Toxikos
Toxicology Consultants

ABN 30 095 051 791
PO Box 74
Caulfield East
Victoria, 3145
Ph: +61 3 9569 3088
Fax: +61 3 9569 3618

# Assessment of additional Whole Effluent Testing relevant to Bell Bay Pulp Mill Effluent.

**Prepared for: Lawson Harding**
**Gunns Ltd**

**Prepared by: John Frangos M App Sc (Toxicol.)**

**Toxikos document: TC250210-JF**
**25th February 2010**

## Introduction & Scope

The Marine Impact Assessment report (Toxikos Document: TR101006-RJF, January 2007) included a summary and assessment of whole effluent testing coordinated by Ecotox Services Australasia (ESA 2006 a,b) on pulp mill effluents from Thailand and South America.

Since then additional testing has been conducted using a sample collected from a Brazilian pulp mill using similar technologies to that proposed a Bell Bay (Permit Condition 34). Two reports were provided to Toxikos:

- ESA (2009a) “Toxicity Assessment of Pulp Mill Effluent for the Proposed Tasmanian Pulp Mill – Brazil Pulp Mill Sample report number PR0490, dated December 2009.

- ESA (2009b) “Toxicity Assessment of a Brazilian Pulp Mill Sample to Gametophytes of the Kelp Ecklonia radiata”, report number TR0490/1, dated December 2009”.

The tests reported by ESA (2006a, b) & (2009a,b) now consider at least four taxonomic groups and nine species. The number of species tested is greater than the minimum species typically required in a valid WET program. For instance ANZECC (2000 pg 8.3-66) states that a minimum of three species from different trophic levels is generally required.

Table 1 summarises the tests conducted with a Brazilian pulp mill sample by Ecotox Services Australasia (ESA 2009 a,b) on behalf of the Gunns Ltd. The summaries have been generated in a consistent manner to those produced within Toxikos (2007, in particularly Table 9.2a,b). The tests conducted by ESA are consistent with the *State Policy on Water Quality Management* monitoring requirements and with tests recommended by both ANZECC and the National Pulp Mills Research Program (NPMRP, ANZECC 2000, Stauber 1994).

Four tests and three additional test species; *Ecklonia radiata*, a brown alga found in deep water around the northern Tasmanian coastline, *Pagrus auratus* (Pink Snapper - commonly found in Tasmanian coastal waters) and a freshwater water flea (*Ceriodaphnia dubia*) were included in the latest round of testing.

**Table 1: Toxicity tests conducted by Ecotox Services Australasia [a] to assess toxicity of pulp mill effluents**

| Taxonomic group Species | Endpoints | Test Type | Listed by ANZECC as a major Australian bioassay [b] | Recommended by the National Pulp Mill Research Program [b] |
|---|---|---|---|---|
| **Bacteria** | | | | |
| Microtox – *Vibrio fischeri* | Light output (luminescence) | Acute 5, 15, 30 minute | ✓ (15 min) | |
| **Algae** | | | | |
| Marine microalga – *Nitzschia closterium* | Growth rate (cell division rate) | Chronic 3 day (72 hour) | ✓ | ✓ |
| Marine macroalga – *Ecklonia radiata* | Germination success | Sublethal 3 day (72 hour) | ✓ | |
| Marine macroalga – *Ecklonia radiata* | Growth rate | Sublethal 14 day | | |
| Marine macroalga – *Hormosira banksii* | Germination success | Sublethal 3 day (72 hour) | ✓ | ✓ (2 hour) |
| **Invertebrates** | | | | |
| Sea urchin *Heliocidaris tuberculata* | Fertilisation success. | Sub-lethal 1 hour exposure plus 20 minute fertilisation time. | ✓ | ✓ (1-2 hour) |
| Sea urchin *Heliocidaris tuberculata* | Developmental toxicity (larval abnormality) | Sub-lethal 3 day (72 hour) | ✓ | |
| Doughboy scallop *Mimachlamys asperrima* | Developmental toxicity (larval abnormality) | Sub-lethal 2 day (48 hour) | ✓ | ✓ |
| Amphipod *Allorchestes compressa* | Survival | Acute 4 day (96 hour) | ✓ | |
| **Fish** | | | | |
| Striped trumpeter *Latris lineata* | Imbalance including larval survival. | Acute 4 day (96 hour) | ✓ | Tasmanian blenny (*Parablennius tasmanianus*) 96-h larval survival test |
| Pink snapper *Pagrus auratus* | Imbalance including larval survival | Acute 4 day (96 hour) | ✓ | As above |

[a] Ecotox Services Australasia (ESA 2006a,b and ESA 2009a,b).

[b] ANZECC (2000, Vol 2 section 8.3.6). A tick (✓) indicates that the test is listed, a blank cell indicates that the test was not considered.

## Test results

According to the *State Policy on Water Quality Management* (Section 21) the objective of aquatic toxicity tests with effluents are to ensure:

- Acute toxicity (using the endpoint of the $IC_{50}$, $EC_{50}$, or $LC_{50}$) will not be observed in discharge effluent (i.e. 100% effluent does not cause mortality), and/or
- Chronic toxicity (using the NOEC as the endpoint) does not occur at the edge of the mixing zone.

Tables 2 summarises the results of ESA (2009a,b). A red line is drawn across the table at the lowest no observed effect concentration of any test, indicating the dilution that is not expected to cause adverse effects to receiving waters. All three rounds of testing conducted to date meet the objective.

Consistent with the NPMRP and Canadian findings, marine invertebrate tests with reproductive endpoints (48-h doughboy scallop larval assay, 72-h sea urchin larval development assay) were found to be sensitive to the effects of the two pulp mill effluents.

## Conclusion:

Assuming the Bell Bay pulp mill produces an effluent similar to the sample tested from a Brazilian pulp mill the Bell Bay effluent would be expected to meet the water quality objectives for whole effluent toxicity.

Toxikos
Toxicology Consultants

**Table 2: Summary of WET testing results for pulp mill effluent - Eucalypt (Brazil 2009)**

Cells shaded yellow indicate the response at this test concentration is significantly different to control

1:100 dilution $DV_{100}$ Lowest NOEC

| Test Concentration % Effluent | Acute | | | | Sub-lethal [a] | | | | | | Chronic |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Microtox acute toxicity test | 48-hr *Cerio-daphnia dubia* | 96-h juvenile amphipod survival | 96-h larval fish imbalance | 1 hr 20 min Sea urchin fertilisation | 72-h sea urchin larval development | 48-hr doughboy scallop larval development | 72-h *Hormosira* macro-algal germination | 72-h *Ecklonia radiata* macro-algal germination | 14-d *Ecklonia radiata* macro algal growth | 72-h Nitzschia micro-algal growth test |
| | % light output | % Survival | % Fertilised Eggs | Mean healthy fish | % Fertilised Eggs | % Fertilised Eggs | % Fertilised Eggs (normal larvae) | % Fertilised Eggs (germinated) | % Fertilised Eggs | Length (µm) | % Cell yield |
| **Eucalypt** | | | | | | | | | | | |
| **0 (FSW)** | 97.5±5.0 | 100±0.0 | 95.0±10.0 | 97.5±5.0 | 93.3±1.7 | 92.8±1.7 | 79.8±3.3 | 74.3±7.3 | 93.5±2.1 | 26.0±2.5 | 39.1±3.5 |
| **0 (ASW)** | 89.2±12.5 | 100±0.0 | 90.0±11.6 | 89.2±12.5 | 93.0±1.4 | 93.0±2.6 | 77.5±4.5 | 74.3±10.5 | 95.5±2.7 | 24.7±2.3 | 4.9±0.3 |
| **1.6** | 92.5±9.6 | 100±0.0 | 95.0±10.0 | 92.5±9.6 | 93.8±2.2 | 93.0±2.9 | 77.8±5.1 | 82.5±7.1 | 95.3±2.1 | 24.8±2.3 a | 31.8±2.7 [b] |
| **3.1** | 80±21.6 | 100±0.0 | 100±0.0 | 80.0±21.6 | 93.8±3.1 | 94.5±2.7 | 78.0±1.8 | 77.5±5.8 | 94.3±2.2 | 25.3±1.0 | 36.0±0.8 [b] |
| **6.3** | 95.0±5.8 | 100±0.0 | 90.0±11.6 | 95.0±5.8 | 92.5±2.7 | 92.5±2.1 | 77.0±4.4 | 76.5±7.9 | 95.0±2.9 | 25.0±1.3 | 36.0±0.8 |
| **12.5** | 95.0±10.0 | 100±0.0 | 95.0±10.0 | 95.0±10.0 | 93.5±3.1 | 93.5±3.1 | 77.0±3.2 | 77.8±8.9 | 94.8±1.7 | 25.0±0.8 | 40.1±5.7 |
| **25** | 87.5±5.0 | 100±0.0 | 95.0±10.0 | 87.5±5.0 | 92.3±1.5 | 94.8±2.8 | 76.8±4.0 | 73.0±7.4 | 92.8±2.5 | 25.1±1.0 | 46.3±1.6 |
| **50** | 95.0±10.0 | 100±0.0 | 90.0±11.6 | 95.0±10.0 | 94.5±2.7 | 91.0±2.2 | 74.8±1.5 | 75.8±9.1 | 94.0±3.4 | 25.5±1.1 | 47.5±4.0 |
| **100** | 82.5±20.6 | 100±0.0 | 75.0±25.2 | 82.5±20.6 | 92.5±2.7 | **43.0±7.8** | **57.8±10.1** | 68.5±3.7 | 94.0±1.8 | 23.8±1.2 | 45.8±3.3 |

[a] Sublethal describes the nature of the endpoint of the test. These tests are commonly considered short term tests for predicting chronic toxicity. Thus they are commonly used for assessing hazards and risks due to chronic exposure (ANZECC 2000, US EPA 2002).

[b]. Significantly reduced cell yield was observed at 1.6% and 6.3% effluent but not at higher concentrations of effluent. However the effect is unlikely to be due to the treatment with effluent as the response is not concentration related. Previous studies with pulp mill effluents & copper have not reported similar findings (i.e. non concentration related responses Stauber 1994, 1996, Eriksen et al 2001). Salinity has a significant effect on the growth rates of Nitzschia closterium (doubling is linearly related to salinity Eriksen 2001), so in order to calculate the effect of sample toxicity on Nitzschia, the sample salinity must be taken into account (Stauber 1994, Eriksen 2001). This is normally controlled by measuring variation in cell division rates per day (1.4±0.2 doublings per day). Given the above the most likely explanation for the variation in cell division rates is a physical difference between the individual cultures (i.e. non effluent related effect).

## References

Eriksen, R., S., Nowak, B., and van Dam, R., A. (2001). Copper speciation and toxicity in a contaminated estuary. Supervising Scientist Report 163, Supervising Scientist, Darwin. http://www.environment.gov.au/ssd/publications/ssr/pubs/ssr163-printquality.pdf

Ecotox Services Australasia (ESA) (2006c). Toxicity assessment of pulp mill effluent for the proposed Tasmanian pulp-mill – Pine pulping campaign. ESA Test Report PR0177 April 2006.

Ecotox Services Australasia (ESA) (2006d). ). Toxicity assessment of pulp mill effluent for the proposed Tasmanian pulp-mill. ESA Test Report PR0177 June 2006.

ESA (2009a) "Toxicity Assessment of Pulp Mill Effluent for the Proposed Tasmanian Pulp Mill – Brazil Pulp Mill Sample report number PR0490, dated December 2009.

ESA (2009b) "Toxicity Assessment of a Briazillian Pulp Mill Sample to Gametophytes of the Kelp Ecklonia radiata", report number TR0490/1, dated December 2009".

Stauber, J.L., Tsai, J., Vaughan, G.T., Peterson, S.M., Brockbank, C.I., 1994. Algae as indicators of toxicity of the effluent from bleached eucalypt kraft pulp mills. In: National Pulp Mills Research Program Technical Report No. 3. CSIRO, Canberra, p. 146.

Stauber, J., L., Ahsanullah, M., Nowak, B., and Florence, T., M. (1996). Toxicity assessment of waters from Macquarie Harbour, Western Tasmania using algae, invertebrates and fish, Mount Lyell Remediation Research and Demonstration Program. Supervising Scientist Report 112, Supervising Scientist, Canberra.

US EPA (2002). Short-term methods for estimating the chronic toxicity of effluents and receiving waters to marine and estuarine organisms. Third Edition. EPA-821-R-02-014. United States Environmental Protection Agency. Office of Water.