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Research notes on possible dilution factors for the proposed Bell Bay Pulp Mill based on ecotoxicity results for a Brazilian pulp mill's effluent

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Executive Summary

Gunns Ltd. has requested Toxikos to provide preliminary modelling of the dilution factors that would apply to the proposed Bell Bay pulp mill effluent assuming the pulp mill effluent had an equivalent toxicity to the Brazilian pulp mill effluent previously evaluated for ecotoxicity through a sampling and analysis plan (SAP) approved by the Department of Environment, Water, Heritage and the Arts (DEWHA) as part of the Bell Bay mill statutory approval process.

Toxikos provided Gunns with version I of the research note (entitled TL071010-TJF), which was reviewed by Dr. Graeme Batley from the CSIRO. Toxikos has subsequently refined the report to this version (version 2), which takes into account the comments and suggestions that were made.

In Australia the standard technique for estimating trigger values for industrial discharges or chemical substances is a statistical distribution method called species

sensitivity distribution (SSD). The BurrliOZ software has been developed and used by ANZECC to determine Australian guideline values to protect Australian aquatic ecosystems (normally at a protection level of 95%).

The latest available version of the software was used in this exercise to predict the 95% and 99% protection levels of species (or dilution factor for a particular effluent) when using EC_{10} and EC_{50}[1] data for the Brazilian effluent.

The current ANZECC guidelines use chronic no observed effect concentration (NOEC) data to derive high reliability trigger values. There has been debate on the merits of using NOEC toxicity data for regulatory purposes (OECD 1998, DEWHA 2009a, ANZECC 2000, Hobbs and Warne 2008). Hobbs and Warne (2008) have summarised the limitations of using NOEC values:

- They are largely determined by concentrations chosen by the tester.
- The NOEC does not actually correspond to “no effect.” Instead, it typically corresponds to an EC_{10}-EC_{30}.
- They can be easily manipulated and do not encourage high quality work.
- Trigger values derived using NOECs do not have as clear a definition as those derived using EC_x data.

The OECD (1998) also concluded that using NOECs as the main summary parameter of aquatic toxicity tests is inappropriate mainly because NOECs are set by the study design (i.e. in this case, many NOECs were set at 100% effluent, because it is the highest concentration tested; however aquatic organisms may be able to tolerate much higher concentrations of chemicals in the effluent). It was recommended that regulators and other government bodies should move towards regression-based estimation, in which EC_x end-points are often preferred (OECD 1998, DEWHA 2009a, ANZECC 2000).

EC_{10} data is thus gaining more importance than NOEC data in terms of applicability for setting trigger values and discharge limits, as EC_{10}s are a statistical representation of concentrations that would result in 10% of species exhibiting adverse effects. ANZECC (2000) did not use EC_{10} data mainly because of the lack of this data in the general literature; NOECs were much more readily available at the time.

[1] EC_{10} data was used for chronic and sub-lethal endpoints, EC_{50} data was used for acute endpoints after application of an acute-to-chronic ratio of 10.

Because several EC_{10}s and EC_{50}s were reported as >100% of the effluent in the Brazilian dataset, two different approaches were used when plotting these data points. The first conservatively assumes all values reported as >100% are 101%. The other assumes all values are equal to 200%. The distributions assuming values >100% equal 200% fit the Burr Type III and log-logistic distribution better than when assuming values >100% equal 101%, but increased the trigger values slightly.

There were a total of two acute end-points deemed applicable for generation of a distribution; these had to be converted to chronic end-points. To convert acute values into chronic estimates, ANZECC (2000) generally applied a chemical-specific acute-to-chronic ratio (ACR) to acute EC_{50} data. Where chemical-specific ACRs were not available, a default ACR of 10 was applied. Thus the same approach was taken in this exercise by applying an ACR of 10 to acute EC_{50} data.

The ACR can be refined by determining the "true" ACR through a literature search.

There is some uncertainty associated with using the Burr Type III distribution with only seven data points (CSIRO 2000), and sometimes the log-normal or log-logistic distribution may provide a better fit of the data. Thus the 1st and 5th percentiles were visually estimated from the log-logistic distribution curves (these percentiles correspond to the 99th and 95th percent protection levels, respectively), and results from both methods presented. The resulting trigger values estimated from the log-logistic distribution were slightly greater than when using the Burr Type III distribution.

Table E1 provides the dilution factors and discharge 'limits' calculated for each scenario considered (assuming all values reported >100% are 101%[2]). Depending on the protection level and the distribution selected the dilution factors ranged between 9-33.

[2] The 101% values are depicted in Table E1, because the resulting discharge 'limits' are slightly lower than when using 200%. Table 2 shows the trigger values (i.e. discharge 'limits') for both assumptions.

Table E1 – Dilution factors and discharge limits calculated using Brazilian EC_{10} and EC_{50} data in Burrlioz

Species protection level	Distribution Used	Discharge 'limit' (%)	Dilution factor
95%	Burr Type III	9	11
	Log-logistic [a]	11	9
99%	Burr Type III	3	33
	Log-logistic [a]	5	20

[a] As the current version of the BurrliOZ software does not display the trigger value for the log-logistic distribution, this was visually estimated from the graphs.

Background

The Chief Scientist's Report to the then Minister for the Environment and Water Resources included a section on Whole-Effluent Toxicity (WET) (Point 61). The review comment noted that prior analyses on samples from Thailand and Chile were "*not designed with sufficient scientific rigour to demonstrate whether or not the effluent would adversely affect biota in the marine environment*". Subsequently the Ministers Approval Decision (EPBC2007/3385) included a specific condition (No. 35) to address the Chief Scientist's and others concerns regarding the original WET analyses.

Gunns Ltd. subsequently addressed Condition 35 as a component of their overall Hydrodynamic Modelling Project by (1) providing the Department (DEWHA) with a detailed Sampling and Analysis Plan (SAP) for their review and eventual formal approval and (2) obtaining access to a similar mill and notifying the Department of the mill's suitability and identity before implementing the SAP.

The SAP was successfully implemented in late 2009 with a suite of eight assays, which were all successfully carried out. In addition to the approved schedule of assays, Gunns placed a number of other assays on the analytical schedule to be undertaken if sufficient sample material was available, in order to provide further objective evidence of the effluent's quality beyond that required to comply with the approved SAP. The additional tests beyond the SAP were:

- 1-hr sea urchin fertilisation success using *Heliocidaris tuberculate*
- 72-hr macro-algal germination assay using *Ecklonia radiata*
- 14-d macro-algal gametophyte growth assay using *Ecklonia radiata*
- 48-hr acute *Ceriodaphnia dubia* immobilisation test.

This document provides a suitable interpretation of the information obtained by the Condition 35 study in the context of dilution requirements for the Hydrodynamic Modelling Project, assuming the characteristics of the selected Brazilian mill are analogous to that proposed for Bell Bay, Tasmania.

Introduction to BurrliOZ

The BurrliOZ software was used by ANZECC to derive Australian guideline values for the protection of aquatic ecosystems and is therefore widely accepted by Australian environmental protection authorities.

The software uses a maximum likelihood method to determine which particular member of the Burr Type III statistical distribution best fits the toxicity data, and calculates the concentration protective of any specified percentage of species. The original method was developed by Shao (2000), and the software was developed by the CSIRO Environmetrics Group for Environment Australia for ANZECC. The input data into the software must normally be chronic no observed effect concentration (NOEC) data, collected for a range of species. The maximum likelihood method used in the BurrliOZ software to select the statistical distribution that best fits the data can become unreliable when the dataset contains less than eight data values (ANZECC 2000). Generally, however, statistical methods can be applied if data are available for five or more species belonging to at least four different taxonomic groups (ANZECC 2000, pg. 8.3-97).

The latest available version of the software (1.0.14, dated 26th of July 2001) was used in generating the statistical distributions for this exercise.

BurrliOZ modelling results

Table 1 shows the toxicity data available for the Brazilian pulp mill effluent; only EC_{10} data was used from chronic and sub-lethal tests, and EC_{50} data was used from acute tests to construct a number of Burr Type III and log-logistic distributions. The data was divided into acute, sub-lethal, and chronic end-points, based on guidance in ANZECC (2000) and US EPA (2002).

ANZECC (2000, vol. 2, pg. 8.3-4) generally only uses endpoints measuring functions of life (e.g. mortality, reproductive impairment, hatchability, immobilization and inhibition of growth) when deriving trigger values for aquatic ecosystems. Thus behavioural (e.g. mobility, motility, burial rate, ventilation rates, swimming rate, etc) and biochemical (e.g. inhibition of bioluminescence, induction and activity of a range of enzymes, changes in DNA, etc) end-points are excluded.

The construction of the statistical distribution is complicated by several factors:

- The BurrliOZ software requires the data to be entered as concentrations (in mg/L or µg/L). All available toxicity data has been reported as a percentage of the effluent. This was dealt with by assigning the percentage effluent to a matching number reported as a concentration. Thus, 30% became 30 mg/L and 100% became 100 mg/L. The concentration that results in a 95 (or 99) percent protection level of species (the discharge limit) was then reported by BurrliOZ in mg/L, but was changed back to a percentage of the effluent by Toxikos.

- Several EC_{10}s are reported as >100% of the effluent. This signifies that the undiluted effluent was not toxic enough to cause an effect significantly different from controls. As the actual percentage that would result in effects is unknown (i.e. it could be as little as 101% or it could be greater than 500%), two different approaches were used when plotting these data points. The first conservatively assumes all values reported as >100% are 101%. The other assumes all values are equal to 200%. Although this method is unlikely to provide an accurate number for an acceptable discharge limit, it aids in evaluating the magnitude of the difference that could be expected.

- Toxicity data was sub-divided into three end-points: acute, sub-lethal and chronic. Sub-lethal 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). However, acute EC_{10} data was converted to chronic data using acute-to-chronic ratio of 10. As both acute EC_{50}s were greater than 100%, application of an ACR of 10 may overestimate the toxicity of the effluent to these organisms.

The BurrliOZ software was used in this exercise to predict the 95% and 99% protection levels of species when using Brazilian effluent EC_{10} data from chronic studies and EC_{50} data from acute studies converted to chronic endpoints with an ACR of 10.

In most cases, the 95% protection level applies to ecosystems that can be classified as slightly-moderately disturbed, although the higher protection level of 99% can sometimes be applied to slightly-moderately disturbed ecosystems if the goal is no change in biodiversity. In ANZECC, the higher protection level is used as the default level for ecosystems with high conservation value (ANZECC 2000, pg. 3.4-3). The agreed-upon level of protection for an ecosystem is ultimately up to a particular state jurisdiction or catchment manager.

Table 1: Toxicity data for Brazilian pulp mill effluent (reported as % effluent) [c]

	Test species	Brazilian effluent			
		EC_{10}	NOEC	LOEC	EC_{50}
Acute	*Allorchestes compressa* (amphipod, 96-h survival)	65	100	>100	>100
Acute	*Pagrus auratus* (fish, 96-h imbalance)	>100	100	>100	>100
Acute	*Ceridaphnia dubia* (water flea, 48-h survival) [a]	>100	100	>100	>100
Sub-lethal	*Heliocidaris tuberculata* (sea urchin, 1.3-h fertilisation) [a]	>100	100	>100	>100
Sub-lethal	*Heliocidaris tuberculata* (sea urchin, 72-h larval development)	62	50	100	97
Sub-lethal	*Mimachlamys asperrima* (doughboy scallop, 48-h larval development)	69	100	>100	>100
Sub-lethal	*Ecklonia radiata* (alga, 72-h germination) [a]	>100	100	>100	>100
Sub-lethal	*Ecklonia radiata* (alga, 14-day growth) [b]	>100	100	>100	>100
Sub-lethal	*Hormosira banksii* (alga, 72-h germination)	90	100	>100	>100
Chronic	*Nitzschia closterium* (diatom, 72-h growth)	>100	100	>100	>100

[a] Shaded cells represent toxicity test results which were excluded from generating BurrliOZ distributions. The *Ceriodaphnia* result was excluded as it is not appropriate to include results for a freshwater organism together with all the marine tests. The sea urchin fertilisation result was excluded, as only the most sensitive endpoint for a species should be used to construct the distribution. Thus only the results from the sea urchin larval development test were used. As the results from the *Ecklonia* tests were identical, the sub-lethal test with the longest duration was included in the analysis.

[b] Effects of growth were determined by comparison to controls. Growth was not significantly different at test concentrations that in controls, thus both the EC_{10} and LOEC were >100%.

[c] Values have been reported to the nearest whole number, so as not to convey a certain level of precision in the analysis, as there are errors associated with estimation of $E(L)C_x$ values.

Modelled data

The discharge limits (95% protection level) from the distribution results for the 101% and 200% assumption would be approximately 9-11% and 11-13%, respectively (Figures 1 and 2), depending on the distribution used to fit the data[3]. Increasing the assumed percentage of effluent from 101 to 200% stretches out the distribution slightly, giving a better fit of the data. However, it seems the effects on the discharge limit are minor. The discharge limits protective of 99% of species would be approximately 3-5% and 3-6%, respectively.

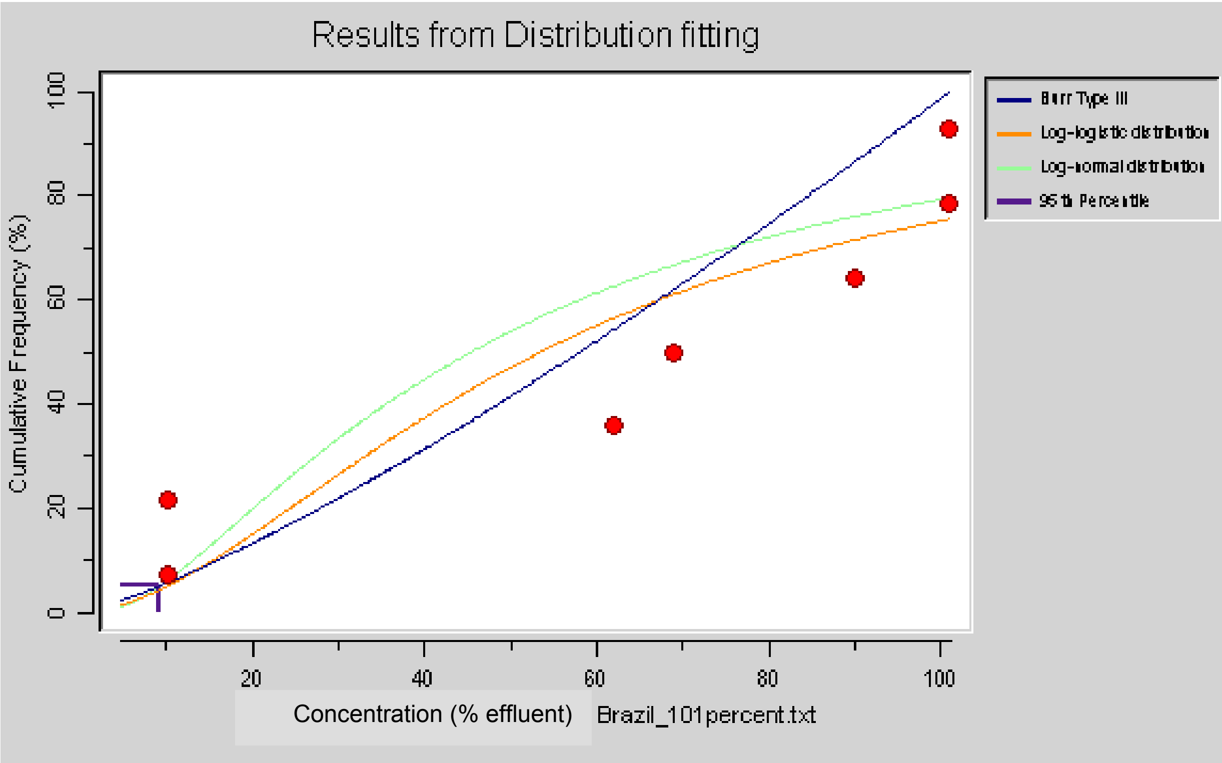

Figure 1: BurrliOZ distribution for Brazilian effluent results if >100% is taken to be 101% (using an ACR of 10 for acute studies).

There were seven data points in this distribution, from five different taxonomic groups. Note that the software advises caution in interpretation of results with less than eight species.

Burr Type III distribution:
Trigger value for 95% protection level = 9% effluent
Trigger value for 99% protection level = 3% effluent

Log-logistic distribution:
Trigger value for 95% protection level = 11% effluent
Trigger value for 99% protection level = 5% effluent

[3] Values have been reported to the nearest whole number, so as not to convey a certain level of precision in the analysis, as there are errors associated with estimation of E(L)C_x values.

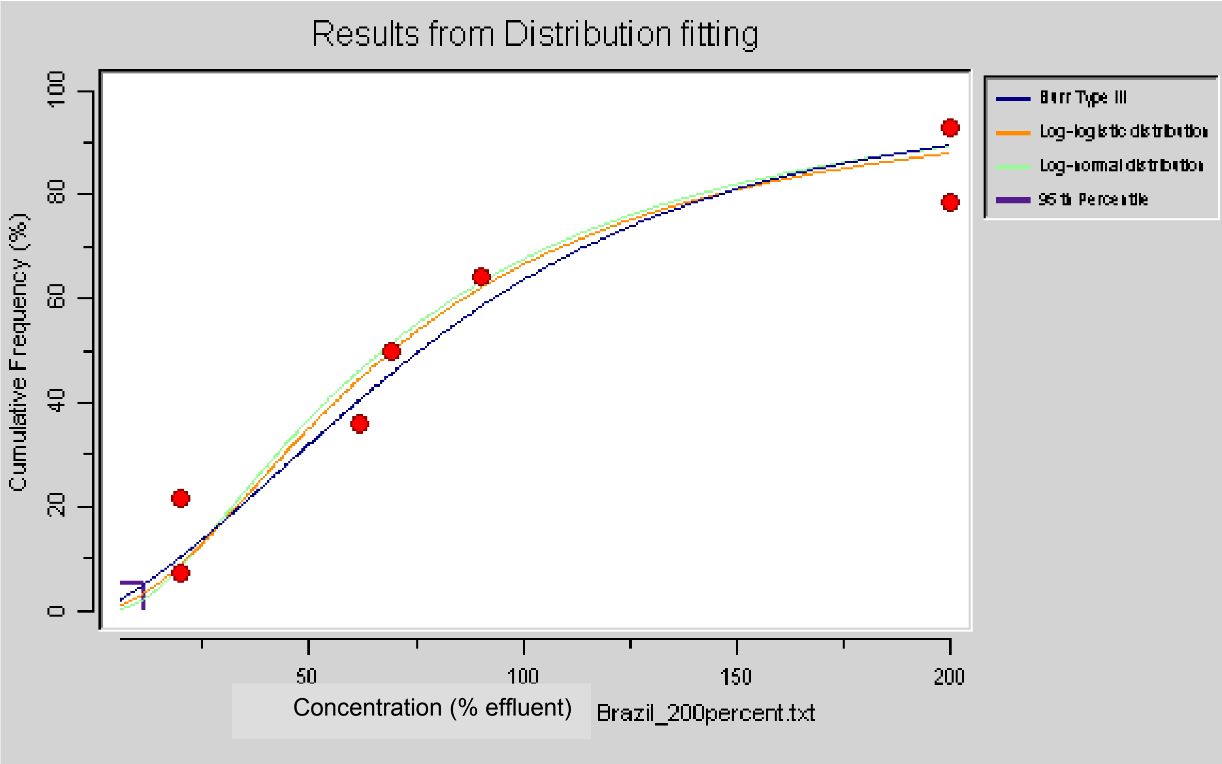

Figure 2: BurrliOZ distribution for Brazilian effluent results if >100% is taken to be 200% (using an ACR of 10 for acute studies).

There were seven data points in this distribution, from five different taxonomic groups. Note that the software advises caution in interpretation of results with less than eight species.

Burr Type III distribution:
Trigger value for 95% protection level = 11% effluent
Trigger value for 99% protection level = 3% effluent

Log-logistic distribution:
Trigger value for 95% protection level = 13% effluent
Trigger value for 99% protection level = 6% effluent

Table 2 summarises all the results from this exercise.

Table 2: Discharge limits (% effluent) predicted from Brazilian EC_{10} and EC_{50} data

Protection Level	Distri-bution Used	>100% assumed to be:	Predicted discharge limit (%)	Limitations/Comments
95 % protection	Burr Type III	101%	9	➢ 2 out of 7 (29%) values >100%. ➢ Only 7 data points to create distributions (8 is usually min. required). ➢ Assuming 200% for values >100% provides a better fit of the data than assuming 101%; this only has a very minor effect on the discharge limit. ➢ An ACR of 10 was applied to the two acute EC_{50}s given as >100%. Use of this ACR is conservative and may overestimate the chronic toxicity of the effluent.
		200%	11	
	Log-logistic	101%	11	
		200%	13	
99% protection	Burr Type III	101%	3	
		200%	3	
	Log-logistic	101%	5	
		200%	6	

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