Appendix D Whole of Effluent Toxicity (WET) Testing

APPENDIX D WET TESTING

Subsequent to the conclusion of the chemical selection process described in Appendix A Whole Effluent Toxicity (WET) testing was conducted on Hydrosure 0-3670R (Champion Chemicals Pty Ltd) to identify the potential toxicity of the effluent following discharge to the marine environment. Toxicity of Hydrosure 0-3670R was evaluated using product obtained from the manufacturer, diluted in seawater obtained from the Wheatstone operational area. A series of dilutions of the Hydrosure 0-3670R in seawater were prepared to test a range of product concentrations in order to determine the relative toxicity to individual species.

Measures of toxicity from all of the test species were combined to develop environmental performance objectives, standards and measurement criteria for comparison with the anticipated concentrations of the flooding fluid at the time of flooding (500 ppm of Hydrosure 0-3670R). These assumptions do not take into account any degradation of the active constituents within the hydrotest flooding fluid while held within the pipe.

A1.0 WET Testing Methods

WET testing involves exposing organisms to various concentrations of an effluent or flooding fluid and then measuring a pre-determined experimental endpoint (e.g. mortality, growth, or reproductive characteristics) after a selected period of time (ANZECC & ARMCANZ 2000). WET testing was conducted on Hydrosure 0-3670R diluted with seawater and the dilution water was sourced from waters in the vicinity of the trunkline route (Latitude -21.28850^{o}, Longitude 114.51600^{o}). The water was collected directly from an onsite vessel in accordance with ANZECC & ARMCANZ (2000) procedures for water sampling. Water samples were packed in ice and sent via air freight directly to the NATA accredited ecotoxicity testing laboratory (Ecotox Service Australasia). Full details of the ecotoxicity methodology are presented in Chevron Australia (2013a).

WET testing was undertaken on five locally relevant species from four different taxonomic groups using the recommended protocols from ANZECC and ARMCANZ (2000). The Wheatstone marine project development area lies at the southern extent of NWS marine region which is considered to represent tropical waters as classified by IMCRA 4.0 (Commonwealth of Australia 2006). The waters in this region contain both tropical and temperate organisms so the selection of species from both regions for WET testing was considered relevant. WET testing included mostly tropical species from a range of trophic levels (primary producer, herbivore and carnivore), where chronic (where available) and acute tests for toxicity were applied.

WET tests were conducted using two experimental components. The first being a range finding test in which the effective concentration range for each experimental end-point and species was determined. Once the effective concentration range was determined, the definitive ecotoxicological assays were conducted over this concentration range to capture the complete dose response relationship. Since Hydrosure 0-3670R is a mixture containing both the oxygen scavenger and the biocide for chemical treatment, only one assay in each test species was necessary to evaluate the toxicity of the product.

Concentration range varied depending on the test species and experimental end-point. The concentrations used for each test can be found in (Table D 1). In all experiments, the ratio of concentrations selected was relative to the following proportions: 100, 50, 25, 12.5 and 6.25 %. Four replicates (N=4) were used in all experiments for each test concentration.

Table D 1: Whole Effluent Toxicity Testing of Hydrotest Medium Discharges in Commonwealth Waters

Species	Test	Type	End-Point	Temp ˚C	Conc.
Nitzschia closterium (Algae)	72 hr Growth Inhibition	Chronic	Cell yield	21 ± 1	0.2–10.0 mg/L
Saccostrea echinata (Mollusc)	48 hr Larval Abnormality	Chronic	Normal development rate	29 ± 1	31.3–2000.0 µg/L
Heliocidaris tuberculata (Echinoderm)	72 hr Larval Development	Chronic	Normal development rate	20 ± 1	78.1–5000.0 µg/L
Melita plumulosa (Crustacean)	96 hr Acute Toxicity	Acute*	Survival	20 ± 1	0.03–1.00 mg/L
Lates calcifer (Fish)	96 hr Acute Toxicity	Acute*	Imbalance	25 ± 2	6.3–100.0 mg/L

*ACR=10, where ACR is the Acute to Chronic Ratio used as a divisor for transformation of acute values into chronic test values.

Two main procedures are currently used for developing single species toxicity measures based on these ecotoxicity tests, hypothesis testing and point estimation techniques. Hypothesis testing using Dunnett's test (Dunnett 1955) compares each test concentration in order to determine the lowest test concentration that is significantly different from the dilution water control (the Lowest Observed Effect Concentration - LOEC); the No Observed Effect Concentration (NOEC) is then inferred to be the highest test concentration below the LOEC. Point estimation techniques use regression analysis of the dose response curve to derive a figure such as EC_p (Effect Concentration), the concentration that causes a stated effect in ‘p’ percent of the test organisms.

There is debate over the appropriateness of each estimate of single species toxicity when extrapolating results to the wider aquatic ecosystem; however the prevailing guidance in ANZECC and ARMCANZ (2000) appears to advocate the use of NOEC (hypothesis tested). Both endpoints have been reported in the presentation of results. Test results for locally relevant species used for the WET testing program are described in Table D 2. Chronic tests were selected where available for that taxonomic group.

Table D 2: WET testing results for flooding fluid / medium treated containing Hydrosure

Species	Duration (hrs)	EC10 (mg/L)	EC50 (mg/L)	LOEC (mg/L)	NOEC (mg/L)
Nitzschia closterium (Algae)	72	1.5 *	3.3 (3.0–3.58)	2.50	1.30
Saccostrea echinata (Mollusc)	48	0.29 (0.24–0.33)	0.54 (0.52–0.56)	0.50	0.250
Heliocidaris tuberculata (Echinoderm)	72	1.30 (1.27–1.32)	1.71 (1.70–1.74)	2.50	1.25
Melita plumulosa (Crustacean)[#]	96	0.08 (0.04–0.11)	0.14 (0.10–0.16)	0.25	0.13
Lates calcifer (Fish)[#]	96	13.5 (12.3–18.0)	17.5 (17.1–18.0)	25.0	12.5

*95% confidence limits are not reliable; Numbers in brackets represent the 95% fiducial limits.
[#] Toxicity test is defined as an acute test.

Single species toxicity assessments for flooding fluid, Hydrosure 0-3670R, showed toxicity in all species tested. This result was expected given that the active substances of the chemical treatment (oxygen scavenger and biocide) are designed to interfere with natural (bio)-chemical processes. For Hydrosure the species rank toxicity (NOEC) from most observed toxicity to least was: crustacean > oyster (mollusc) > sea urchin (echinoderm) = microalgae > fish.

A2.0 Environmental Criteria

Environmental criteria have been developed to be applied to a discharge of a large volume of flooding fluid (approximately 220 000 m^3) over a duration of approximately 6 - 8 days. Discharges associated with other activities are significantly smaller and for a shorter duration. Application of the same environmental criteria to these smaller discharges can be viewed as a more conservative approach.

Single species WET test laboratory results can be extrapolated to effects in the wider aquatic ecosystem using a risk based approach. By investigating the statistical distribution of all of the single species toxicities, guideline values can be developed to estimate the maximum concentrations unlikely to cause adverse environmental effects. Environmental criteria to be applied to the discharge were developed from the results of WET testing (Table D 2) using the recommended methods in ANZECC and ARMCANZ (2000), with the three following modifications:

1. The ANZECC and ARMCANZ (2000) trigger development methods are intended for application to long-term outfalls that result in chronic effects, with long-term continuous exposure. Any NOECs derived from an acute toxicity test (*Melita plumulosa* and *Lates calcarifer* in the current tests) can be converted to a chronic value by dividing by the ACR. The discharge of flooding fluid is not a chronic

discharge (approximately 192 hrs at approximately -40 m LAT) so an acute toxicity trigger is more appropriate. Therefore the ACR was not applied to the acute toxicity test results; test results defined as chronic were retained for use without transformation.

2. Effluent toxicity is based on a concentration by time interaction. There are some difficulties in extrapolating results from a laboratory (where concentrations can be maintained throughout the duration) to application in the field (where concentrations at a single point will vary over time depending on the metocean conditions). For this reason, the best available approach for a concentration that varies over time was to compare the fixed laboratory value with the median field concentration of the chemical over a defined duration.
3. As a methodology for chronic discharge, the triggers developed using the ANZECC and ARMCANZ (2000) guidelines do not specify a prescribed duration of exposure. Again this is not appropriate for a short term exposure of a potentially acutely toxic discharge, as is the case here. It is appropriate that if an acute intensity trigger is to be used, this must be associated with specific exposure duration. The durations of exposures in the laboratory tests ranged from 48–96 hrs. The discharge will be subject to hydrodynamic conditions that result in higher median concentrations over short durations than at longer durations. Therefore the most conservative approach (i.e. the highest median value in the field) is to establish an exposure duration for trigger comparison based on the minimum test duration, in this case 48 hrs

The latest available version of the BurrliOZ software (TclPro Application V8.3.2, last modified: 25 July 2001; Copyright 2000 Ajuba Solutions) supplied with the National Water Quality Management Strategy Paper No. 4 package (ANZECC & ARMCANZ 2000) was used to analyse the ecotoxicity of Hydrosure 0-3670R. The NOEC values were used as the statistical endpoints from the single species ecotoxicity testing for estimation of the species sensitivity distribution (SSD), fitted using BurrliOZ. Full details of the methodology are included in MScience (2013). Calculation of environmental criteria for the flooding medium using the SSD and raw NOEC values are presented in Table D 3.

Table D 3: Species protection concentrations for Hydrosure 0-3670R based on the NOEC SSD from WET testing

	PC99% (ppm or mg/l)	PC95% (ppm or mg/l)	PC90% (ppm or mg/l)	PC80% (ppm or mg/l)
Hydrosure (based on NOEC)	0.06	0.10	0.15	0.23

The 99% species protection concentration is suggested by ANZECC and ARMCANZ (2000) for development of environmental criterion for *high conservation ecosystems* or chemicals that have a tendency to bioaccumulate. This would result in an environmental criterion trigger of 0.06 mg/L (or ppm). Since Hydrosure 0-3670R has a negligible risk for bioaccumulation the 95 % level of species protection may also be applied. In this instance the environmental criterion trigger would be 0.10 mg/L (or ppm). These criteria have been developed for chronic discharges and do not recognise the duration of the exposure to the flooding fluid.

In order to establish a conservative environmental criterion for application in Commonwealth waters, the data from Table D 3 and the three modifications listed above were applied. The environmental criterion applied to modelling and assessment was therefore defined as follows:

- Over a 48 hour period, the median concentration of Hydrosure 0-3670R is not to exceed 0.06 mg/L.

As the Commonwealth Conditions for approval (EPBC/2008/4469) do not define spatial zones of environmental protection, this criterion applies to all spatial points around the discharge. Since the concentration of Hydrosure 0-3670R in the flooding fluid is 500 ppm, significantly greater than 0.06 ppm, there is no scenario in which the discharge will be able to comply with this environmental criterion at all locations and times around the discharge. As such, this criterion will be used to interrogate the results of the modelling to define a mixing zone, outside of which the environmental criterion will be considered to have been met.

As Hydrosure 0-3670R has a negligible risk for bioaccumulation (Appendix A) and the discharge is to occur over a short period (less than 192 hrs), no additional environmental criteria have been set.

Unpublished data for the degradation profile for Hydrosure 0-3670R during a 12 month field simulation test shows approximately 20% reduction in activity over 12 months at 10 °C (Figure D 1). Based on this data, the application of the results of the WET testing program to develop environmental criteria is considered to represent a conservative approach. That is, while the Hydrosure toxicity to local species remains constant (e.g. the environmental criterion), the concentration of the toxic components in the flooding fluid discharge (e.g. those used in the modelling) will likely be reduced from the concentrations known at the time of flooding (i.e. 500 ppm). Therefore the extent of the mixing zone defined by modelling is predicted to be larger than the actual mixing zone during discharge.

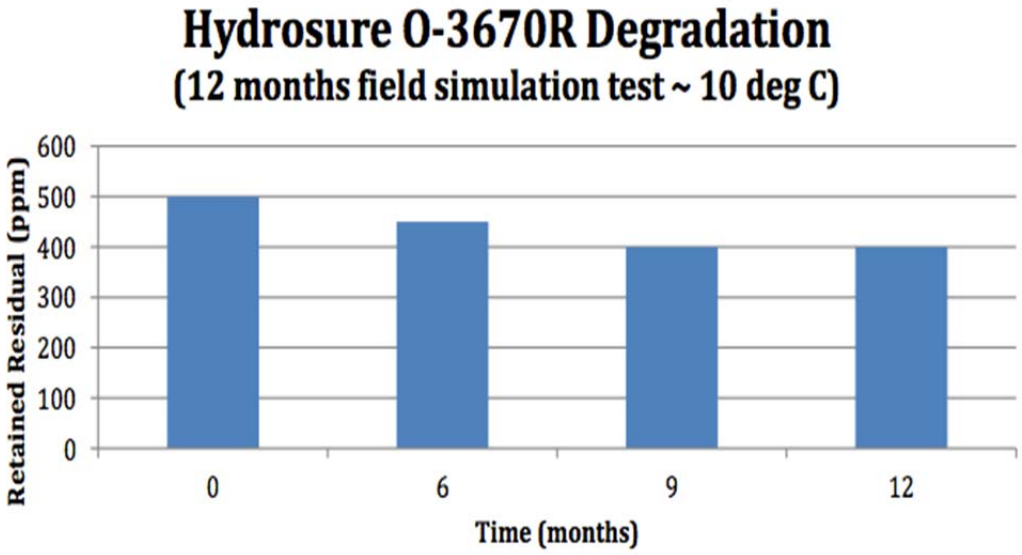

Figure D 1: Hydrosure 0-3670R degradation profile (source: Champion Technologies 2011)

In addition to Hydrosure 0-3670R, the flooding medium is also planned to contain Fluorescein dye (50 ppm). The ecological information in the Fluorescein MSDS report the product is not expected to be hazardous to the environment (Champion Technologies 2011) and estimates of the lethal concentration of sodium fluorescein solution (Walthall and Stark 1999) were higher than the concentrations proposed for use in FCGT activities of the trunkline (50 ppm). Based on the routine use of Fluorescein in offshore projects, WET testing and environmental criteria were not considered relevant for this compound.