

Expert witness statement of Dr Rick (Frederick) Krasso Expert of Gunns

**In the matter of the Bell Bay Pulp Mill Project: A project of State Significance
Resource Planning and Development Commission inquiry**

Proponent: Gunns Limited

1 Name and address

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2 Area of expertise

My area of expertise is as an ecotoxicologist, providing laboratory toxicity testing services for the assessment of toxicity that may be associated with environmental samples.

My qualifications and experience are detailed in Attachment 1.

I managed and participated in the ecotoxicological testing of two pulp mill effluent samples, and a test with sodium chlorate, on behalf of Gunns. I have over 15 years experience in the field of ecotoxicological testing. I was employed as an ecotoxicologist with the NSW Environment Protection Authority for 8 years, and as a Laboratory Manager for the Sinclair Knight Merz Ecotoxicology laboratory for a further 2 years prior to establishing Ecotox Services Australasia in early 2001. I have been involved in the development of several toxicity tests using Australian organisms, including for the National Pulp Mills Research Program.

3 Scope

I was the author of the three reports provided as appendices 58 to 60 in the Draft IIS prepared by Gunns. Since the public exhibition of the Draft IIS I have also completed two new test programs which are described below.

3.1 Instructions

(a) Work included in Draft IIS

Ecotox Services Australasia Pty Ltd (ESA) was engaged by Gunns in May 2005, and again in March 2006, to test the toxicity of two pulp mill effluent samples. Effluent samples tested were from a pulp mill in Thailand processing eucalypt pulp for the June 2005 testing program, and a South American mill processing radiata pine pulp for the April 2006 test program.

Gunns engaged the consultants Elecrowatt-Ekono (Thailand) Ltd to collect and ship the Thai pulp mill effluent sample to the ESA laboratory in Sydney, using sample bottle provided by ESA. Gunns also engaged the Santiago office of the consultancy Gutterage Haskins Davey (GHD) to collect and deliver an effluent sample from the South American pulp mill, also using sample bottles provided by ESA.

ESA undertook the toxicity testing of the Thai sample in June 2005, and the South American sample in April 2006. ESA was engaged to provide a comprehensive test report (including descriptions of methodology used), which are provided by Gunns in Appendices 58 and 59 of the Draft IIS. ESA was requested to include a 'plain English' version of the Executive Summary for both reports in order that non-scientists may understand the findings.

Two of the toxicity tests performed on the effluent samples were sub-contracted to CSIRO Centre for Environmental Contaminants Research at the Lucas Heights Research Laboratories, NSW.

ESA was again engaged by Gunns in June 2006 to undertake a single toxicity test with sodium chlorate using the 72-h germination test with the brown macro-alga (seaweed) *Hormosira banksii*. This test was reported using our 1-page Summary Test Report format, which is given in Appendix 60 in the Draft IIS.

(b) Work since public exhibition of the Draft IIS

Most recently, ESA was engaged by Gunns, following technical advice from Toxikos Pty Ltd, to further test the effects of chlorate on the brown macroalga *Hormosira banksii*. This was initiated in September 2006 (with test commencing on 10 October), and involved a repeat of the June 2006 study together with extending the assay to 14 and 21-days duration. The macroalgal gametes used in this October study were from parental stock that may have been stressed by unseasonally high temperatures in Sydney, which is why a repeat test was initiated in mid November 2006.

3.2 Process and methodology

In undertaking the toxicity tests with the two pulp mill effluent samples, ESA and CSIRO performed the following tests:

- Microtox assay using the marine bacterium *Vibrio fischeri*
- 72-h micro-algal growth inhibition test using *Nitzschia closterium*
- 72-h macro-algal germination assay using *Hormosira banksii*
- Sea urchin fertilisation success using *Heliocidaris tuberculata*
- 72-h larval development using the sea urchin *Heliocidaris tuberculata*
- 48-h larval development using the doughboy scallop *Mimachlamys asperrima*
- 96-h survival of the juvenile amphipod *Allorchesttes compressa*
- 96-h larval fish imbalance test using the striped trumpeter *Latris lineata*

The bioassays were performed at the ESA laboratory in Lane Cove, with the exception of the Microtox and *N. closterium* micro-algal assays which were performed by CSIRO Centre for Environmental Contaminants Research in Sydney.

The suite of toxicity tests used for the effluent test program was forwarded by Gunns to DPIWE for comment and approval prior to initiating the program. The assays were selected on the basis that these tests were based on the suite assessed by the National Pulp Mills Research Program and may be used in the future to meet license conditions should the pulp mill be constructed.

For each toxicity test, a dilution series of the effluents was prepared. In addition to testing the dilution series, an additional test concentration of 1% was included, which

represented the concentration of effluent at a 1 in 100 dilution zone. The ESA reports referred to the 1 in 100 dilution as that expected at the edge of the 'mixing zone'. The defined statutory mixing zone, as determined under the State Policy on Water Quality Management, is yet to be determined. Therefore to avoid confusion with statutory mixing zone, in this Statement reference is made to the 1 in 100 dilution zone.

ESA and CSIRO analysed the toxicity test data to ensure the individual tests met quality assurance criteria, and to determine median toxicity estimates (ie, LC, EC or IC50 estimates), No Observed Effect Concentrations (NOECs) and Lowest Observed Effect Concentrations (LOECs) for each assay.

At the request of Gunns, a comprehensive test report was prepared for each of the pulp mill effluent samples tested. Summary reports prepared by CSIRO for the Microtox and *Nitzschia* assays were appended to the ESA reports.

The toxicity tests with chlorate were undertaken using the following assay:

- 72-h macro-algal germination assay using *Hormosira banksii*

The test undertaken with sodium chlorate in June 2006 tested a range of nine chlorate concentrations up to 784mg/L, but failed to detect toxicity up to the maximum concentration. Consequently a 72-h EC50 and reliable NOEC and LOEC could not be determined for this assay. The assay was repeated in October 2006 and again in November 2006, but on these occasions the assays were extended to 14 and 21 days in duration in order to determine if chlorate had any inhibitory effects on rhizoid growth. The extension to 14 and 21-days duration was non-routine, and to my knowledge, has not previously been undertaken as a toxicity test (with the exception of a few trial assays in our laboratory). No routine quality assurance measures could be applied to this non-routine assay, apart from those performed for the routine 72-h germination test. However, the performance of a positive control at the 72-h stage does provide some confidence in the relative health and representativeness of the gametes used.

3.3 Reports reviewed

As our toxicity testing program involved laboratory testing of pulp mill effluent samples and a single test using sodium chlorate, no reports were required to be reviewed for the undertaking of the tests.

In June 2006 ESA was provided with a copy of the Emission Limits Guidelines Volume 2 and asked to make comment as to whether the toxicity tests performed and the levels of toxicity determined met the Emission Limits Guidelines for any future pulp mills. The toxicity tests recommended by the Emission Limits Guidelines are based on those assessed by the National Pulp Mills Research Program. My opinion is that the test suite used for the Thai and South American pulp mill met the Emission Limits Guidelines testing requirements, and providing that the assumptions in Section 3.4 of this Statement are met, that the effluent produced by the proposed mill is likely to meet the Emission Limits Guidelines with respect to ecotoxicity.

Finally I was requested to review several submissions and reports made to the RPDC concerning the Draft IIS. Where relevant, I have addressed concerns raised in these submissions and reports in Section 4.3 of this Statement.

3.4 Assumptions

Conclusions regarding absence of acute and short-term sub-lethal toxicity at the edge of the 1 in 100 dilution zone assumed:

1. That the concentration of effluent at the edge of the statutory mixing zone for any future license conditions is at least 1 in 100,

2. That the Thai and South American pulp mill samples are representative of those that may be produced by the proposed Bell Bay pulp mill, and
3. That the Thai and South American samples tested are typical of the effluent produced by those mills and the proposed Bell Bay pulp mill with respect to temporal variation.

3.5 Limitations and exclusions

The conclusions of the toxicity test program are limited by the assumptions given in Section 3.4 of this Statement.

4 Findings

4.1 Summary of findings from Draft IIS

I adopt the findings of the three Ecotox Services Australasia reports provided in Appendices 58 to 60 of the Draft IIS.

The Test Reports for the Thai and South American pulp mill effluents presented toxicity test data (including median toxicity estimates, NOECs and LOECs), brief descriptions of test results, and an assessment as to whether or not acute or sub-lethal toxicity may be expected at the edge of a 1 in 100 dilution zone. The test data are reproduced here in Tables 1 and 2.

Table 1. Summary of toxicity test data for the Thai pulp mill final effluent sample.

Toxicity Test	Endpoint	Value (%)
Sea urchin fertilisation	1h-EC50	>100
	NOEC	30
	LOEC	100
72-h sea urchin larval development	72-h EC50	>100
	NOEC	100
	LOEC	>100
48-h doughboy scallop larval development	48-h EC50	37.3 (35.3-39.5)
	NOEC	10
	LOEC	30
96-h juvenile amphipod survival	96-h LC50	>100
	NOEC	100
	LOEC	>100
72-h <i>Hormosira</i> macro-algal germination	72-h EC50	>100
	NOEC	100
	LOEC	>100
96-h larval fish imbalance test	96-h EC50	>100
	NOEC	100
	LOEC	>100
72-h <i>Nitzschia</i> micro-algal growth test	72-h IC50	>100
	NOEC	50
	LOEC	100
Microtox acute toxicity test	5, 15 and 30-m EC50	>90
	NOEC	36
	LOEC	45

Table 2 Summary of toxicity test data for the South American pulp mill final effluent sample.

Toxicity Test	Endpoint	Value (%)
Sea urchin fertilisation	1h-EC50	>100
	NOEC	25
	LOEC	50
72-h sea urchin larval development	72-h EC50	53.0 (51.7-54.4)
	NOEC	25
	LOEC	50
48-h doughboy scallop larval development	48-h EC50	47.4 (46.1-48.8)
	NOEC	25
	LOEC	50
96-h juvenile amphipod survival	96-h LC50	>100
	NOEC	100
	LOEC	>100
72-h <i>Hormosira</i> macro-algal germination	72-h EC50	>100
	NOEC	100
	LOEC	>100
96-h larval fish imbalance test	96-h EC50	>100
	NOEC	100
	LOEC	>100
72-h <i>Nitzschia</i> micro-algal growth test	72-h IC50	>100
	NOEC	50
	LOEC	100
Microtox acute toxicity test	5, 15 and 30-m EC50	>90
	NOEC	36
	LOEC	45

It is important to note that the test concentrations used differed between the Thai and the latter South American sample. The results gained from the Thai sample (using a 1-in-3 dilution series) were used to determine the test concentrations for the South American sample (which used a 1-in-2 dilution series). The concentrations used affect the possible NOEC and LOEC estimates. If for example the test concentration range 100, 30, 10, 3 and 1% was tested, and a significant effect was only found in the 100% (ie undiluted) treatment, then the NOEC and LOEC estimates would be 30 and 100%, respectively. Had 50% effluent concentration been tested, it may be that no effect would have been detected at 50%, and then the NOEC would be 50% rather than 30%. In this way, the test concentrations selected at the commencement of a test determine the NOEC and LOEC

estimates. For this reason, the test concentrations were altered for the testing of the South American sample.

Gunns (Richard Fawkes, Pers. Com) have indicated that the dilution of effluent at the edge of the statutory mixing zone shall be at least 1 in 100 (ie 1 percent effluent). Assuming the effluent from the pulp mill proposed for Bell Bay is the same as that sampled and tested from the Thai and South American pulp mills, and that those samples were representative of effluent quality over time, then based on the results presented herein, no acute or sub-lethal toxicity would be expected to be observed at the edge of the 1 in 100 dilution zone.

In addition, a toxicity test was undertaken in June 2006 with sodium chlorate reagent using the 72-h germination test with the brown macro-alga *Hormosira banksii*. A summary test report was provided to Gunns. The test indicated that there were no significant inhibitory effects at 784 mg/L, the highest concentration tested. This result indicated that the 72-h germination test end point was relatively insensitive compared with other macro- and micro-algal studies in the literature.

4.2 Summary of findings of work since public exhibition of the Draft IIS

On the advice and experimental design developed by Toxikos Pty Ltd, Gunns requested that the tests be repeated, but with an extension of the test to assess rhizoid growth over a 14 and 21-day exposure period. The tests were also undertaken in the presence of sodium nitrate at differing concentrations in order to test the hypothesis that increased background nitrate stimulates the production of nitrate reductase, which may reduce the toxicity exhibited by chlorate. This was undertaken on 10 October 2006, and again on 16 November 2006.

For the October 2006 study, the 14-day EC50 (with 95% confidence limits), NOEC and LOEC was determined to be 884 (744-1010), 200 and 1000 µg chlorate/L. The 21-day EC50 (with 95% confidence limits), NOEC and LOEC was determined to be 749 (671-831), 100 and 200 µg chlorate/L. These results are for sodium chlorate without the addition of nitrate. A copy of this summary report for chlorate-only data is annexed to this Statement. This test also assessed the effect of nitrate on chlorate toxicity. This dataset was difficult to interpret (with several interrupted dose-response relationships), with some ambiguity in the NOEC and LOEC estimates, and recorded an unexpected effect due to the presence of nitrate. The gametes used for this assay may have been from parental stock subjected to heat stress, following un-seasonally high temperatures coinciding with midday low tides. These gametes were obtained from the first parental stock that were successfully spawned following several attempts over a 2-3 week period immediately following the succession of hot days. The test results may be questionable or equivocal given the potential stress on the parental stock, and a repeat test was scheduled. A report on these October assays is yet to be finalised.

A repeat of the assays was initiated on 16 November 2006. The test report for these tests has not been finalised, however a summary of the test data are provided in Tables 3, 4 and 5. In summary, the results of the November tests showed the following:

- The NOEC and LOEC for chlorate alone (without added nitrate) for rhizoid growth over both 14 and 21 days was 100 and 200µg/L, respectively. This was the same result as the 21-day data for the October study (but without the ambiguity of interrupted dose-responses)
- The June, October and November studies all demonstrate that the 72-h germination end-point is relatively insensitive to chlorate.
- The addition of nitrate appears to have a stimulatory effect on rhizoid growth, as would generally be expected. This is contradictory to the October data set.
- The addition of nitrate, particularly 500µg/L, reduces the inhibitory effect of chlorate, relative the respective chlorate-only treatments. This is consistent with

the hypothesis that elevated nitrate stimulated the production of nitrate reductase, which may be used by the organism to detoxify chlorate.

Table 3 Summary of toxicity test data for chlorate with the 72-h germination test using the brown macro-alga *Hormosira banksii*, with and without the addition of nitrate.

	NOEC (µg/L)	LOEC (µg/L)	EC50 (µg/L)
Background	1000	>10000	>10000
50 µg/L Nitrate	1000	>10000	>10000
500 µg/L Nitrate	1000	>10000	>10000

Note: Comparisons are made to respective control

Table 4 Summary of toxicity test data for chlorate on rhizoid growth of the brown macro-alga *Hormosira banksii* over a 14-day exposure period, with and without the addition of nitrate.

	NOEC (µg/L)	LOEC (µg/L)	EC50 (µg/L)
Background	100	200	>10000
50 µg/L Nitrate	200	1000	1965
500 µg/L Nitrate	200	1000	8478

Note: Comparisons are made to respective control

Table 5 Summary of toxicity test data for chlorate on rhizoid growth of the brown macro-alga *Hormosira banksii* over a 21-day exposure period, with and without the addition of nitrate.

	NOEC (µg/L)	LOEC (µg/L)	EC50 (µg/L)
Background	100	200	1451
50 µg/L Nitrate	100	200	661.0
500 µg/L Nitrate	200	1000	1423

Note: Comparisons are made to respective control

4.3 Response to community concerns and key submissions

The following is a response to general concerns with respect to the work undertaken by Ecotox Services Australasia. In addition I have provided a response to the submission of Associate Professor Barbara Nowak entitled 'Review of the report on toxicity assessment of a pulp mill effluent for the proposed Tasmanian pulp mill', and by Uniquet entitled 'Peer review regarding an Integrated Impact Statement Part A- Review of Volume 17 of the Gunns' report' which are the only submissions received that had as their sole purpose a critical review of our reports. No comment is offered on concerns outside our brief as a service testing laboratory.

(a) Thai Eucalypt Pulp Mill Effluent Sample Testing and General Issues

(1) Sample age at commencement of testing

Our report provided in Appendix 59 of the Draft IIS identified sample age at time of arrival at the laboratory as an issue that may affect interpretation of test data.

To help determine if the sample altered in toxicity over the approximate 96-h period that the toxicity tests were undertaken, an acute Microtox test was performed on the sample each day over this period. No significant changes in toxicity of the effluent to Microtox were observed during this period.

It should be noted that no such logistical impediments would exist for samples taken from the proposed Bell Bay Pulp Mill and shipped to any Australian capital city. To remove any doubt regarding sample holding times, it is recommended that should the pulp mill be constructed, sample holding times be determined and specified in operating licences.

(2) Acute and sub-lethal toxicity tests- defining test types

The sea urchin fertilisation success test and the 48-h doughboy scallop larval development tests are sub-lethal tests. However there has been some confusion evident in the Tasmanian Whole of Government submission as to whether the sea urchin fertilisation success test and the doughboy scallop larval development tests are acute or sub-lethal. These definitions are significant in that the Emission Limits Guideline relate to the toxicity test endpoint used.

The Emission Limits Guideline (Volume 2, page 33, paragraph 'g') correctly refer to both the sea urchin fertilisation test and the doughboy scallop larval development tests as sub-lethal tests. The development of both of these tests was sponsored by the National Pulp Mills Research Program, and the protocol documents published by the National Pulp Mills Research Program name these tests as sub-lethal (Krassoi *et al.*, 1996, Simon and Laginestra, 1997).

(3) Conclusions regarding effects at the edge of the 1 in 100 dilution zone

The description of the results for most assays as being of no or negligible toxicity (except for the doughboy scallop test result) is an assessment based on the LC/ EC/ IC 50 estimates, which except for the Doughboy scallop assay, were all greater than 100%. One of the primary uses for these estimates is for the relative assessment of toxicity, comparing between species or between samples.

It is for the regulatory authorities to determine what degree of toxicity is allowable at the end-of-pipe or at the edge of the mixing zone in emission licenses, which in accordance with the ANZECC approach would use NOEC estimates rather than LOEC and LC/ EC/ IC 50 estimates. The Emission Limits Guideline (2004, Volume 2, Table 6) indicate that the limits for toxicity are based on the following:

- Acute toxicity tests: Effect in undiluted effluent should be less than 50% (ie, an EC50 of >100%).
- Chronic Toxicity tests (ie, sub-lethal): NOEC not to exceed at the edge of the statutory mixing zone.

Given these discharge limits, and if the assumptions regarding sample representativeness held, then the Emission Limits Guideline would have been met by the Thai and South American samples.

(4) Issues with Toxicity Testing with Fish

Gunns requested that fish be included among the suite of test organisms, if possible, in order to address likely concerns of the fishing industry, among others.

Fish are being increasingly included in among the suite of toxicity test for whole effluent toxicity test programmes conducted in Australia, particularly with the establishment of numerous fish hatcheries and the gaining of animal testing licenses by laboratories such as ours. However the availability of toxicity tests with fish remains constrained by availability of larval fish. This situation affected

the toxicity test with the Thai pulp mill sample, as no larval fish were available at the time of sample arrival in Australia. TAFI was identified as a source of Stripped Trumpeter larvae, and larval fish were made available on 8 June, some 5 days following the commencement of the other assays. Stripped trumpeter had not previously been used for toxicity testing, and there was some risk that the fish may not be amenable to handling. However the fish were found to be tolerant of laboratory handling, and presented promise of being a suitable test organism to complement others used in the suite of tests.

Approval for Ecotox Services Australasia (consistent with other institutions) undertaking fish toxicity tests was granted on the condition that we reduce the overall number of fish used in testing. This requirement prevents the use of reference toxicant tests. The RPDC and DPIW may wish to note these constraints when determining any future emission licenses, and provide some latitude given the difficulty in obtaining larval fish for toxicity testing and in performing reference toxicant tests.

(b) South American pine pulp mill effluent sample testing

(1) Representativeness of the sample

Comments have been made regarding the representativeness of the South American effluent sample, given that the South American sample may be more dilute than the proposed Bell Bay pulp mill effluent. This assertion has been made based on the estimated volume of water used per tonne of pulp produced. As a service testing laboratory, Ecotox Services Australasia is not in a position to make comment on this issue.

(c) Chlorate toxicity test with *Hormosira banksii*

Ecotox Services Australasia was commissioned by Gunns to undertake a 72-h germination assay using the brown alga *Hormosira banksii* with analytical grade sodium chlorate. Reporting was in the form of a 1-page summary report, which provided basic dose-response relationship data and quality assurance data.

Gunns selected this assay as no other routine bioassays with brown alga were available at the time of testing. The test using the 72-h germination end-point proved to be relatively insensitive.

It should also be noted that additional studies have since been initiated with sodium chlorate, extending the 72-h germination endpoint with *H. banksii* to assess rhizoid growth over a 14 and 21 day exposure period (see Section 4.2 of this Statement).

(d) Review by Associate Professor Barbara Nowak

(1) Recommendations of the Review

Assoc. Prof. Nowak highlights that no sub-chronic or chronic tests, genotoxic tests, bioaccumulation tests, sediment toxicity tests, fish residue tests, compositional analyses of effluents and proposed monitoring programs were presented. All of these items were outside ESAs scope of testing. ESA was engaged to undertake a suite of routine laboratory based acute and sub-lethal assays on two pulp mill effluent samples. The suite of tests used by ESA were based on those developed by the National Pulp Mills Research Program for the testing of pulp mill effluents, conformed with the tests required by the RPDC for testing effluents against emission limits, and undertaken in consultation by Gunns with DPIWE.

(2) Review comments regarding the samples tested

The Nowak Review makes the comment that the testing was limited to a single sample from both the Thai and South American pulp mills, with no replication in time, meaning that no conclusions regarding temporal variability of the effluents. The ESA reports acknowledge this issue in that our conclusions specifically state the assumption that the effluent from the pulp mill proposed for Bell Bay is the same as that sampled and tested from the Thai and South American pulp mills, and that the sample was representative of effluent quality over time. As such, based on these assumptions and on the results presented herein, no acute or sub-lethal toxicity would be expected to be observed at the edge of the mixing zone. In other words, the assumption of representativeness of the sample is subject to the sample taken being representative of the sample over time. The conclusions drawn were correct.

(3) Comments regarding duration of toxicity tests

The Nowak Review regards all of the toxicity tests undertaken by ESA as being acute, given that they were all of up to 96-h in duration. It is incorrect to define end-point type based on exposure duration alone. ESA supports the view expressed by the National Pulp Mills Research Program authors, the Emission Limits Guidelines and ANZECC/ARMCANZ (2000) Water and Sediment Quality Guidelines (with the exception of ANZECCs mistaken tag of bivalve larval assays and sea urchin fertilisation test as being acute) regarding the sub-lethal nature of the sea urchin fertilisation, sea urchin larval development and the doughboy scallop larval development tests. Indeed the 72-h growth inhibition test with the micro-alga *Nitzschia closterium* is regarded as a chronic test (ANZECC 2000).

The Nowak Review stated that the toxicity testing program should have included sub-chronic toxicity tests. No such tests were included in the Emission Limits Guidelines document for the assessment of pulp mill effluents. Sub-chronic tests are not available in Australia on a routine basis.

(4) Comments regarding exposure systems

The Nowak Review comments that the static exposure tests undertaken by ESA may underestimate toxicity, and that a more *realistic* approach would have been to use flow-through or static-renewal exposure systems. My general response is that the use of flow-through or static-renewal exposure systems would be quite *un-realistic* given the microscopic nature of the test organisms.

(5) Comments regarding Routes of Exposure

The toxicity tests performed used water-only exposure pathways, as would be required with effluents assessed against the Emission Limits Guidelines. The assessment of sediments and associated routes of exposure are outside the scope of testing for the tests performed.

(6) Comments regarding assumptions and conclusions

The Nowak Review states that some of the conclusions are overstated. The Review questions whether there would be no sub-lethal toxicity at the edge of the 1 in 100 dilution zone should exposure period be longer than 96-h. Clearly the ESA conclusions are based on the short-term sub-lethal tests performed for the program. It may have been possible to run several of the assays beyond 96-h, but then these tests would be non-routine in nature and have to be assessed in the absence of reliable QA limits and historical data for the extended end-points.

(e) Review by Uniquist

(1) Structure of the Uniquist Report

Uniquet have highlighted several aspects that they assume were to be addressed by the toxicity test studies. Those aspects highlighted in Table 1 of the Uniquet report are outside our brief. Similarly for Table 2, the first part entitled "Continued reduction of organochlorins" and the last part entitled "Monitoring" are outside our brief. The remaining sections of Table 2 are relevant to our reports.

(2) General comments of Volume 17

Uniquet comment that "longer term sub lethal impacts including mechanistic assays may be needed". Such assays are outside of scope of our brief, and are not required by the Emission Limits Guidelines. It should also be noted that such assays are non-routine in nature and therefore may not be appropriate for regulatory purposes (ie setting license limits based on non-routine assays).

Uniquet point out that they prepared their report without any terms of reference, and that many of the issues raised may well have been outside ESAs scope of work. They are correct in this assessment.

(3) *Question asked by RPDC of Uniquet: Adequacy of how the Draft IIS (Volume 17) addresses the issues as set out in the Guidelines*

Referring to Uniquet Table 3, the following issues raised were outside our brief:

- Long-term assays (beyond 96-h exposure time)
- Mutagenicity
- Cumulative effects
- Antagonistic and synergistic effects
- Mechanistic assays (eg endocrine disruption)

The Uniquet report concedes that the long-term effects are available in some research laboratories, and not available commercially at this stage. Indeed these assays are non-routine, and are not required under the discharge limits for the Emission Limits Guidelines.

Table 3 also incorrectly refers to the sea urchin fertilisation success test as being acute, when it is in fact a sub-lethal endpoint.

(4) *Question asked by RPDC of Uniquet: Is it adequate for the RPDCs assessment purposes?*

Uniquet make the comment that the effluent from the proposed Bell Bay Mill is fresh (with respect to salinity). ESA undertook a salinity adjustment procedure which involved adding artificial sea salts to the Thai and South American samples so that the effects of toxicants are not confounded with those of salinity. It should be noted that the salinity of the test solutions at 1%, the concentration of effluent at the edge of the 1 in 100 dilution zone, is similar to that of seawater. It should also be noted also that the discharge limits given in the Emission Limits Guidelines for acute toxicity assume that the samples are salinity adjusted. If the samples were not salinity adjusted, then no sample (clean or contaminated) could possibly meet the discharge limits of $EC_{50} < 100\%$ (ie less than 50% effect in undiluted effluent) due purely to the effects of reduced salinity.

The 48-h larval development test with the doughboy scallop is a sub-lethal assay (refer to Section 4.3 (a) 3 of this Statement). Uniquet appear to be unsure as to the status of this assay. It is important to note that the discharge limits given in the Emission Limits Guideline for chronic toxicity cite the sub-lethal assays as developed by the National Pulp Mills Research Program as surrogates for chronic tests, in the absence of any established long-term chronic assays.

(5) *Question asked by RPDC of Uniquest: Validity of methodology and findings*

Uniquest state “ The methods used and the conclusion may be valid for the tests conducted, however they only apply to the range of test organisms and methods used. There was no discussion on how the data may relate to the field environment”. Such assessments are outside ESAs brief as a service testing laboratory.

The report also states “ The report states that no acute or sub-acute (ESA used the term sub-lethal) toxicity would be expected at this dilution (ie 1 in 100, edge of the mixing zone) and the comments are valid, but no comment was made of what could happen in the mixing zone”. The Emission Limits Guidelines does not require a specific assessment of effects within the mixing zone, except to say that the effluent must have an EC50 >100% at the end of pipe.

(6) *Question asked by RPDC of Uniquest: Identify any major/critical errors or omissions in the Draft IIS and identify what further work is required.*

Uniquest suggest that if the project proceeds that sub-lethal effects on fish reproduction and mechanistic effects (eg endocrine disruption) be considered along side physico chemical and chemical parameters. As Uniquest points out, such assays are currently only available at some research laboratories on a non-commercial basis. Caution should be exercised when undertaking such assays in a regulatory context, given that they are usually only offered in a University environment, in the absence of Standard Operating Procedures, without QA limits, probably using shared facilities and student labour and in the absence of a NATA endorsed quality system.

5 Provisional opinion

The opinions that I have expressed in this report are based on my experience and the experience and advice provided to me by Gunns and the consultants engaged to carry out specialist studies for the Bell Bay Pulp Mill Project. Subject to any limitations and exclusions identified in this statement, my opinions are complete and accurate in every respect.

I am satisfied through my inquiries that the opinions I have expressed are reasonable in regard to the acute and short-term ecotoxicological toxicity tests performed by Ecotox Services Australasia Pty Ltd.

6 Declaration

I have made all the inquiries that I believe are desirable and appropriate and no matters of significance which I regard as relevant have, to my knowledge, been withheld from the Commission.



13 December 2006

7 References cited in this statement

Krassoi, F.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. 56pp

Simon, J. and Laginestra, E. (1997) Bioassay for testing sublethal toxicity in effluents, using gametes of the sea urchin *Heliocidaris tuberculata*. National Pulp Mills Research Program, Technical Report No. 20, CSIRO, Canberra. 42pp.

RPDC (2004) Recommended environmental emission limit guidelines for any new bleached eucalypt kraft pulp mill in Tasmania. Volume 2, August 2004. Resource Planning and Development Commission (Tasmania). 45pp.

Attachment 1

Qualifications

1 Qualifications

B. App. Sc (Applied Biology), UTS 1997

Ph. D (Marine Ecology), UTS 2001

2 Professional associations

Sustaining Member, Australasian Society for Ecotoxicology

Corporate Member, Australian Water Association

3 Relevant publications

Bailey, H.C., Krassoi, R., Elphick, J.R., Mulhall, A., Hint, P., Tedmanson, L. and Lovell, A. (2000) Application of *Ceriodaphnia cf. dubia* for whole effluent toxicity tests in the Hawkesbury-Nepean watershed, New South Wales, Australia: method development and validation. *Environmental Toxicology and Chemistry*.

Krassoi, F. R., Anderson, I.J. and Everett, D.A. (2000) Larval abnormalities in doughboy scallops *Chlamys asperrima* L. in response to test conditions and copper, zinc, chromium, cadmium, sodium dodecyl sulphate and 4-chlorophenol. *Australasian Journal of Ecotoxicology* 3: 65-74.

Krassoi, F.R. (In press) Sediment toxicity assessment using the larvae of the doughboy scallop *Chlamys asperrima* and the Sydney rock oyster *Saccostrea commercialis*. *Australasian Journal of Ecotoxicology*. 21 pp.

Stauber, J., Gunthorpe, L., Woodworth, J., Munday, B., Krassoi, R. and Simon, J. (1996). Comparative toxicity of effluents from ECF and TCF bleaching of eucalypt kraft pulps. *Appita* 49 (3):184-188.

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Julli, M. and Krassoi, F. R. (1995). Acute and chronic toxicity of the thiocarbamate herbicide, Molinate, to the cladoceran *Moina australiensis* sars. *Bulletin of Environmental Contamination and Toxicology* 54: 690-694.

Nelson, P.J., Stauber, J.L., Gunthorpe, L., Deavin, J.G., Munday, B.L., Krassoi, R. and Simon, J. (1995). Study shows ECF, TCF effluents have long-term toxic impact on sea-life. *Pulp and Paper (USA)*, August 1995: 103-109.

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Brown, K.R., Krassoi, F. R., Angus, C., and Underwood, P. (1991). The mangroves of Wolli creek, Sydney. *Wetlands (Australia)*7: 27-29.

Krassoi, F. R., Everett, D.A. and Anderson, I.J. (1996). Methods for estimating sublethal toxicity of single compounds and effluents to the doughboy scallop *Chlamys asperrimus* (Mollusca:Pectinidae) L. National Pulp Mills Research Program Technical Report Series No. 17, CSIRO Canberra, 48 pp.

Stauber, J.L., Krassoi, R., Simon, J. and Laginestra, E. (1996). Toxicity to marine organisms of effluents from EFC and TCF bleaching of eucalypt kraft pulps. National Pulp Mills Research Program Technical Report Series No. 19, CSIRO Canberra, 32 pp.

4 Employment history, achievements and projects worked on

2001 to present: Director, Ecotox Services Australasia

- . Establishment of the first independent NATA endorsed ecotoxicity testing facility in Australasia

1998 to 2000: Laboratory Manager, Sinclair Knight Merz Ecotoxicology Laboratory

- . Management of the construction and testing facilities of the Ecotoxicology Laboratory in the grounds of the University of Technology Sydney
- . Produced a Laboratory Procedures Manual for testing, calibration and maintenance of equipment, and laboratory QA/QC procedures.
- . Oversee the testing programme of the ecotoxicological assessment of 17 sewage treatment plant discharges into the Hawkesbury Nepean River system.
- . Implementation and oversight of staff training programme in accordance with QA/QC procedures.
- . Co-ordination of the laboratory testing programme, introducing new test procedures to service client requirements.

1991 to 1998: Ecotoxicologist, A/Information and Liaison Officer, NSW Environment Protection Authority (currently NSW Department of Environment and Conservation)

- . Received a grant from the National pulp Mills Research program to develop a toxicity test with the Doughboy scallop, protocol published 1996, conferred Outstanding Performance Award by the NSW EPA.
- . Over seven years, conducted a vast number of ecotoxicological tests using several species of cladoceran (water flea), both acute and chronic.
- . Ecotoxicological test and culture expertise gained with freshwater and marine fish and shrimp.
- . Participated in the OECD inter laboratory ring test using cladocerans, conferred Outstanding performance Award by the EPA.
- . Routinely prepared affidavits and Statements of Environmental Effects for prosecutions initiated by the EPA
- . Appeared as an Expert Witness on behalf of the EPA in several Land and Environment Court proceedings.
- . Appeared as an Expert Witness in the Commission of Inquiry over peat mining in Wingecarribee swamp, Southern Highlands.
- . Routinely attended fish kill incidents and chemical/waste spills, and advised EPA officers of potential environmental risk and harm.
- . Implemented in-situ ecotoxicological techniques, including Toxicity Identification and Evaluation to investigate sources of a major fish kill in the Cooks River, 1997.
- . For the first time in Australia, applied a range of marine test species and Toxicity Identification and Evaluation procedures to assess the toxicity of an effluent discharged off the Kurnell Peninsula, resulting in the formulation of more appropriate discharge licenses. Test methodology presented at the INSTA 8 conference, Perth 1997.

- . Developed a sediment elutriate toxicity test using the doughboy scallop and the Sydney rock oyster, which was applied to several known contaminated sites in the Sydney region.
- . Developed methods for cryopreservation of oyster and scallop gametes, for more time and cost-efficient test performance.
- . Presented the findings of several investigations and test method development at scientific conferences.
- . Conducted an investigation into the toxicity of the pesticide chlorpyrifos, comparing standard laboratory test species with a purpose built stream mesocosm. Initial findings presented at the Australasian Society for Ecotoxicology 1997 conference, Brisbane.
- . Developed and enhanced methods for the laboratory rearing of marine and freshwater aquatic organisms, to NATA standard, for routine toxicity testing.
- . Administered the budget of the Ecotoxicology section.
- . Published several papers on the toxicity of chemicals to a range of marine and freshwater species, and test methodology

Expert witness statement of Dr
Rick (Frederick) Krasso
Expert of Gunns

Attachment 2

Summary Test Report for the effect of chlorate (without added
nitrate) on 72-h germination and rhizoid growth at Days 14 and
21

The following Summary Test Report was provided at the request of Toxikos Pty Ltd who required a chlorate-only NOEC estimate by 15 November 2006 for the derivation of a site-specific guideline level for chlorate using a species sensitivity distribution. The comprehensive Test Report describing the complete data set, including responses to chlorate in the presence of elevated nitrate, was not finalised at the time of submission of this Statement.

Toxicity Test Report: TR0262/1

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Client:	Toxikos Pty Ltd PO Box 74 Caulfield East VIC 3145	ESA Job #:	PR0177
Attention:	Dr Roger Drew	Date Sampled:	N/A
Contract #:		Date Received:	N/A
		Sampled By:	N/A
		Quote #	PL0262_q01

Lab ID No.:	Sample Name:	Sample Description:
N/A	Sodium chlorate	99+% ACS Reagent, Sigma-Aldrich

Test Performed:	72-hr Macroalgal germination test using <i>Hormosira banksii</i>
Test Protocol:	ESA SOP 116, based on Gunthorpe <i>et al.</i> (1997) and Kevekordes and Clayton (1996)
Deviations from Protocol:	Test was continued on from the routine 72-h endpoint to 21-d exposure. Test solutions were renewed on 24 to 72-h renewal periods. Rhizoid length was determined at Day 14 and at test termination on Day 21 using a dissecting microscope with a calibrated eyepiece graticule. The concentration of chlorate in the highest treatment was determined analytically at the commencement of each renewal period.
Source of Test Organisms:	Field collected from Bilgola Beach on 9 October 2006. Parental stock may have been subject to heat stress following exposure in late September to un-seasonally high temperatures (ambient temperature in mid 30's) coinciding with midday low tides. Stock were collected at approximate intervals of 3-days from 20 September following this period and spawning attempted in the laboratory. Spawning had been unsuccessful until 10 October 2006.
Test Initiated:	10 October 2006 at 1800 h

Sample: Sodium chlorate		Sample: Sodium chlorate		Sample: Sodium chlorate	
Nominal Concentration (µg ClO ₃ /L)	% Germinated at 72-h (Mean ± SD)	Nominal Concentration (µg ClO ₃ /L)	Rhizoid Length (µm) at 14-d (Mean ± SD)	Nominal Concentration (µg ClO ₃ /L)	Rhizoid Length (µm) at 21-d (Mean ± SD)
0 (control)	98.3 ± 2.4	0 (control)	429 ± 32	0 (control)	590 ± 29
2	94.0 ± 2.7	2	410 ± 24	2	536 ± 23
10	94.5 ± 3.5	10	376 ± 19	10	498 ± 45*,**
50	94.3 ± 2.1	50	378 ± 65	50	520 ± 49*
100	96.0 ± 1.8	100	362 ± 31*	100	544 ± 33
200	96.5 ± 1.3	200	374 ± 25	200	509 ± 23*,**
1000	97.8 ± 2.1	1000	188 ± 17*,**	1000	198 ± 24*,**
10,000	95.3 ± 1.5	10,000	136 ± 17*,**	10,000	117 ± 11*,**
72-h EC50 = >10,000 µg ClO₃/L		14-d IC50 = 884 (744-1010) µg ClO₃/L		21-d IC50 = 749 (671-831) µg ClO₃/L	
NOEC = 10,000 µg ClO₃/L¹		NOEC = 200 µg ClO₃/L		NOEC = 100 µg ClO₃/L	
LOEC = >10,000 µg ClO₃/L¹		LOEC = 1000 µg ClO₃/L		LOEC = 200 µg ClO₃/L	

¹ There was no reduction in *Hormosira* germination success in any of the chlorate treatments tested (Dunnetts Test, 1-tailed, P=0.05 and P=0.01, df= 3,12).

* Significant reduction in rhizoid length compared with control treatments (Dunnetts Test, 1-tailed, P=0.05, df=7,24)

** Significant reduction in rhizoid length compared with control treatments (Dunnetts Test, 1-tailed, P=0.01, df=7,24)

Toxicity Test Report: TR0262/1

(page 2 of 3)

Notes on the determination of NOEC and LOEC estimates for this test: An interrupted dose response relationship was observed for both the 14 and 21-day test endpoints, rendering the determination of the NOEC and LOEC more difficult. USEPA guidance documents are used here for guidance for undertaking statistical analysis and determination of IC50, NOEC and LOEC estimates. USEPA (Section 9.1.1.2, 2002) defines the No Observed Effect Concentration (NOEC) as “the highest concentration of toxicant to which organisms are exposed in a full life-cycle or partial life-cycle (short-term) test, that causes no observable adverse effects on the test organisms (ie, the highest concentration of toxicant in which the values for the observed responses are not statistically significant from the controls)”. Dunnett’s Procedure is used to determine the NOEC (Section 9.6.1.1, USEPA 2002), if assumptions of data normality and homogeneity of variance can be met by the data. Assuming that the test data are either continuous or dis-continuous, and that toxicity decreases with decreasing concentrations of chlorate, the highest chlorate concentration which was not significantly different to the control was considered to be the NOEC estimate. It follows then, maintaining these assumptions, that the LOEC estimate is the next highest test concentration above the NOEC estimate.

USEPA 2002 does not provide specific guidance with respect to determining NOEC (and following that, the LOEC) estimates from datasets exhibiting an interrupted dose response relationship. This question is however addressed in USEPA 2000a, *Method Guidance and Recommendations for Whole Effluent Toxicity Testing*. USEPA (2000a) recommends careful interpretation involving an assessment of test sensitivity, using the Percent Minimum Significant Difference (PMSD) estimate. USEPA (2000b) provides acceptable high and low PMSD estimate band for a number of its toxicity tests as determined by inter-laboratory ring-tests. When a given test’s level of sensitivity falls within the upper and lower acceptable limits (*i.e.* The test is on the normal or lesser side of a scale of sensitivity), it is justified to accept a lower NOEC estimate than may be determined from an interrupted dose response relationship. However, a test that falls below the lower sensitivity band limits (*i.e.* Is on the more sensitive side of the scale) may use the value at the upper end on the interrupted dose response relationship in order to account for high test sensitivity.

The PMSD ($P=0.05$) for the 14 and 21-day rhizoid length test endpoints for the chlorate tests were 13.2 and 9.4, respectively. Given that the extension of the 72-h germination success test with *Hormosira banksii* to 14 and 21-d is non-routine, no data are available for the variability of the assay over time. Thus an acceptable range for the PMSD estimate can not be determined without extensive further testing using these extended test endpoints. USEPA (2000b) gives the lower PMSD value for a range of assays using growth related endpoints, and the range is between 6.3 and 12% (with upper band ranging from 23 to 37%). By comparing the PMSD estimates from the chlorate tests with those given by USEPA (2000b), it is apparent that the chlorate tests with *H. banksii* were on the sensitive end of the scale. This result may provide additional evidence for retaining the highest non-significant chlorate test concentration in the interrupted dose-response relationship as the NOEC.

QA/QC Parameter	Criterion	This Test	Criterion met?
Control minimum % germinated	>70 %	98.3%	Yes
Test Temperature limits	18.0 ± 1 °C	18.0-18.5°C	Yes
Reference Toxicant within cusum chart limits	48.6-181.0 µg Cu ²⁺ /L	113.6 µg Cu ²⁺ /L	Yes

Test Report Authorised by:



Dr Rick Krassoi, Director on 16 November 2006

Results are based on the samples in the condition as received by ESA. This report shall not be reproduced except in full.

References

Gunthorpe, L., Nottage, M., Palmer, D. and Wu, R (1997) Testing for sublethal toxicity using gametes of *Hormosira banksii*. protocol. National Pulp Mills Research Program, Technical Report No. 22, CSIRO, Canberra. 31pp

Kevekordes, K. and Clayton, M.N. (1996). Using developing embryos of *Hormosira banksii* (Phaeophyta) as a marine bioassay system. *International Journal of Plant Science*, 157: 582-585

USEPA (2000a) Method guidance and recommendations for Whole Effluent Toxicity (WET) Testing (40 CFR Part 136), United States Environmental Protection Agency, Office of Water, Washington DC. EPA-821-B-00-004.

USEPA (2000b) Understanding and accounting for method variability in whole effluent toxicity applications under the National Pollutant Discharge Elimination System Program, United States Environmental Protection Agency, Office of Wastewater Management, Washington DC. EPA-833-R-00-003.

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