming the location of species distribution curves, and so should be applicable to converting either LC10 to an EC10 or LC50 to an EC50 equivalent. We explicitly measured how large this transformation could be based on the LC/EC50 data, finding an ACR = 2 appropriate. Transforming from a LC50 to an EC10 equivalent as per ANZECC/ARMCANZ (2000) guidelines, however, also assumes something about the shape of the cumulative distribution of species' sensitivities and that this is captured in the ACR. In our approach the magnitude/appropriateness of the ACR can be tested formally using the data; this cannot be tested using the ANZECC guideline approach. In response to this suggestion we included the ANZECC approach in the revised report. Just as the reviewer suggested, it led to a similar, if slightly larger estimate for the PC99(95). The NOEC method led to lower concentrations than either SSD estimates.
Section 3.1 and SSD calculations throughout	3. The larval fish imbalance test is an **acute, sub-lethal** test, not a chronic or chronic surrogate test. Because it is only a 96-h exposure, following ANZECC/ARMCANZ(2000) guidelines, fish tests that are <7 days (even larval ones) are not considered to be chronic tests. An ACR needs to also be applied to the fish data before use in the SSD (if used).	Agreed, changes made. The SSD and comparison of LC50 with EC50 were repeated, treating the fish data as acute.
	Choice of Test Species	
Section 5.2 (last line)	(about combining urchin data)... It is not clear whether the arithmetic or geometric mean was used. Usually geometric means are used when combining ecotoxicology data, but either in this case would be appropriate.	Arithmetic means were used to combine the two urchin data. In any case, both were very similar (confirming the reviewer's prediction about the likely similarity of the two urchin tests).

Changes Made	CSIRO Comments	RPS Response
	Experimental Design and Bioassays	
Section 6.1	It wasn't clear whether any pre-adjustment of the simulated effluents was required, e.g. pH and filtering. I had to search through the detailed appendices to find the final pH and salinity of each of the simulated effluents at each dilution. It would be good to include these basic water quality parameters in the body of the report.	Filtering or pH adjustment was not required. A line has been added to indicate the starting pH of test solutions.
Section 3.1; Tables 24, 25	It should have been made clear in Section 3.1 which tests were acute and chronic. This would have avoided the later error in the data manipulations. The larval fish imbalance test is an **acute, sub-lethal** test, not a chronic or chronic surrogate test. It measures imbalance which is a euphemism for survival in some respects to overcome animal ethics issues. Because it is only a 96-h exposure, following ANZECC/ARMCANZ(2000) guidelines, fish tests that are <7 days are not considered chronic. I have never seen fish 96-h tests, even larval ones, used as chronic tests in SSDs. International and national practice is that they should be treated as acute, sub-lethal tests only and have an ACR applied to their EC50s to derive a chronic value for use in risk assessment, SSDs, etc.	Agreed. The larval fish imbalance test has now been treated as an acute test throughout, and an ACR applied to the LC50 and LC10 for the SSD analysis. The dot point list of tests in Section 3.1 has been changed to indicate up front whether tests were considered acute or chronic. Tables 24 and 25 also indicate this.
Tables 6, 8	Table 6 – size and age of barramundi (larvae) should be stated.	Agreed. Added to Table 6, also size and age of polychaetes added to Table 8.
Section 4.0 (first paragraph)	Quality Assurance: State which tests are NATA accredited and which are not, otherwise it gives a false impression that all these toxicity tests were NATA accredited.	Both urchin tests, the oyster larval test and the fish imbalance are NATA accredited. This information has been added to the end of the first paragraph in Section 4.0.
Table 2; Appendix 3 (ESA report) ammended	Table 9: What was the initial algal cell density used? This has a major impact on the sensitivity of algal growth tests. Also note that the algal test validity criteria here is >20,000 cells/ml at 72 h, however in Table 2, $>2x10^6$ cells/mL (100-fold higher is used) and in the actual test reports in the appendix, >60,000 cells/ml is the validity criteria. Which one is correct? The actual algal toxicity tests did not meet the upper limit of >2 x 10^6 cell/ml.	The initial cell density at t=0 was 10,000 cells per mL. The correct criteria is >20,000 cells /mL at 72-hr; table 2 has been changed. The criteria given in the Test Report of 6 cells/0.1uL was a typo (it should have been 2) and has been re-issued with the correct value. Algal tests all met the criteria of 20,000 cells /ml.
	Statistical Analyses and Use of the Toxicity Test Data	
Section 5.4 Figure 4	The authors have instead applied the ACR to acute LC10 data, which is a more conservative approach (and sometimes used) but is not strictly according to ANZECC/ARMCANZ (2000).	As above for second point in General Comments. While we think the approach taken is valid, we have also repeated the analyses using the suggested ANZECC/ARMCANZ approach of correcting the LC50 by the ACR to generate an EC10 equivalent.

Changes Made	CSIRO Comments	RPS Response
Section 5.4 and calculations throughout	The other problem is that the acute fish test was wrongly classified as a chronic test and no ACR was applied. While this probably won't affect the final outcome, if SSDs are going to be used, then I recommend that all the SSDs be rerun using the three acute data (LC/EC50s) divided by an appropriate ACR, then combined with the three chronic EC10 or NOEC data as per international and national best practice.	Agreed. Have repeated the analyses, treating the fish data as acute. As suggested, it did not make a large difference to the overall results.
Section 6.3 (Table 23)	Is it possible to redo this comparison comparing the averaged LC50/EC50s from the 3 acute bioassays, with the averaged EC10s (or geometric mean of NOECs) from the 3 chronic bioassays and reach the same conclusion that an ACR of 10 is excessive?	Yes, this also has been done and we additionally tested whether an ACR = 2.5 was appropriate as well (it was not).
	Major Issues	
Section 7.3	1. When you look at the Appendices in detail, it is obvious that for most tests and effluents (particularly the two sea urchin tests, the oyster tests and fish tests) there was an 'all or nothing response'… This is a major issue for this data and should be discussed. Conclusions regarding the variability between the tests in Section 6.2 are therefore incorrect and this section needs to be re-written.	Agreed. This was an unavoidable shortcoming in these data. The dilution series run was a standard 1 in 2 dilutions, but for some of the tests it would have been good to have some additional dilutions; unfortunately, this could not be known before the tests were run. This point has now been noted (see Section 7.3), and the NOEC + safety factor approach to determining a safety concentration adopted (next comment). The conclusions about the variability are nonetheless still correct (there were still differences in the variability of the estimates derived from the tests), but the previous discussion about this in Section 7.3 has been removed for brevity.
Section 6.5	(points 2 and 3). All this taken together suggests that these ecotox data are not really suitable for standard statistical analyses or SSDs or at least their limitations should have been considered and discussed in the report. I recommend that instead of SSDs, the application of a safety factor (perhaps 10) to the lowest NOEC from the most sensitive species, to derive a 'safe' dilution. This 'safe' dilution could then be compared with the PC99(95) estimates from the corrected SSDs.	A very useful suggestion which we have adopted. The safe dilution generated from the minimum NOEC approach is lower than either of the SSD derived estimates but, taking a precautionary approach, we have based our recommendation for a safe limit on that (NOEC-derived) estimate.
	Minor Issues	
Tables 16 to 22	1. It would have been useful to have more details and discussion of the ecotoxicity test results in the body of the report. A summary table of actual LC/EC50 and EC10 and NOEC data for each effluent for each test would supplement the small figure which is difficult to read in the printed version of the report and would better highlight the problems of the raw data.	Agreed. Added Tables 16 to 22 with this information

Changes Made	CSIRO Comments	RPS Response
Section 6.1	2. Some comments on QA being achieved, including water quality parameters throughout the tests, would also be useful. For example, the pH in all tests at all dilutions ranged from 7.8–8.3, suggesting that pH wasn't an issue. Similarly, a summary of the salinities at each effluent dilution would more clearly show that only at salinities above 43‰ were adverse effects observed.	Lines have been added to the first paragraph to reflect this, though we have kept the bulk of the details in the appendices for brevity sake.
Section 6.2	3. In Section 6.2, 1st para, you mean 'the fish imbalance bioassay **LC50** was low…' not the bioassay was low. Similarly for sea urchin, etc. in the sentence above.	Yes! Changes have been made to both sentences to reflect this.
Table 25	4. Correct Table 17 – fish imbalance is acute. How confident are you in using this many significant figures and quoting three standard errors for mean EC10 given the all or nothing response, where the real EC50 and EC10 values could be anywhere between 0 and 100% effluent?	Agreed. Table corrected. Very confident about the standard errors; standard errors are a measure of how different the estimate of a parameter would be if the same testing process were repeated many times. The general approach taken to significant figures here was to report to a relatively high level throughout to allow these to be used for calculations, but for the final estimate (the number of dilutions required for a safe concentration) report to one significant figure (e.g. 1 in 7 dilution; one in 40 dilution).
Table 26	5. Redo SSDs and Table 18.	Redone.
Section 8.0 (second paragraph)	6. I think you are being too precise with your PC99s, etc that are given to 3 significant figures.	As above, higher numbers of significant figures were reported for estimates which may be used in calculations. But only one significant figure has now been reported in the text for the final recommendations of the safe numbers of dilutions required.
	Discussion	
Section 7.1	Section 7.1: I don't think you can rule out either explanation in paragraph 1.	Agreed. Removed the previous last line of the paragraph to help indicate this.
Previous Section 7.2 omitted	Section 7.2: This gives the reader a false sense of security about the precision of the EC10 estimates. The problems with the lack of intermediate responses and the way the raw data are used to calculate the EC50 and EC10s from 'all or nothing' responses negates the implied precision.	Disagree that the precision is not high for these estimates – standard errors were correctly based on the variance among the 12 repeated tests for each bioassay; the error *within* tests which the reviewer refers to is irrelevant to this calculation. But the point about the underlying 'all or nothing' response in the data is an important one and dealt with in responses above. To avoid confusion, the previous Section 7.2 which discussed precision of tests generally has been removed in this final version.

APPENDIX 2

Summary Results Sheets From WET Testing

Toxicity Test Report: TR0341/1

Client:	RPS 290 Churchill Ave Subiacco WA 6008	**ESA Job #:** **Date Sampled:** **Date Received:**	PR0341 28 July 2008* 29 July 2008
Attention:	Dr Craig Styan	**Sampled By:**	Client
Client Ref #:	M08504	**ESA Quote #:**	PR0341_q01&q02

* Sampling date of seawater to be used for production of brine

Lab ID No.:	Sample Name:	Sample Description:
2817	***1***- Brine Only	Brine prepared by evaporation of seawater
2817	***2***- Veolia Brine Effluent + Backwash	Brine prepared by evaporation of seawater, with additives as specified by client
2817	***3***- Veolia Brine Effluent + Backwash + CIP (variant a)	Brine prepared by evaporation of seawater, with additives as specified by client
2817	***4***- Veolia Brine Effluent + Backwash + CIP (variant d)	Brine prepared by evaporation of seawater, with additives as specified by client
2817	***5***- ITT Brine Effluent + Backwash	Brine prepared by evaporation of seawater, with additives as specified by client
2817	***6***- ITT Brine Effluent + Backwash + CIP (variant b)	Brine prepared by evaporation of seawater, with additives as specified by client
2817	***7***- Osmoflow Brine Effluent + Backwash	Brine prepared by evaporation of seawater, with additives as specified by client
2817	***8***- Osmoflow Brine Effluent + Backwash + CIP (variant c)	Brine prepared by evaporation of seawater, with additives as specified by client
2817	***9***- Osmoflow Brine Effluent + Backwash + CIP (variant d)	Brine prepared by evaporation of seawater, with additives as specified by client
2817	***10***- GE Brine Effluent + Backwash	Brine prepared by evaporation of seawater, with additives as specified by client
2817	***11***- GE Brine Effluent + Backwash + CIP (variant e)	Brine prepared by evaporation of seawater, with additives as specified by client
2817	***12***- Backwash only (NaCLO 0.53mg/L)	Brine prepared by evaporation of seawater, with additives as specified by client

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
Comments on Solution Preparation:	A simulated brine of 60‰ was prepared by evaporating seawater collected off Barrow Island (lab ID 2817) in 80L HDPE carboys. The Barrow Island seawater was filtered to 0.45µm using a peristaltic pump and Sartorius Sartobran filter capsule prior to being poured into the carboys. Evaporation was assisted by heating the seawater to 30°C with the aid of aquarium heaters, and with vigorous aeration using aquarium aerators. The salinity of the seawater was monitored daily, and upon reaching 60‰, the solution were poured off into 2.5L amber glass winchers for holding under refrigeration at 4oC until required for testing. The process took approximately 4 weeks. The test solutions were prepared on the day/evening prior to initiating the toxicity tests given the complex and time-consuming nature of the brine additive preparation procedure. A full breakdown of additives for each brine sample is provided in the annex to this report.
Source of Test Organisms:	Field collected from South Maroubra, NSW, on 17 September 2008
Test Initiated:	17 September 2008 at 1800h

Toxicity Test Report: TR0341/1

Sample 1 **Concentration (%)**	**% Normal larvae (Mean ± SD)**
0 (control)	92.5 ± 2.7
6.25	93.8 ± 2.8
12.5	93.8 ± 2.2
25	93.8 ± 3.1
50	0.0 ± 0.0
100	0.0 ± 0.0

72 hr IC10 = 26.5 (25.8 – 26.7)%
72 hr EC50 = 35.4%
(Geometric Mean Method)
NOEC = 25%
LOEC = 50%

Sample 2 **Concentration (%)**	**% Normal larvae (Mean ± SD)**
0 (control)	92.5 ± 2.7
6.25	93.5 ± 2.1
12.5	93.0 ± 3.9
25	93.8 ± 2.8
50	0.0 ± 0.0
100	0.0 ± 0.0

72 hr IC10 = 26.5 (25.9 – 26.7)%
72 hr EC50 = 35.4%
(Geometric Mean Method)
NOEC = 25%
LOEC = 50%

Sample 3 **Concentration (%)**	**% Normal larvae (Mean ± SD)**
0 (control)	92.5 ± 2.7
6.25	93.5 ± 2.1
12.5	94.5 ± 3.5
25	94.0 ± 3.4
50	0.0 ± 0.0
100	0.0 ± 0.0

72 hr IC10 = 26.5 (25.7 – 26.8)%
72 hr EC50 = 35.4%
(Geometric Mean Method)
NOEC = 25%
LOEC = 50%

.

Sample 4 **Concentration (%)**	**% Normal larvae (Mean ± SD)**
0 (control)	92.5 ± 2.7
6.25	93.8 ± 2.8
12.5	94.8 ± 4.4
25	93.0 ± 2.2
50	0.0 ± 0.0
100	0.0 ± 0.0

72 hr IC10 = 26.4 (25.7 – 26.8)%
72 hr EC50 = 35.2 (35.0-35.4)%
(TSK trim value= 0.0%)
NOEC = 25%
LOEC = 50%

Sample 5 **Concentration (%)**	**% Normal larvae (Mean ± SD)**
0 (control)	92.5 ± 2.7
6.25	93.0 ± 1.6
12.5	94.3 ± 3.3
25	92.0 ± 2.5
50	0.0 ± 0.0
100	0.0 ± 0.0

72 hr IC10 = 26.2 (25.6 – 26.7)%
72 hr EC50 = 35.0 (34.8-35.3)%
(TSK trim value= 0.0%)
NOEC = 25%
LOEC = 50%

Sample 6 **Concentration (%)**	**% Normal larvae (Mean ± SD)**
0 (control)	92.5 ± 2.7
6.25	93.0 ± 3.6
12.5	93.5 ± 2.4
25	93.8 ± 2.6
50	0.0 ± 0.0
100	0.0 ± 0.0

72 hr IC10 = 26.5 (25.9 – 26.7)%
72 hr EC50 = 35.4%
(Geometric Mean Method)
NOEC = 25%
LOEC = 50%

Sample 7 **Concentration (%)**	**% Normal larvae (Mean ± SD)**
0 (control)	92.5 ± 2.7
6.25	94.5 ± 3.1
12.5	91.8 ± 1.7
25	94.0 ± 2.6
50	0.0 ± 0.0
100	0.0 ± 0.0

72 hr IC10 = 26.4 (25.8 – 26.7)%
72 hr EC50 = 35.0 (34.8-35.3)%
(TSK trim value= 0.0%)
NOEC = 25%
LOEC = 50%

Sample 8 **Concentration (%)**	**% Normal larvae (Mean ± SD)**
0 (control)	92.5 ± 2.7
6.25	94.0 ± 3.2
12.5	92.5 ± 2.7
25	91.8 ± 1.7
50	0.0 ± 0.0
100	0.0 ± 0.0

72 hr IC10 = 26.2 (25.6 – 26.5)%
72 hr EC50 = 34.8 (34.8-35.1)%
(TSK trim value= 0.0%)
NOEC = 25%
LOEC = 50%

Sample 9 **Concentration (%)**	**% Normal larvae (Mean ± SD)**
0 (control)	92.5 ± 2.7
6.25	93.5 ± 2.4
12.5	94.3 ± 3.0
25	92.3 ± 3.3
50	0.0 ± 0.0
100	0.0 ± 0.0

72 hr IC10 = 26.3 (25.5 – 26.8)%
72 hr EC50 = 35.1 (34.8-35.3)%
(TSK trim value= 0.0%)
NOEC = 25%
LOEC = 50%

Toxicity Test Report: TR0341/1

Sample 10 Concentration (%)	% Normal larvae (Mean ± SD)
0 (control)	92.5 ± 2.7
6.25	93.8 ± 2.2
12.5	93.8 ± 3.5
25	92.3 ± 2.9
50	0.0 ± 0.0
100	0.0 ± 0.0

72 hr IC10 = 26.3 (25.6 – 26.70%
72 hr EC50 = 35.1 (34.8-35.3)%
(TSK trim value= 0.0%)
NOEC = 25%
LOEC = 50%

Sample 11 Concentration (%)	% Normal larvae (Mean ± SD)
0 (control)	92.5 ± 2.7
6.25	93.3 ± 2.2
12.5	93.8 ± 3.0
25	92.0 ± 2.2
50	0.0 ± 0.0
100	0.0 ± 0.0

72 hr IC10 = 26.2 (25.7 – 26.7)%
72 hr EC50 = 35.1 (34.8-35.3)%
(TSK trim value= 0.0%)
NOEC = 25%
LOEC = 50%

Sample 12 Concentration (%)	% Normal larvae (Mean ± SD)
0 (control)	92.5 ± 2.7
6.25	93.8 ± 3.1
12.5	93.0 ± 2.2
25	92.8 ± 2.8
50	0.0 ± 0.0
100	0.0 ± 0.0

72 hr IC10 = 26.4 (25.6 – 26.6)%
72 hr EC50 = 35.2 (35.1-35.4)%
(TSK trim value= 0.0%)
NOEC = 25%
LOEC = 50%

QA/QC Parameter	Criterion	This Test	Criterion met?
Control mean % normal	>70 %	92.5 ± 2.7%	Yes
Test Temperature limits	20.0 ± 1ºC	20.0 ± 1ºC	Yes
Reference Toxicant within cusum chart limits	7.3-12.2 μg Cu^{2+}/L	9.7 μg Cu^{2+}/L	Yes

Test Report Authorised by: Dr Rick Krassoi, Director on 23 September 2008

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: TR0341/2

Client:	RPS 290 Churchill Ave Subiacco WA 6008	**ESA Job #:** **Date Sampled:** **Date Received:**	PR0341 28 July 2008* 29 July 2008
Attention:	Dr Craig Styan	**Sampled By:**	Client
Client Ref #:	M08504	**ESA Quote #:**	PR0341_q01&q02

* Sampling date of seawater to be used for production of brine

Lab ID No.:	Sample Name:	Sample Description:
2817	***1***- Brine Only	Brine prepared by evaporation of seawater
2817	***2***- Veolia Brine Effluent + Backwash	Brine prepared by evaporation of seawater, with additives as specified by client
2817	***3***- Veolia Brine Effluent + Backwash + CIP (variant a)	Brine prepared by evaporation of seawater, with additives as specified by client
2817	***4***- Veolia Brine Effluent + Backwash + CIP (variant d)	Brine prepared by evaporation of seawater, with additives as specified by client
2817	***5***- ITT Brine Effluent + Backwash	Brine prepared by evaporation of seawater, with additives as specified by client
2817	***6***- ITT Brine Effluent + Backwash + CIP (variant b)	Brine prepared by evaporation of seawater, with additives as specified by client
2817	***7***- Osmoflow Brine Effluent + Backwash	Brine prepared by evaporation of seawater, with additives as specified by client
2817	***8***- Osmoflow Brine Effluent + Backwash + CIP (variant c)	Brine prepared by evaporation of seawater, with additives as specified by client
2817	***9***- Osmoflow Brine Effluent + Backwash + CIP (variant d)	Brine prepared by evaporation of seawater, with additives as specified by client
2817	***10***- GE Brine Effluent + Backwash	Brine prepared by evaporation of seawater, with additives as specified by client
2817	***11***- GE Brine Effluent + Backwash + CIP (variant e)	Brine prepared by evaporation of seawater, with additives as specified by client
2817	***12***- Backwash only (NaCLO 0.53mg/L)	Brine prepared by evaporation of seawater, with additives as specified by client

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
Comments on Solution Preparation:	A simulated brine of 60‰ was prepared by evaporating seawater collected off Barrow Island (lab ID 2817) in 80L HDPE carboys. The Barrow Island seawater was filtered to 0.45µm using a peristaltic pump and Sartorius Sartobran filter capsule prior to being poured into the carboys. Evaporation was assisted by heating the seawater to 30°C with the aid of aquarium heaters, and with vigorous aeration using aquarium aerators. The salinity of the seawater was monitored daily, and upon reaching 60‰, the solution were poured off into 2.5L amber glass winchers for holding under refrigeration at 4oC until required for testing. The process took approximately 4 weeks. The test solutions were prepared on the day/evening prior to initiating the toxicity tests given the complex and time-consuming nature of the brine additive preparation procedure. A full breakdown of additives for each brine sample is provided in the annex to this report.
Source of Test Organisms:	Farm-reared, Wallis Lakes, NSW
Test Initiated:	17 September 2008 at 1930h

Toxicity Test Report: TR0341/2

Sample 1 **Concentration (%)**	**% Alive/Normal larvae (Mean ± SD)**
0 (control)	72.9 ± 5.9
6.25	74.6 ± 4.9
12.5	77.0 ± 5.6
25	73.5 ± 6.1
50	0.0 ± 0.0
100	0.0 ± 0.0

48 hr IC10 = 25.5 (24.8 – 25.8)%
48 hr EC50 = 34.9 (34.4-35.4)%
(TSK trim value = 0.0%)
NOEC = 25%
LOEC = 50%

Sample 2 **Concentration (%)**	**% Alive/Normal larvae (Mean ± SD)**
0 (control)	72.9 ± 5.9
6.25	75.2 ± 4.9
12.5	74.6 ± 4.5
25	75.2 ± 3.6
50	0.0 ± 0.0
100	0.0 ± 0.0

48 hr IC10 = 25.6 (25.1 – 25.7)%
48 hr EC50 = 35.4 %
(Geometric Mean Method)
NOEC = 25%
LOEC = 50%

Sample 3 **Concentration (%)**	**% Alive/Normal larvae (Mean ± SD)**
0 (control)	72.9 ± 5.9
6.25	74.6 ± 4.5
12.5	75.8 ± 4.3
25	72.3 ± 4.1
50	0.0 ± 0.0
100	0.0 ± 0.0

48 hr IC10 = 25.4 (24.8 – 25.7)%
48 hr EC50 = 34.7 (34.0-35.3)%
(TSK trim value = 0.0%)
NOEC = 25%
LOEC = 50%

Sample 4 **Concentration (%)**	**% Alive/Normal larvae (Mean ± SD)**
0 (control)	72.9 ± 5.9
6.25	75.8 ± 6.4
12.5	71.7 ± 4.1
25	71.1 ± 7.5
50	0.0 ± 0.0
100	0.0 ± 0.0

48 hr IC10 = 25.3 (7.3 – 25.8)%
48 hr EC50 = 33.5 (32.5-34.5)%
(TSK trim value = 0.0%)
NOEC = 25%
LOEC = 50%

Sample 5 **Concentration (%)**	**% Alive/Normal larvae (Mean ± SD)**
0 (control)	72.9 ± 5.9
6.25	74.6 ± 4.1
12.5	69.3 ± 4.1
25	74.6 ± 6.3
50	0.0 ± 0.0
100	0.0 ± 0.0

48 hr IC10 = 25.5 (24.8 – 25.7)%
48 hr EC50 = 34.2 (33.4-35.0)%
(TSK trim value = 0.0%)
NOEC = 25%
LOEC = 50%

Sample 6 **Concentration (%)**	**% Alive/Normal larvae (Mean ± SD)**
0 (control)	72.9 ± 5.9
6.25	74.6 ± 4.9
12.5	75.2 ± 9.2
25	75.2 ± 5.3
50	0.0 ± 0.0
100	0.0 ± 0.0

48 hr IC10 = 25.6 (24.8 – 25.7)%
48 hr EC50 = 35.4%
(Geometric Mean Method)
NOEC = 25%
LOEC = 50%

Toxicity Test Report: TR0341/2

Sample 7 **Concentration (%)**	**% Alive/Normal larvae (Mean ± SD)**
0 (control)	72.9 ± 5.9
6.25	75.2 ± 7.1
12.5	65.2 ± 10.7
25	78.8 ± 4.1
50	0.0 ± 0.0
100	0.0 ± 0.0

48 hr IC10 = 25.4 (2.6 – 25.7)%
48 hr EC50 = 34.0 (33.2-34.9)%
(TSK trim value = 0.0%)
NOEC = 25%
LOEC = 50%

Sample 8 **Concentration (%)**	**% Alive/Normal larvae (Mean ± SD)**
0 (control)	72.9 ± 5.9
6.25	69.9 ± 3.1
12.5	73.5 ± 8.4
25	71.7 ± 7.1
50	0.0 ± 0.0
100	0.0 ± 0.0

48 hr IC10 = 25.5 (13.0 – 25.7)%
48 hr EC50 = 35.2 (34.9-35.4)%
(TSK trim value = 1.6%)
NOEC = 25%
LOEC = 50%

Sample 9 **Concentration (%)**	**% Alive/Normal larvae (Mean ± SD)**
0 (control)	72.9 ± 5.9
6.25	71.7 ± 2.3
12.5	79.4 ± 7.9
25	74.6 ± 7.9
50	0.0 ± 0.0
100	0.0 ± 0.0

48 hr IC10 = 25.6 (24.7 – 25.7)%
48 hr EC50 = 35.4%
(Geometric Mean Method)
NOEC = 25%
LOEC = 50%

Sample 10 **Concentration (%)**	**% Alive/Normal larvae (Mean ± SD)**
0 (control)	72.9 ± 5.9
6.25	75.8 ± 7.0
12.5	71.1 ± 4.3
25	76.4 ± 8.3
50	0.0 ± 0.0
100	0.0 ± 0.0

48 hr IC10 = 25.6 (24.8 – 25.7)%
48 hr EC50 = 35.0 (34.5-35.5)%
(TSK trim value = 0.0%)
NOEC = 25%
LOEC = 50%

Sample 11 **Concentration (%)**	**% Alive/Normal larvae (Mean ± SD)**
0 (control)	72.9 ± 5.9
6.25	72.9 ± 2.3
12.5	74.6 ± 7.4
25	73.5 ± 5.1
50	0.0 ± 0.0
100	0.0 ± 0.0

48 hr IC10 = 25.6 (24.9 – 25.7)%
48 hr EC50 = 35.4%
(Geometric Mean Method)
NOEC = 25%
LOEC = 50%

Sample 12 **Concentration (%)**	**% Alive/Normal larvae (Mean ± SD)**
0 (control)	72.9 ± 5.9
6.25	75.8 ± 7.5
12.5	71.7 ± 6.2
25	73.5 ± 8.9
50	0.0 ± 0.0
100	0.0 ± 0.0

48 hr IC10 = 25.5 (15.8 – 25.7)%
48 hr EC50 = 34.2 (33.4-35.0)%
(TSK trim value = 0.0%)
NOEC = 25%
LOEC = 50%

QA/QC Parameter	**Criterion**	**This Test**	**Criterion met?**
Control mean % survival	>70%	72.9 ± 5.9%	Yes
Test Temperature limits	25.0 ± 1ºC	25.0 ± 1ºC	Yes
Reference Toxicant within cusum chart limits	18.9-25.3 µg Cu^{2+}/L	22.3 µg Cu^{2+}/L	Yes

Toxicity Test Report: TR0341/2

Test Report Authorised by:  Dr Rick Krassoi, Director on 23 September 2008

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

NATA Accredited Laboratory Number: 14709

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Toxicity Test Report: TR0341/3

Client:	RPS 290 Churchill Ave Subiacco WA 6008	**ESA Job #:** **Date Sampled:** **Date Received:**	PR0341 28 July 2008* 29 July 2008
Attention:	Dr Craig Styan	**Sampled By:**	Client
Client Ref #:	M08504	**ESA Quote #:**	PR0341_q01&q02

* Sampling date of seawater to be used for production of brine

Lab ID No.:	Sample Name:	Sample Description:
2817	***1***- Brine Only	Brine prepared by evaporation of seawater
2817	***2***- Veolia Brine Effluent + Backwash	Brine prepared by evaporation of seawater, with additives as specified by client
2817	***3***- Veolia Brine Effluent + Backwash + CIP (variant a)	Brine prepared by evaporation of seawater, with additives as specified by client
2817	***4***- Veolia Brine Effluent + Backwash + CIP (variant d)	Brine prepared by evaporation of seawater, with additives as specified by client
2817	***5***- ITT Brine Effluent + Backwash	Brine prepared by evaporation of seawater, with additives as specified by client
2817	***6***- ITT Brine Effluent + Backwash + CIP (variant b)	Brine prepared by evaporation of seawater, with additives as specified by client
2817	***7***- Osmoflow Brine Effluent + Backwash	Brine prepared by evaporation of seawater, with additives as specified by client
2817	***8***- Osmoflow Brine Effluent + Backwash + CIP (variant c)	Brine prepared by evaporation of seawater, with additives as specified by client

Test Performed:	96-hr fish imbalance toxicity test using the barramundi *Lates calcarifer*
Test Protocol:	ESA SOP 117, based on USEPA (1994, 1996)
Deviations from Protocol:	Nil
Comments on Solution Preparation:	A simulated brine of 60‰ was prepared by evaporating seawater collected off Barrow Island (lab ID 2817) in 80L HDPE carboys. The Barrow Island seawater was filtered to 0.45µm using a peristaltic pump and Sartorius Sartobran filter capsule prior to being poured into the carboys. Evaporation was assisted by heating the seawater to 30°C with the aid of aquarium heaters, and with vigorous aeration using aquarium aerators. The salinity of the seawater was monitored daily, and upon reaching 60‰, the solution were poured off into 2.5L amber glass winchers for holding under refrigeration at 4oC until required for testing. The process took approximately 4 weeks. The test solutions were prepared on the day/evening prior to initiating the toxicity tests given the complex and time-consuming nature of the brine additive preparation procedure. A full breakdown of additives for each brine sample is provided in the annex to this report.
Source of Test Organisms:	Hatchery-reared, SA
Test Initiated:	19 September 2008 at 1700h

Toxicity Test Report: TR0341/3

Sample 1 **Concentration (%)**	**% Un-Affected (Mean ± SD)**
0 (control)	100.0 ± 0.0
6.25	100.0 ± 0.0
12.5	100.0 ± 0.0
25	100.0 ± 0.0
50	0.0 ± 0.0
100	0.0 ± 0.0

96 hr IC10 = 32.6 (32.6 – 32.6)%
96 hr EC50 = 35.4%
(Geometric Mean Method)
NOEC = 25%
LOEC = 50%

Sample 2 **Concentration (%)**	**% Un-Affected (Mean ± SD)**
0 (control)	100.0 ± 0.0
6.25	100.0 ± 0.0
12.5	100.0 ± 0.0
25	100.0 ± 0.0
50	0.0 ± 0.0
100	0.0 ± 0.0

96 hr IC10 = 32.6 (32.6 – 32.6)%
96 hr EC50 = 35.4%
(Geometric Mean Method)
NOEC = 25%
LOEC = 50%

Sample 3 **Concentration (%)**	**% Un-Affected (Mean ± SD)**
0 (control)	100.0 ± 0.0
6.25	100.0 ± 0.0
12.5	100.0 ± 0.0
25	100.0 ± 0.0
50	0.0 ± 0.0
100	0.0 ± 0.0

96 hr IC10 = 32.6 (32.6 – 32.6)%
96 hr EC50 = 35.4%
(Geometric Mean Method)
NOEC = 25%
LOEC = 50%

Sample 4 **Concentration (%)**	**% Un-Affected (Mean ± SD)**
0 (control)	100.0 ± 0.0
6.25	100.0 ± 0.0
12.5	100.0 ± 0.0
25	100.0 ± 0.0
50	50.0 ± 11.6*
100	0.0 ± 0.0

96 hr IC10 = 42.4 (40.9 – 44.1)%
96 hr EC50 = 50.0 (42.8-58.4)%
(TSK trim value= 0.0%)
NOEC = 25%
LOEC = 50%

Sample 5 **Concentration (%)**	**% Un-Affected (Mean ± SD)**
0 (control)	100.0 ± 0.0
6.25	100.0 ± 0.0
12.5	100.0 ± 0.0
25	100.0 ± 0.0
50	0.0 ± 0.0
100	0.0 ± 0.0

96 hr IC10 = 32.6 (32.6 – 32.6)%
96 hr EC50 = 35.4%
(Geometric Mean Method)
NOEC = 25%
LOEC = 50%

Sample 6 **Concentration (%)**	**% Un-Affected (Mean ± SD)**
0 (control)	100.0 ± 0.0
6.25	100.0 ± 0.0
12.5	100.0 ± 0.0
25	100.0 ± 0.0
50	25.0 ± 50.0
100	0.0 ± 0.0

96 hr IC10 = 40.1 (28.1 – 48.8)%
96 hr EC50 = 42.1 (36.8-48.1)%
(TSK trim value= 0.0%)
NOEC = 50%
LOEC = 100%

Sample 7 **Concentration (%)**	**% Un-Affected (Mean ± SD)**
0 (control)	100.0 ± 0.0
6.25	100.0 ± 0.0
12.5	100.0 ± 0.0
25	75.0 ± 30.0
50	0.0 ± 0.0
100	0.0 ± 0.0

96 hr IC10 = 22.8 (19.9 – 38.5)%
96 hr EC50 = 29.7 (26.0-34.0)%
(TSK trim value= 0.0%)
NOEC = 25%
LOEC = 50%

Sample 8 **Concentration (%)**	**% Un-Affected (Mean ± SD)**
0 (control)	100.0 ± 0.0
6.25	100.0 ± 0.0
12.5	100.0 ± 0.0
25	95.0 ± 10.0
50	0.0 ± 0.0
100	0.0 ± 0.0

96 hr IC10 = 26.1 (22.7 – 36.5)%
96 hr EC50 = 34.2 (31.9-36.6)%
(TSK trim value= 0.0%)
NOEC = 25%
LOEC = 50%

Toxicity Test Report: TR0341/3

QA/QC Parameter	Criterion	This Test	Criterion met?
Control mean % un-affected	≥90%	100 ± 0.0%	Yes
Test Temperature limits	25.0 ± 1ºC	25.0 ± 1ºC	Yes
Reference Toxicant within cusum chart limits	0.96-1.32 mg Cu^{2+}/L	1.30 mg Cu^{2+}/L	Yes

Test Report Authorised by: Dr Rick Krassoi, Director on 23 September 2008

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

NATA Accredited Laboratory Number: 14709

Toxicity Test Report: TR0341/4

Client:	RPS 290 Churchill Ave Subiacco WA 6008	**ESA Job #:** **Date Sampled:** **Date Received:**	PR0341 28 July 2008* 29 July 2008
Attention:	Dr Craig Styan	**Sampled By:**	Client
Client Ref #:	M08504	**ESA Quote #:**	PR0341_q01&q02

* Sampling date of seawater to be used for production of brine

Lab ID No.:	Sample Name:	Sample Description:
2817	***1***- Brine Only	Brine prepared by evaporation of seawater
2817	***2***- Veolia Brine Effluent + Backwash	Brine prepared by evaporation of seawater, with additives as specified by client
2817	***3***- Veolia Brine Effluent + Backwash + CIP (variant a)	Brine prepared by evaporation of seawater, with additives as specified by client
2817	***4***- Veolia Brine Effluent + Backwash + CIP (variant d)	Brine prepared by evaporation of seawater, with additives as specified by client
2817	***5***- ITT Brine Effluent + Backwash	Brine prepared by evaporation of seawater, with additives as specified by client
2817	***6***- ITT Brine Effluent + Backwash + CIP (variant b)	Brine prepared by evaporation of seawater, with additives as specified by client
2817	***7***- Osmoflow Brine Effluent + Backwash	Brine prepared by evaporation of seawater, with additives as specified by client
2817	***8***- Osmoflow Brine Effluent + Backwash + CIP (variant c)	Brine prepared by evaporation of seawater, with additives as specified by client

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
Comments on Solution Preparation:	A simulated brine of 60‰ was prepared by evaporating seawater collected off Barrow Island (lab ID 2817) in 80L HDPE carboys. The Barrow Island seawater was filtered to 0.45µm using a peristaltic pump and Sartorius Sartobran filter capsule prior to being poured into the carboys. Evaporation was assisted by heating the seawater to 30°C with the aid of aquarium heaters, and with vigorous aeration using aquarium aerators. The salinity of the seawater was monitored daily, and upon reaching 60‰, the solution were poured off into 2.5L amber glass winchers for holding under refrigeration at 4oC until required for testing. The process took approximately 4 weeks. The test solutions were prepared on the day/evening prior to initiating the toxicity tests given the complex and time-consuming nature of the brine additive preparation procedure. A full breakdown of additives for each brine sample is provided in the annex to this report.
Source of Test Organisms:	Hatchery-reared, Cairns, QLD
Test Initiated:	19 September 2008 at 1630h

Toxicity Test Report: TR0341/4

Sample 1	
Concentration (%)	**% Survival (Mean ± SD)**
0 (control)	95.0 ± 10.0
6.25	100.0 ± 0.0
12.5	100.0 ± 0.0
25	90.0 ± 11.6
50	75.0 ± 10.0
100	0.0 ± 0.0
96 hr IC10 = 27.62 (17.3 – 51.7)%	
96 hr LC50 = 56.6 (48.3-66.2)%	
(TSK trim value= 0.0%)	
NOEC = 50%	
LOEC = 100%	

Sample 2	
Concentration (%)	**% Survival (Mean ± SD)**
0 (control)	95.0 ± 10.0
6.25	100.0 ± 0
12.5	80.0 ± 0.0
25	90.0 ± 11.6
50	75.0 ± 10.0
100	0.0 ± 0.0
96 hr IC10 =11.5 (10.0 – 45.4)%	
96 hr LC50 = 50.5 (41.5-61.4)%	
(TSK trim value= 0.0%)	
NOEC = 50%	
LOEC = 100%	

Sample 3	
Concentration (%)	**% Survival (Mean ± SD)**
0 (control)	95.0 ± 10.0
6.25	90.0 ± 11.6
12.5	90.0 ± 11.6
25	95.0 ± 10.0
50	80.0 ± 16.3
100	0.0 ± 0.0
96 hr IC10 = 38.4 (0.0 – 60.5)%	
96 hr LC50 = 63.7 (56.2-72.2)%	
(TSK trim value= 3.5%)	
NOEC = 50%	
LOEC = 100%	

Sample 4	
Concentration (%)	**% Survival (Mean ± SD)**
0 (control)	95.0 ± 10.0
6.25	85.0 ± 19.2
12.5	100.0 ± 0.0
25	85.0 ± 10.0
50	75.0 ± 10.0
100	0.0 ± 0.0
96 hr IC10 = 24.1 (0.0 – 55.1)%	
96 hr LC50 = 57.8 (48.8-68.4)%	
(TSK trim value= 2.6%)	
NOEC = 50%	
LOEC = 100%	

Sample 5	
Concentration (%)	**% Survival (Mean ± SD)**
0 (control)	95.0 ± 10.0
6.25	85.0 ± 10.0
12.5	80.0 ± 0.0
25	90.0 ± 11.6
50	50.0 ± 20.0*
100	0.0 ± 0.0
96 hr IC10 = 5.8 (2.8 – 46.5)%	
96 hr LC50 = 49.3 (37.9-64.2)%	
(TSK trim value= 10.5%)	
NOEC = 25%	
LOEC = 50%	

Sample 6	
Concentration (%)	**% Survival (Mean ± SD)**
0 (control)	95.0 ± 10.0
6.25	95.0 ±10.0
12.5	85.0 ± 19.2
25	85.0 ± 10.0
50	35.0 ± 30.0*
100	0.0 ± 0.0
96 hr EC10 = 29.0 (9.3 – 37.9)%	
96 hr LC50 = 39.4 (32.3-48.2)%	
(TSK trim value= 0.0%)	
NOEC = 25%	
LOEC = 50%	

* Significantly lower % survival compared with the control treatment (Dunnett's Test, 1 tailed, P=0.05).

Sample 7	
Concentration (%)	**% Survival (Mean ± SD)**
0 (control)	95.0 ± 10.0
6.25	90.0 ± 20.0
12.5	95.0 ± 10.0
25	95.0 ± 10.0
50	70.0 ± 11.6
100	0.0 ± 0.0
96 hr IC10 =53.4 (50.9 – 55.6)%	
96 hr LC50 = 58.9 (51.1-68.0)%	
(TSK trim value= 1.8%)	
NOEC = 50%	
LOEC = 100%	

Sample 8	
Concentration (%)	**% Survival (Mean ± SD)**
0 (control)	95.0 ± 10.0
6.25	90.0 ± 11.6
12.5	80.0 ± 16.3
25	95.0 ± 10.0
50	30.0 ± 25.8*
100	0.0 ± 0.0
96 hr IC10 = 41.6 (29.0 – 55.7)%	
96 hr LC50 = 40.9 (33.2-50.5)%	
(TSK trim value= 5.3%)	
NOEC = 25%	
LOEC = 50%	

* Significantly lower % survival compared with the control treatment (Dunnett's Test, 1 tailed, P=0.05).

Toxicity Test Report: TR0341/4

QA/QC Parameter	Criterion	This Test	Criterion met?
Control mean % survival	≥90 %	95.0 ± 10.0%	Yes
Test Temperature limits	25.0 ± 1ºC	24.0 ± 1ºC	Yes
Reference Toxicant within cusum chart limits	5.6-23.7 mg SDS/L	7.5 mg SDS/L	Yes

Test Report Authorised by: Dr Rick Krassoi, Director on 23 September 2008

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

Toxicity Test Report: TR0341/5

Client:	RPS 290 Churchill Ave Subiacco WA 6008	**ESA Job #:** **Date Sampled:** **Date Received:**	PR0341 28 July 2008* 29 July 2008
Attention:	Dr Craig Styan	**Sampled By:**	Client
Client Ref #:	M08504	**ESA Quote #:**	PR0341_q01&q02

* Sampling date of seawater to be used for production of brine

Lab ID No.:	Sample Name:	Sample Description:
2817	***1***- Brine Only	Brine prepared by evaporation of seawater
2817	***2***- Veolia Brine Effluent + Backwash	Brine prepared by evaporation of seawater, with additives as specified by client
2817	***3***- Veolia Brine Effluent + Backwash + CIP (variant a)	Brine prepared by evaporation of seawater, with additives as specified by client
2817	***4***- Veolia Brine Effluent + Backwash + CIP (variant d)	Brine prepared by evaporation of seawater, with additives as specified by client
2817	***5***- ITT Brine Effluent + Backwash	Brine prepared by evaporation of seawater, with additives as specified by client
2817	***6***- ITT Brine Effluent + Backwash + CIP (variant b)	Brine prepared by evaporation of seawater, with additives as specified by client
2817	***7***- Osmoflow Brine Effluent + Backwash	Brine prepared by evaporation of seawater, with additives as specified by client
2817	***8***- Osmoflow Brine Effluent + Backwash + CIP (variant c)	Brine prepared by evaporation of seawater, with additives as specified by client
2817	***9***- Osmoflow Brine Effluent + Backwash + CIP (variant d)	Brine prepared by evaporation of seawater, with additives as specified by client
2817	***10***- GE Brine Effluent + Backwash	Brine prepared by evaporation of seawater, with additives as specified by client
2817	***11***- GE Brine Effluent + Backwash + CIP (variant e)	Brine prepared by evaporation of seawater, with additives as specified by client
2817	***12***- Backwash only (NaCLO 0.53mg/L)	Brine prepared by evaporation of seawater, with additives as specified by client

Test Performed:	72-hr Sea urchin fertilisation test using *Heliocidaris tuberculata*
Test Protocol:	ESA SOP 104, based on USEPA (1988, 2002) and Simon and Laginestra (1997)
Deviations from Protocol:	Nil
Comments on Solution Preparation:	A simulated brine of 60‰ was prepared by evaporating seawater collected off Barrow Island (lab ID 2817) in 80L HDPE carboys. The Barrow Island seawater was filtered to 0.45µm using a peristaltic pump and Sartorius Sartobran filter capsule prior to being poured into the carboys. Evaporation was assisted by heating the seawater to 30°C with the aid of aquarium heaters, and with vigorous aeration using aquarium aerators. The salinity of the seawater was monitored daily, and upon reaching 60‰, the solution were poured off into 2.5L amber glass winchers for holding under refrigeration at 4oC until required for testing. The process took approximately 4 weeks. The test solutions were prepared on the day/evening prior to initiating the toxicity tests given the complex and time-consuming nature of the brine additive preparation procedure. A full breakdown of additives for each brine sample is provided in the annex to this report.
Source of Test Organisms:	Field collected from South Maroubra, NSW, on 17 September 2008
Test Initiated:	17 September 2008 at 1730h

Toxicity Test Report: TR0341/5

Sample 1	
Concentration (%)	**% Fertilised (Mean ± SD)**
0 (control)	91.5 ± 2.1
6.25	90.3 ± 1.0
12.5	91.8 ± 1.7
25	91.8 ± 3.1
50	0.0 ± 0.0
100	0.0 ± 0.0

72 hr IC10 = 26.3 (25.7 – 26.4)%
72 hr EC50 = 35.3 (35.3-35.4)%
(TSK trim value= 0.3%)
NOEC = 25%
LOEC = 50%

Sample 2	
Concentration (%)	**% Fertilised (Mean ± SD)**
0 (control)	91.5 ± 2.1
6.25	93.3 ± 3.6
12.5	91.0 ± 1.4
25	91.8 ± 3.1
50	0.0 ± 0.0
100	0.0 ± 0.0

72 hr IC10 = 26.2 (25.5 – 26.5)%
72 hr EC50 = 34.8 (34.5-35.2)%
(TSK trim value= 0.0%)
NOEC = 25%
LOEC = 50%

Sample 3	
Concentration (%)	**% Fertilised (Mean ± SD)**
0 (control)	91.5 ± 2.1
6.25	90.8 ± 1.5
12.5	92.3 ± 1.7
25	91.8 ± 2.9
50	0.0 ± 0.0
100	0.0 ± 0.0

72 hr IC10 = 26.3 (25.8 – 26.4)%
72 hr EC50 = 35.4%
(Geometric Mean Method)
NOEC = 25%
LOEC = 50%

Sample 4	
Concentration (%)	**% Fertilised (Mean ± SD)**
0 (control)	91.5 ± 2.1
6.25	90.8 ± 1.0
12.5	92.3 ± 1.7
25	92.3 ± 2.6
50	0.0 ± 0.0
100	0.0 ± 0.0

72 hr IC10 = 26.3 (25.9 – 26.4)%
72 hr EC50 = 35.4%
(Geometric Mean Method)
NOEC = 25%
LOEC = 50%

Sample 5	
Concentration (%)	**% Fertilised (Mean ± SD)**
0 (control)	91.5 ± 2.1
6.25	93.3 ± 3.6
12.5	92.8 ± 2.5
25	91.8 ± 1.7
50	0.0 ± 0.0
100	0.0 ± 0.0

72 hr IC10 = 26.2 (25.8 – 26.5)%
72 hr EC50 = 35.2 (34.9-35.4)%
(TSK trim value= 0.0%)
NOEC = 25%
LOEC = 50%

Sample 6	
Concentration (%)	**% Fertilised (Mean ± SD)**
0 (control)	91.5 ± 2.1
6.25	91.5 ± 1.3
12.5	91.5 ± 2.4
25	93.8 ± 2.6
50	0.0 ± 0.0
100	0.0 ± 0.0

72 hr IC10 = 26.3 (26.0 – 26.5)%
72 hr EC50 = 35.4%
(Geometric Mean Method)
NOEC = 25%
LOEC = 50%

Sample 7	
Concentration (%)	**% Fertilised (Mean ± SD)**
0 (control)	91.5 ± 2.1
6.25	91.5 ± 1.3
12.5	92.0 ± 3.4
25	92.0 ± 2.2
50	0.0 ± 0.0
100	0.0 ± 0.0

72 hr IC10 = 26.3 (25.9 – 26.5)%
72 hr EC50 = 35.4%
(Geometric Mean Method)
NOEC = 25%
LOEC = 50%

Sample 8	
Concentration (%)	**% Fertilised (Mean ± SD)**
0 (control)	91.5 ± 2.1
6.25	91.0 ± 1.4
12.5	92.8 ± 2.8
25	92.0 ± 2.2
50	0.0 ± 0.0
100	0.0 ± 0.0

72 hr IC10 = 26.3 (25.8 – 26.4)%
72 hr EC50 = 35.4%
(Geometric Mean Method)
NOEC = 25%
LOEC = 50%

Sample 9	
Concentration (%)	**% Fertilised (Mean ± SD)**
0 (control)	91.5 ± 2.1
6.25	91.5 ± 1.3
12.5	93.0 ± 2.9
25	91.5 ± 1.9
50	0.0 ± 0.0
100	0.0 ± 0.0

72 hr IC10 = 26.3 (25.8 – 26.5)%
72 hr EC50 = 35.2 (35.0-35.4)%
(TSK trim value= 0.0%)
NOEC = 25%
LOEC = 50%

Toxicity Test Report: TR0341/5

Sample 10		Sample 11		Sample 12	
Concentration (%)	**% Fertilised (Mean ± SD)**	**Concentration (%)**	**% Fertilised (Mean ± SD)**	**Concentration (%)**	**% Fertilised (Mean ± SD)**
0 (control)	91.5 ± 2.1	0 (control)	91.5 ± 2.1	0 (control)	91.5 ± 2.1
6.25	91.0 ± 1.4	6.25	92.8 ± 2.8	6.25	91.8 ± 2.9
12.5	92.0 ± 2.9	12.5	93.0 ± 2.7	12.5	92.5 ± 1.3
25	91.8 ± 1.7	25	94.5 ± 3.7	25	93.3 ± 3.4
50	1.0 ± 2.0*	50	3.3 ± 4.3*	50	0.5 ± 1.0*
100	0.0 ± 0.0	100	0.0 ± 0.0	100	0.0 ± 0.0
72 hr IC10 = 27.2 (25.4 – 27.8)%		**72 hr IC10 = 27.9 (25.6 – 28.9)%**		**72 hr IC10 = 27.1 (25.7 – 27.7)%**	
72 hr EC50 = 35.6 (35.4-35.9)%		**72 hr EC50 = 36.2 (35.8-36.7)%**		**72 hr EC50 = 35.5 (35.3-35.7)%**	
(TSK trim value= 0.0%)		**(TSK trim value= 0.0%)**		**(TSK trim value= 0.0%)**	
NOEC = 25%		**NOEC = 25%**		**NOEC = 25%**	
LOEC = 50%		**LOEC = 50%**		**LOEC = 50%**	

* Significantly lower % fertilized eggs compared with the control treatment (Dunnett's Test, 1 tailed, P=0.05, df=6,21).

QA/QC Parameter	Criterion	This Test	Criterion met?
Control mean % normal	>70 %	91.5 ± 2.1%	Yes
Test Temperature limits	20.0 ± 1ºC	20.0 ± 1ºC	Yes
Reference Toxicant within cusum chart limits	28.8-61.2 μg Cu^{2+}/L	41.2 μg Cu^{2+}/L	Yes

Test Report Authorised by: Dr Rick Krassoi, Director on 23 September 2008

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

NATA Accredited Laboratory Number: 14709

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Toxicity Test Report: TR0341/6

Client:	RPS	**ESA Job #:**	PR0341
	290 Churchill Ave	**Date Sampled:**	28 July 2008*
	Subiacco WA 6008	**Date Received:**	29 July 2008
Attention:	Dr Craig Styan	**Sampled By:**	Client
Client Ref #:	M08504	**ESA Quote #:**	PR0341_q01&q02

* Sampling date of seawater to be used for production of brine

Lab ID No.:	Sample Name:	Sample Description:
2817	***1***- Brine Only	Brine prepared by evaporation of seawater
2817	***2***- Veolia Brine Effluent + Backwash	Brine prepared by evaporation of seawater, with additives as specified by client
2817	***3***- Veolia Brine Effluent + Backwash + CIP (variant a)	Brine prepared by evaporation of seawater, with additives as specified by client
2817	***4***- Veolia Brine Effluent + Backwash + CIP (variant d)	Brine prepared by evaporation of seawater, with additives as specified by client
2817	***5***- ITT Brine Effluent + Backwash	Brine prepared by evaporation of seawater, with additives as specified by client
2817	***6***- ITT Brine Effluent + Backwash + CIP (variant b)	Brine prepared by evaporation of seawater, with additives as specified by client
2817	***7***- Osmoflow Brine Effluent + Backwash	Brine prepared by evaporation of seawater, with additives as specified by client
2817	***8***- Osmoflow Brine Effluent + Backwash + CIP (variant c)	Brine prepared by evaporation of seawater, with additives as specified by client
2817	***9***- Osmoflow Brine Effluent + Backwash + CIP (variant d)	Brine prepared by evaporation of seawater, with additives as specified by client
2817	***10***- GE Brine Effluent + Backwash	Brine prepared by evaporation of seawater, with additives as specified by client
2817	***11***- GE Brine Effluent + Backwash + CIP (variant e)	Brine prepared by evaporation of seawater, with additives as specified by client
2817	***12***- Backwash only (NaCLO 0.53mg/L)	Brine prepared by evaporation of seawater, with additives as specified by client

Test Performed:	72-hour marine algal growth test using *Isochrysis aff. Galbana*
Test Protocol:	ESA SOP 110, based on USEPA (1996), APHA (1998) and Stauber *et al*. (1994)
Deviations from Protocol:	Nil
Comments on Solution Preparation:	A simulated brine of 60‰ was prepared by evaporating seawater collected off Barrow Island (lab ID 2817) in 80L HDPE carboys. The Barrow Island seawater was filtered to 0.45µm using a peristaltic pump and Sartorius Sartobran filter capsule prior to being poured into the carboys. Evaporation was assisted by heating the seawater to 30°C with the aid of aquarium heaters, and with vigorous aeration using aquarium aerators. The salinity of the seawater was monitored daily, and upon reaching 60‰, the solution were poured off into 2.5L amber glass winchers for holding under refrigeration at 4oC until required for testing. The process took approximately 4 weeks. The test solutions were prepared on the day/evening prior to initiating the toxicity tests given the complex and time-consuming nature of the brine additive preparation procedure. A full breakdown of additives for each brine sample is provided in the annex to this report.
Source of Test Organisms:	CSIRO Microalgae Supply Service, TAS
Test Initiated:	18 September 2008 at 12:30h

Toxicity Test Report: TR0341/6

Sample 1		Sample 2		Sample 3	
Concentration (%)	**Cell Density x10^4 (Mean ± SD)**	**Concentration (%)**	**Cell Density x10^4 (Mean ± SD)**	**Concentration (%)**	**Cell Density x10^4 (Mean ± SD)**
0 (control)	44.0 ± 3.7	0 (control)	44.0 ± 3.7	0 (control)	44.0 ± 3.7
6.25	45.0 ± 1.8	6.25	44.5 ± 0.6	6.25	37.5 ± 3.7*
12.5	48.3 ± 6.1	12.5	47.5 ± 5.9	12.5	46.8 ± 2.6
25	47.0 ± 1.6	25	46.0 ± 2.2	25	46.3 ± 1.3
50	36.3 ± 0.5*	50	33.8 ± 1.7*	50	37.3 ± 2.5*
100	6.8 ± 1.5*	100	3.8 ± 1.5*	100	4.3 ± 1.7*
72 hr IC10 = 36.7 (31.1 – 39.4)% **72 hr IC50 = 72.4 (69.6 – 74.6)%** **NOEC = 25%** **LOEC = 50%**		**72 hr IC10 = 34.7 (27.3 – 37.8)%** **72 hr IC50 = 68.3 (64.7 – 71.7)%** **NOEC = 25%** **LOEC = 50%**		**72 hr IC10 = 40.6 (18.0 – 56.3)%** **72 hr IC50 = 73.1 (68.5 – 76.4)%** **NOEC = 25%** **LOEC = 50%**	

* Significantly lower cell density compared with the control treatment (Dunnett's Test, 1 tailed, P=0.05, df=6,21).

Sample 4		Sample 5		Sample 6	
Concentration (%)	**Cell Density x10^4 (Mean ± SD)**	**Concentration (%)**	**Cell Density x10^4 (Mean ± SD)**	**Concentration (%)**	**Cell Density x10^4 (Mean ± SD)**
0 (control)	44.0 ± 3.8	0 (control)	44.0 ± 3.7	0 (control)	44.0 ± 3.7
6.25	44.8 ± 2.1	6.25	46.8 ± 2.6	6.25	44.5 ± 1.7
12.5	47.8 ± 2.2	12.5	45.0 ± 2.2	12.5	45.5 ± 1.9
25	45.5 ± 3.1	25	44.3 ± 1.3	25	47.5 ± 4.7
50	34.3 ± 1.3*	50	33.5 ± 3.1*	50	37.3 ± 4.4*
100	6.0 ± 1.8*	100	5.3 ± 1.0*	100	4.8 ± 1.7*
72 hr IC10 = 35.1 (25.5 – 37.0)% **72 hr IC50 = 70.4 (66.8 – 73.4)%** **NOEC = 25%** **LOEC = 50%**		**72 hr IC10 = 32.9 (24.8 – 38.2)%** **72 hr IC50 = 69.1 (64.1 – 73.5)%** **NOEC = 25%** **LOEC = 50%**		**72 hr IC10 = 39.0 (29.2 – 57.6)%** **72 hr IC50 = 72.4 (65.3 – 77.9)%** **NOEC = 25%** **LOEC = 50%**	

* Significantly lower cell density compared with the control treatment (Dunnett's Test, 1 tailed, P=0.05, df=6,21).

Sample 7		Sample 8		Sample 9	
Concentration (%)	**Cell Density x10^4 (Mean ± SD)**	**Concentration (%)**	**Cell Density x10^4 (Mean ± SD)**	**Concentration (%)**	**Cell Density x10^4 (Mean ± SD)**
0 (control)	44.0 ± 3.7	0 (control)	44.0 ± 3.7	0 (control)	44.0 ± 3.7
6.25	48.3 ± 2.9	6.25	44.0 ± 4.7	6.25	44.5 ± 1.3
12.5	47.0 ± 3.6	12.5	45.0 ± 2.9	12.5	45.5 ± 1.3
25	46.8 ± 4.7	25	43.3 ± 2.6	25	45.0 ± 3.2
50	30.8 ± 4.1*	50	35.3 ± 0.5*	50	32.5 ± 3.4*
100	6.0 ± 1.2*	100	8.5 ± 3.7*	100	4.5 ± 1.3*
72 hr IC10 = 32.4 (21.8 – 36.4)% **72 hr IC50 = 65.2 (55.8 – 72.8)%** **NOEC = 25%** **LOEC = 50%**		**72 hr IC10 = 35.5 (16.8 – 42.0)%** **72 hr IC50 = 74.5 (68.5 – 80.7)%** **NOEC = 25%** **LOEC = 50%**		**72 hr IC10 = 34.1 (23.4 – 37.2)%** **72 hr IC50 = 68.1 (61.6 – 72.3)%** **NOEC = 25%** **LOEC = 50%**	

* Significantly lower cell density compared with the control treatment (Dunnett's Test, 1 tailed, P=0.05, df=6,21).

Toxicity Test Report: TR0341/6

Sample 10		Sample 11		Sample 12	
Concentration (%)	**Cell Density x10^4 (Mean ± SD)**	**Concentration (%)**	**Cell Density x10^4 (Mean ± SD)**	**Concentration (%)**	**Cell Density x10^4 (Mean ± SD)**
0 (control)	44.0 ± 3.7	0 (control)	44.0 ± 3.7	0 (control)	44.0 ± 3.7
6.25	42.8 ± 1.0	6.25	42.0 ± 3.4	6.25	46.3 ± 1.7
12.5	46.0 ± 3.2	12.5	41.0 ± 4.7	12.5	44.5 ± 3.4
25	44.0 ± 4.6	25	44.3 ± 2.1	25	47.0 ± 4.7
50	28.5 ± 3.3*	50	35.0 ± 1.2*	50	34.5 ± 0.6*
100	6.8 ± 1.0*	100	4.0 ± 1.6*	100	6.3 ± 3.9*
72 hr IC10 = 31.7 (22.1 – 34.0)%		**72 hr IC10 = 34.5 (0.0 – 44.2)%**		**72 hr IC10 = 35.4 (26.4 – 36.7)%**	
72 hr IC50 = 64.7 (53.0 – 70.4)%		**72 hr IC50 = 71.0 (66.8 – 74.7)%**		**72 hr IC50 = 70.9 (66.3 – 74.7)%**	
NOEC = 25%		**NOEC = 25%**		**NOEC = 25%**	
LOEC = 50%		**LOEC = 50%**		**LOEC = 50%**	

* Significantly lower cell density compared with the control treatment (Dunnett's Test, 1 tailed, P=0.05, df=6,21).

QA/QC Parameter	Criterion	This Test	Criterion met?
Control mean cell density/0.1µL	≥6	44.0 ± 3.7	Yes
Test Temperature limits	25.0 ± 1ºC	25.0 ± 1ºC	Yes
Reference Toxicant within cusum chart limits	29.6-65.0 µg Cu^{2+}/L	37.1 µg Cu^{2+}/L	Yes

Test Report Authorised by: Dr Rick Krassoi, Director on 23 September 2008

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

Toxicity Test Report: TR0341/7

Client:	RPS 290 Churchill Ave Subiacco WA 6008	**ESA Job #:** **Date Sampled:** **Date Received:**	PR0341 28 July 2008* 29 July 2008
Attention:	Dr Craig Styan	**Sampled By:**	Client
Client Ref #:	M08504	**ESA Quote #:**	PR0341_q01&q02

* Sampling date of seawater to be used for production of brine

Lab ID No.:	Sample Name:	Sample Description:
2817	***9***- Osmoflow Brine Effluent + Backwash + CIP (variant d)	Brine prepared by evaporation of seawater, with additives as specified by client
2817	***10***- GE Brine Effluent + Backwash	Brine prepared by evaporation of seawater, with additives as specified by client
2817	***11***- GE Brine Effluent + Backwash + CIP (variant e)	Brine prepared by evaporation of seawater, with additives as specified by client
2817	***12***- Backwash only (NaCLO 0.53mg/L)	Brine prepared by evaporation of seawater, with additives as specified by client

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
Comments on Solution Preparation:	A simulated brine of 60‰ was prepared by evaporating seawater collected off Barrow Island (lab ID 2817) in 80L HDPE carboys. The Barrow Island seawater was filtered to 0.45µm using a peristaltic pump and Sartorius Sartobran filter capsule prior to being poured into the carboys. Evaporation was assisted by heating the seawater to 30°C with the aid of aquarium heaters, and with vigorous aeration using aquarium aerators. The salinity of the seawater was monitored daily, and upon reaching 60‰, the solution were poured off into 2.5L amber glass winchers for holding under refrigeration at 4oC until required for testing. The process took approximately 4 weeks. The test solutions were prepared on the day/evening prior to initiating the toxicity tests given the complex and time-consuming nature of the brine additive preparation procedure. A full breakdown of additives for each brine sample is provided in the annex to this report.
Source of Test Organisms:	Hatchery-reared, Cairns, QLD
Test Initiated:	25 September 2008 at 2000h

Toxicity Test Report: TR0341/7

Sample 9	
Concentration (%)	**% Survival (Mean ± SD)**
0 (control)	90.0 ± 11.6
6.25	95.0 ± 10.0
12.5	90.0 ± 11.6
25	90.0 ± 11.6
50	60.0 ± 16.3*
100	0.0 ± 0.0
96 hr IC10 = 31.5 (0.0– 40.8)%	
96 hr LC50 = 53.4(45.3-62.9)%	
(TSK trim value= 0.0%)	
NOEC = 25%	
LOEC = 50%	

Sample 10	
Concentration (%)	**% Survival (Mean ± SD)**
0 (control)	90.0 ± 11.6
6.25	90.0 ± 20.0
12.5	95.0 ± 10.0
25	95.0 ± 10.0
50	95.0 ± 10.0
100	0.0 ± 0.0
96 hr IC10 = 52.9 (41.0-55.4)%	
96 hr LC50 = 70.7%	
(Geometric Mean Method)	
NOEC = 50%	
LOEC = 100%	

Sample 11	
Concentration (%)	**% Survival (Mean ± SD)**
0 (control)	90.0 ± 11.6
6.25	90.0 ± 20.0
12.5	95.0 ± 10.0
25	90.0 ± 20.0
50	65.0 ± 10.0
100	0.0 ± 0.0
96 hr IC10 = 33.3 (0.0-42.5)%	
96 hr LC50 = 57.1 (49.3-66.1)%	
(TSK trim value= 0.0%)	
NOEC = 50%	
LOEC = 100%	

* Significantly lower % survival compared with the control treatment (Dunnett's Test, 1 tailed, P=0.05).

Sample 12	
Concentration (%)	**% Survival (Mean ± SD)**
0 (control)	90.0 ± 11.6
6.25	90.0 ± 11.6
12.5	95.0 ± 10.0
25	95.0 ± 10.0
50	70.0 ± 11.6
100	0.0 ± 0.0
96 hr IC10 = 36.6 (16.7-46.5)%	
96 hr LC50 = 59.7 (52.3-68.2)%	
(TSK trim value= 0.0%)	
NOEC = 50%	
LOEC = 100%	

Vacant

Vacant

QA/QC Parameter	**Criterion**	**This Test**	**Criterion met?**
Control mean % survival	≥90%	90.0 ± 0.0%	Yes
Test Temperature limits	25.0 ± 1ºC	25.0 ± 1ºC	Yes
Reference Toxicant within cusum chart limits	5.6-23.7 mg SDS/L	7.2 mg SDS/L	Yes

Test Report Authorised by: Dr Rick Krassoi, Director on 14 October 2008

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

Toxicity Test Report: TR0341/8

Client:	RPS 290 Churchill Ave Subiacco WA 6008	**ESA Job #:** **Date Sampled:** **Date Received:**	PR0341 28 July 2008* 29 July 2008
Attention:	Dr Craig Styan	**Sampled By:**	Client
Client Ref #:	M08504	**ESA Quote #:**	PR0341_q01&q02

* Sampling date of seawater to be used for production of brine

Lab ID No.:	Sample Name:	Sample Description:
2817	***9***- Osmoflow Brine Effluent + Backwash + CIP (variant d)	Brine prepared by evaporation of seawater, with additives as specified by client
2817	***10***- GE Brine Effluent + Backwash	Brine prepared by evaporation of seawater, with additives as specified by client
2817	***11***- GE Brine Effluent + Backwash + CIP (variant e)	Brine prepared by evaporation of seawater, with additives as specified by client
2817	***12***- Backwash only (NaCLO 0.53mg/L)	Brine prepared by evaporation of seawater, with additives as specified by client

Test Performed:	96-hr fish imbalance toxicity test using the barramundi *Lates calcarifer*
Test Protocol:	ESA SOP 117, based on USEPA (1994, 1996)
Deviations from Protocol:	Nil
Comments on Solution Preparation:	A simulated brine of 60‰ was prepared by evaporating seawater collected off Barrow Island (lab ID 2817) in 80L HDPE carboys. The Barrow Island seawater was filtered to 0.45µm using a peristaltic pump and Sartorius Sartobran filter capsule prior to being poured into the carboys. Evaporation was assisted by heating the seawater to 30°C with the aid of aquarium heaters, and with vigorous aeration using aquarium aerators. The salinity of the seawater was monitored daily, and upon reaching 60‰, the solution were poured off into 2.5L amber glass winchers for holding under refrigeration at 4oC until required for testing. The process took approximately 4 weeks. The test solutions were prepared on the day/evening prior to initiating the toxicity tests given the complex and time-consuming nature of the brine additive preparation procedure. A full breakdown of additives for each brine sample is provided in the annex to this report.
Source of Test Organisms:	Hatchery-reared, SA
Test Initiated:	25 September 2008 at 2000h

Toxicity Test Report: TR0341/8

Sample 9 Concentration (%)	% Un-Affected (Mean ± SD)
0 (control)	100.0 ± 0.0
6.25	100.0 ± 0.0
12.5	100.0 ± 0.0
25	100.0 ± 0.0
50	0.0 ± 0.0
100	0.0 ± 0.0

96 hr IC10 = 32.6 (32.6-32.6)%
96 hr EC50 = 35.4%
(Geometric Mean Method)
NOEC = 25%
LOEC = 50%

Sample 10 Concentration (%)	% Un-Affected (Mean ± SD)
0 (control)	100.0 ± 0.0
6.25	100.0 ± 0.0
12.5	100.0 ± 0.0
25	100.0 ± 0.0
50	0.0 ± 0.0
100	0.0 ± 0.0

96 hr IC10 = 32.6 (32.6-32.6)%
96 hr EC50 = 35.4%
(Geometric Mean Method)
NOEC = 25%
LOEC = 50%

Sample 11 Concentration (%)	% Un-Affected (Mean ± SD)
0 (control)	100.0 ± 0.0
6.25	100.0 ± 0.0
12.5	100.0 ± 0.0
25	100.0 ± 0.0
50	0.0 ± 0.0
100	0.0 ± 0.0

96 hr IC10 = 32.6 (32.6-32.6)%
96 hr EC50 = 35.4%
(Geometric Mean Method)
NOEC = 25%
LOEC = 50%

Sample 12 Concentration (%)	% Un-Affected (Mean ± SD)
0 (control)	100.0 ± 0.0
6.25	100.0 ± 0.0
12.5	100.0 ± 0.0
25	100.0 ± 0.0
50	100.0 ± 0.0
100	0.0 ± 0.0*

96 hr IC10 = 32.6 (32.6-32.6)%
96 hr EC50 = 35.4%
(Geometric Mean Method)
NOEC = 25%
LOEC = 50%

Vacant

Vacant

QA/QC Parameter	Criterion	This Test	Criterion met?
Control mean % un-affected	≥90%	100 ± 0.0%	Yes
Test Temperature limits	25.0 ± 1ºC	25.0 ± 1ºC	Yes
Reference Toxicant within cusum chart limits	0.96-1.32 mg Cu^{2+}/L	1.05 mg Cu^{2+}/L	Yes

Test Report Authorised by: Dr Rick Krassoi, Director on 14 October 2008

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

NATA Accredited Laboratory Number: 14709

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Toxicity Test Report: TR0341/9

Client:	RPS 290 Churchill Ave Subiacco WA 6008	**ESA Job #:** **Date Sampled:** **Date Received:**	PR0341 28 July 2008* 29 July 2008
Attention:	Dr Craig Styan	**Sampled By:**	Client
Client Ref #:	M08504	**ESA Quote #:**	PR0341_q01&q02

* Sampling date of seawater to be used for production of brine

Lab ID No.:	Sample Name:	Sample Description:
2817	***1***- Brine Only	Brine prepared by evaporation of seawater
2817	***2***- Veolia Brine Effluent + Backwash	Brine prepared by evaporation of seawater, with additives as specified by client
2817	***3***- Veolia Brine Effluent + Backwash + CIP (variant a)	Brine prepared by evaporation of seawater, with additives as specified by client
2817	***4***- Veolia Brine Effluent + Backwash + CIP (variant d)	Brine prepared by evaporation of seawater, with additives as specified by client
2817	***5***- ITT Brine Effluent + Backwash	Brine prepared by evaporation of seawater, with additives as specified by client
2817	***6***- ITT Brine Effluent + Backwash + CIP (variant b)	Brine prepared by evaporation of seawater, with additives as specified by client
2817	***7***- Osmoflow Brine Effluent + Backwash	Brine prepared by evaporation of seawater, with additives as specified by client
2817	***8***- Osmoflow Brine Effluent + Backwash + CIP (variant c)	Brine prepared by evaporation of seawater, with additives as specified by client
2817	***9***- Osmoflow Brine Effluent + Backwash + CIP (variant d)	Brine prepared by evaporation of seawater, with additives as specified by client
2817	***10***- GE Brine Effluent + Backwash	Brine prepared by evaporation of seawater, with additives as specified by client
2817	***11***- GE Brine Effluent + Backwash + CIP (variant e)	Brine prepared by evaporation of seawater, with additives as specified by client
2817	***12***- Backwash only (NaCLO 0.53mg/L)	Brine prepared by evaporation of seawater, with additives as specified by client

Test Performed:	96-hr acute toxicity test with the polychaete worm (Beach worm) *Diopatra dentata*
Test Protocol:	ESA SOP 107, based on APHA (1998) and USEPA-PSEP (1996)
Deviations from Protocol:	Nil
Comments on Solution Preparation:	A simulated brine of 60‰ was prepared by evaporating seawater collected off Barrow Island (lab ID 2817) in 80L HDPE carboys. The Barrow Island seawater was filtered to 0.45µm using a peristaltic pump and Sartorius Sartobran filter capsule prior to being poured into the carboys. Evaporation was assisted by heating the seawater to 30°C with the aid of aquarium heaters, and with vigorous aeration using aquarium aerators. The salinity of the seawater was monitored daily, and upon reaching 60‰, the solution were poured off into 2.5L amber glass winchers for holding under refrigeration at 4oC until required for testing. The process took approximately 4 weeks. The test solutions were prepared on the day/evening prior to initiating the toxicity tests given the complex and time-consuming nature of the brine additive preparation procedure. A full breakdown of additives for each brine sample is provided in the annex to this report.
Source of Test Organisms:	Hatchery reared, Lake Macquarie NSW
Test Initiated:	25 September 2008 at 2000h

Sample 1	
Concentration (%)	**% Survival (Mean ± SD)**
0 (control)	100.0 ± 0.0
6.25	100.0 ± 0.0
12.5	100.0 ± 0.0
25	100.0 ± 0.0
50	30.0 ± 11.6*
100	0.0 ± 0.0
96 hr IC10 = 40.6 (39.0-42.0)%	
96 hr LC50 = 43.5 (37.8-50.2)%	
(TSK trim value= 0.0%)	
NOEC = 25%	
LOEC = 50%	

Sample 2	
Concentration (%)	**% Survival (Mean ± SD)**
0 (control)	100.0 ± 0.0
6.25	100.0 ± 0.0
12.5	100.0 ± 0.0
25	100.0 ± 0.0
50	50.0 ± 11.6*
100	0.0 ± 0.0
96 hr IC10 = 42.4 (40.9-44.1)%	
96 hr LC50 = 50.0 (42.8-58.4)%	
(TSK trim value= 0.0%)	
NOEC = 25%	
LOEC = 50%	

Sample 3	
Concentration (%)	**% Survival (Mean ± SD)**
0 (control)	100.0 ± 0.0
6.25	100.0 ± 0.0
12.5	100.0 ± 0.0
25	100.0 ± 0.0
50	30.0 ± 20.0*
100	0.0 ± 0.0
96 hr IC10 = 40.6 (39.0-42.0)%	
96 hr LC50 = 43.5 (37.8-50.2)%	
(TSK trim value= 0.0%)	
NOEC = 25%	
LOEC = 50%	

* Significantly lower % surviving juvenile worms compared with the control treatment (Steel's many-one Rank Test, 1 tailed, P=0.05).

Sample 4	
Concentration (%)	**% Survival (Mean ± SD)**
0 (control)	100.0 ± 0.0
6.25	100.0 ± 0.0
12.5	100.0 ± 0.0
25	95.0 ± 10.0
50	15.0 ± 10.0*
100	0.0 ± 0.0
96 hr IC10 = 28.0 (21.6-46.6)%	
96 hr LC50 = 37.9 (33.3-43.1)%	
(TSK trim value= 0.0%)	
NOEC = 25%	
LOEC = 50%	

Sample 5	
Concentration (%)	**% Survival (Mean ± SD)**
0 (control)	100.0 ± 0.0
6.25	100.0 ± 0.0
12.5	100.0 ± 0.0
25	95.0 ± 10.0
50	40.0 ± 16.3*
100	0.0 ± 0.0
96 hr IC10 = 41.5 (40.0-43.0)%	
96 hr LC50 = 46.7 (40.1-54.3)%	
(TSK trim value= 0.0%)	
NOEC = 25%	
LOEC = 50%	

Sample 6	
Concentration (%)	**% Survival (Mean ± SD)**
0 (control)	100.0 ± 0.0
6.25	100.0 ± 0.0
12.5	100.0 ± 0.0
25	95.0 ± 10.0
50	30.0 ± 25.8*
100	0.0 ± 0.0
96 hr IC10 = 40.6 (35.4-43.5)%	
96 hr LC50 = 43.5 (37.8-50.2)%	
(TSK trim value= 0.0%)	
NOEC = 25%	
LOEC = 50%	

* Significantly lower % surviving juvenile worms compared with the control treatment (Steel's many-one Rank Test, 1 tailed, P=0.05).

Sample 7	
Concentration (%)	**% Survival (Mean ± SD)**
0 (control)	100.0 ± 0.0
6.25	100.0 ± 0.0
12.5	100.0 ± 0.0
25	95.0 ± 10.0
50	65.0 ± 19.2*
100	0.0 ± 0.0
96 hr IC10 = 44.1 (41.5-48.1)%	
96 hr LC50 = 55.5 (47.9-64.3)%	
(TSK trim value= 0.0%)	
NOEC = 25%	
LOEC = 50%	

Sample 8	
Concentration (%)	**% Survival (Mean ± SD)**
0 (control)	100.0 ± 0.0
6.25	100.0 ± 0.0
12.5	100.0 ± 0.0
25	100.0 ± 0.0
50	0.0 ± 0.0
100	0.0 ± 0.0
96 hr IC10 = 47.2 (44.8-49.4)%	
96 hr LC50 = 43.5 (37.8-50.2)%	
(TSK trim value= 0.0%)	
NOEC = 25%	
LOEC = 50%	

Sample 9	
Concentration (%)	**% Survival (Mean ± SD)**
0 (control)	100.0 ± 0.0
6.25	100.0 ± 0.0
12.5	100.0 ± 0.0
25	100.0 ± 0.0
50	0.0 ± 0.0
100	0.0 ± 0.0
96 hr IC10 = 47.2 (44.8-49.4)%	
96 hr LC50 = 43.5 (37.8-50.2)%	
(TSK trim value= 0.0%)	
NOEC = 25%	
LOEC = 50%	

* Significantly lower % surviving juvenile worms compared with the control treatment (Steel's many-one Rank Test, 1 tailed, P=0.05).

Toxicity Test Report: TR0341/9

Sample 10	
Concentration (%)	**% Survival (Mean ± SD)**
0 (control)	100.0 ± 0.0
6.25	100.0 ± 0.0
12.5	100.0 ± 0.0
25	95.0 ± 10.0
50	65.0 ± 19.2*
100	0.0 ± 0.0
96 hr IC10 = 44.1 (41.5-48.1)%	
96 hr LC50 = 55.5 (47.9-64.3)%	
(TSK trim value= 0.0%)	
NOEC = 25%	
LOEC = 50%	

Sample 11	
Concentration (%)	**% Survival (Mean ± SD)**
0 (control)	100.0 ± 0.0
6.25	100.0 ± 0.0
12.5	100.0 ± 0.0
25	95.0 ± 10.0
50	30.0 ± 11.6*
100	0.0 ± 0.0
96 hr IC10 = 40.6 (39.0-42.0)%	
96 hr LC50 = 43.5 (37.8-50.2)%	
(TSK trim value= 0.0%)	
NOEC = 25%	
LOEC = 50%	

Sample 12	
Concentration (%)	**% Survival (Mean ± SD)**
0 (control)	100.0 ± 0.0
6.25	100.0 ± 0.0
12.5	100.0 ± 0.0
25	95.0 ± 10.0
50	20.0 ± 16.3
100	0.0 ± 0.0
96 hr IC10 = 39.6 (36.0-41.9)%	
96 hr LC50 = 40.6 (35.9-46.0)%	
(TSK trim value= 0.0%)	
NOEC = 25%	
LOEC = 50%	

* Significantly lower % surviving juvenile worms compared with the control treatment (Steel's many-one Rank Test, 1 tailed, P=0.05).

QA/QC Parameter	Criterion	This Test	Criterion met?
Control mean % survival	>90 %	100%	Yes
Test Temperature limits	25.0 ± 1ºC	25.0 ± 1ºC	Yes
Reference Toxicant within cusum chart limits	Not available*	140 μg Cu^{2+}/L	Yes

*Reference toxicant cusum chart limits are not available due to limited testing with this species.

Test Report Authorised by: Dr Rick Krassoi, Director on 15 October 2008

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