

## **Tasmanian Investigation Test Number 4**

### **Sampling Details**

March 3, 2005. Sampled by Rick Krassoi, Ecotox Services Australia.

### **Location Sampled**

South George

### **Purpose**

Toxicity Identification and Evaluation. The purpose of the following series of tests is to identify and physically characterise the toxicant(s).

*Question 1: Is the toxicant(s) stable through time?*

### **Investigating Laboratory(s)**

Ecotox Services Australia, Sydney  
Advanced Analytical Australia, Sydney

### **Tests Conducted by Ecotox and Advanced Analytical**

Cladoceran test  
Pesticide and Herbicide Screens  
General Screen

### **Sampling Method**

Only the skimmer box method was employed for this suite of tests. Raw water samples were tested on the 4<sup>th</sup>, 7<sup>th</sup> and 9<sup>th</sup> of March 2005. Water was refrigerated between tests.

### **Experiment**

The test involved running three 48 hour dilution series, one after another, over a period of a week.

### **Results**

Initial tests revealed toxicant(s) was present. Toxicant(s) dissipated over the next two tests indicating that the toxicant(s) breaks down through time (Table 6).

Advanced Analytical ran tests for man-made pesticides, man-made herbicides and general screens. Detection limits were around 1 µg/L (i.e. microgram per litre). No man-made chemicals were detected. According to the literature, detection limits were adequate for most chemicals except pyrethroids.

### **Conclusions**

The toxicant(s) break down through time.

### **Status**

All future tests need to be run on fresh samples.

**Table 6:** Cladoceran survival as sample ages.

<b>Test Dates</b>	<b>Concentration</b>	<b>% Survival</b>
4/03/2005	100	0%
	50	90%
7/03/2005	100	0%
	75	10%
	50	100%
9/03/2005	100	100%

## **Tasmanian Investigation Test Number 5**

### **Sampling Details**

March 3, 2005. Sampled by Jim Harris (a member of St Helen's Marine Farmers) after training by Rick Krassoi, Ecotox Services Australia.

### **Location Sampled**

Creek feeding into Lake Augusta (a World Heritage Area).

### **Purpose**

Toxicity Identification and Evaluation. The purpose of the following series of tests is to identify and physically characterise the toxicant(s).

*Question 2: Do the toxicant(s) occur naturally in undisturbed areas?*

### **Investigating Laboratory(s)**

Ecotox Services Australia, Sydney  
Advanced Analytical Australia, Sydney

### **Tests Conducted by Ecotox**

Cladoceran test

### **Sampling Method**

Only the skimmer box method was employed for this suite of tests. Raw water samples were tested on the 4<sup>th</sup> of March 2005.

### **Experiment**

This involved a WET test on the undiluted skimmer box sample.

### **Results**

No toxicant was identified from this area (Table 7).

### **Conclusions**

No naturally occurring toxicant was identified. However, surrounding vegetation was grasses. Test to be repeated downstream of temperate Eucalypts.

### **Status**

Finding to be confirmed.

**Table 7:** Cladoceran survival in skimmer box sample collected from Lake Augusta area.

<b>Date</b>	<b>Concentration</b>	<b>% Survival</b>
4/03/2005	100	100%

**Photo 2:** Skimmer box deployed in creek feeding into Lake Augusta



## Tasmanian Investigation Test Number 6

### Sampling Details

March 3, 2005. Sampled by Rick Krassoi, Ecotox Services Australia.

### Location Sampled

South George

### Purpose

Toxicity Identification and Evaluation. The purpose of the following series of tests is to identify and physically characterise the toxicant(s).

*Question 3: Is the toxicant(s) a metal (like Copper or Zinc)?*

### Investigating Laboratory(s)

Ecotox Services Australia, Sydney  
Advanced Analytical Australia, Sydney

### Tests Conducted by Ecotox

Cladoceran test

### Sampling Method

Only the skimmer box method was employed for this suite of tests.

### Experiment

Raw water samples were tested on the 4<sup>th</sup> of March 2005. This test involves the addition of a chelating agent called EDTA. If a metal is present, then EDTA will settle it out of the water column reducing or removing toxicity. This method is best suited to divalent metals like Copper and Zinc (removing toxicity completely) but will also reduce toxicity associated with tri-valent and mono-valent metals.

### Results

Toxicity was not reduced or removed (Table 8).

### Conclusions

The toxicant(s) is not a metal.

### Status

Hypothesis falsified, no further test required.

**Table 8:** Cladoceran survival after the addition of EDTA.

Date	Concentration	% Survival
4/03/2005	100	0%

## Tasmanian Investigation Test Number 7

### Sampling Details

March 3, 2005. Sampled by Rick Krassoi, Ecotox Services Australia.

### Location Sampled

South George

### Purpose

Toxicity Identification and Evaluation. The purpose of the following series of tests is to identify and physically characterise the toxicant(s).

*Question 4: Is the toxicant(s) volatile (like petroleum products or fragrant oils)?*

### Investigating Laboratory(s)

Ecotox Services Australia, Sydney  
Advanced Analytical Australia, Sydney

### Tests Conducted by Ecotox

Cladoceran test

### Sampling Method

Only the skimmer box method was employed for this suite of tests. Raw water samples were tested on the 4<sup>th</sup> of March 2005.

### Experiment

This test involves bubbling nitrogen through the sample. If a volatile substance is present, then aeration will evaporate it out of the water column reducing or removing toxicity.

### Results

Toxicity was not reduced or removed (Table 9).

### Conclusions

The toxicant(s) is not a volatile substance.

### Status

Hypothesis falsified, no further test required.

**Table 9:** Cladoceran survival after aeration.

Date	Concentration	% Survival
4/03/2005	100	0%

## **Tasmanian Investigation Test Number 8**

### **Sampling Details**

March 3, 2005. Sampled by Rick Krassoi, Ecotox Services Australia.

### **Location Sampled**

South George

### **Purpose**

Toxicity Identification and Evaluation. The purpose of the following series of tests is to identify and physically characterise the toxicant(s).

*Question 5: Is the toxicant(s) dissolved in the water column or attached to particulate matter?*

### **Investigating Laboratory(s)**

Ecotox Services Australia, Sydney

### **Tests Conducted by Ecotox**

Cladoceran test

### **Sampling Method**

Only the skimmer box method was employed for this suite of tests.

### **Experiment**

Raw water samples were tested on the 4<sup>th</sup>, 7<sup>th</sup> and 9<sup>th</sup> of March 2005. These tests involve filtering the sample and testing the material that is removed as well as the remaining filtered water. If the toxicant(s) is attached to particulate matter, then filtration or centrifuge will remove toxicity. If it is dissolved in the water column, filtration will not remove toxicity.

### **Results**

Toxicity was reduced or removed using centrifuge and glass fibre filtration. Various test indicated that the toxicant(s) was not attached to coarse (i.e. clearly visible) material but was attached to very fine particulate matter (Table 10). Filtration reduced toxicity but did not always completely remove it (as indicated by the addition of PBO, discussed in test 10).

### **Conclusions**

The toxicant(s) is predominantly attached to very fine particulate matter.

### **Status**

Tested using a variety of filtration techniques, centrifuge being the best method of removing large particulate matter (suspended solids like soil) from the samples without substantial reduction of toxicity. To be confirmed by Test 10.

**Table 10:** Cladoceran survival after several different filtration methods.

<b>Treatment</b>	<b>Test Date</b>	<b>Concentration</b>	<b>% Survival</b>
Centrifuge	4/03/2005	100	0%
GFB Filtrate	4/03/2005	100	100%
Centrifuge	7/03/2005	100	0%
Centrifuge Pellet	7/03/2005	100	100%
GFB then C18 SPE	4/03/2005	100	100%

GFB is glass fibre filtration; C18 is a carbon based system for removing organic chemicals.



## **Tasmanian Investigation Test Number 9**

### **Sampling Details**

March 3, 2005. Sampled by Rick Krassoi, Ecotox Services Australia.

### **Location Sampled**

South George

### **Purpose**

Toxicity Identification and Evaluation. The purpose of the following series of tests is to identify and physically characterise the toxicant(s).

*Question 6: Is the toxicant(s) an organic chemical?*

### **Investigating Laboratory(s)**

Ecotox Services Australia, Sydney

### **Tests Conducted by Ecotox**

Cladoceran test

### **Sampling Method**

Only the skimmer box method was employed for this suite of tests.

### **Experiment**

Raw water samples were tested on the 9<sup>th</sup> of March 2005. These tests involve filtering a centrifuged sample through a C18 column (a type of activated carbon filter) and testing the material that is removed as well as the filtered water. This test could not be run until the appropriate clean up method had been established (Test Number 8). Toxicity was greatly reduced by this time (Test Number 4) so this test will be repeated with a fresh sample.

The methanol extraction of toxicant(s) from the C18 column concentrates the toxicant(s) from the original sample (usually 1 litre of water) into 2ml of methanol. This methanol is then added back to water allowing the concentration to increase. The add back concentration is the concentration of toxicant(s) compared to the amount present in the original sample. Thus, in Table 11, "4x" means four times the concentration that was in the original sample.

### **Results**

A toxicant(s) was isolated and eluted from the column using methanol. Methanol extraction also removed toxicant(s) from the material trapped by glass fibre filtration (Table 11).

### **Conclusions**

The toxicant(s) is methanol soluble. The toxicant(s) is probably an organic chemical.

**Status**

To be confirmed.

**Table 11:** Cladoceran survival in the concentrated toxicant(s) removed from the C18 column.

<b>Treatment</b>	<b>Test Date</b>	<b>Concentration</b>	<b>% Survival</b>
Lab C18 SPE	9/03/2005	4x	0%
		2x	100%
		1x	100%
C18 Filtrate	9/03/2005	100	100%
GFB Methanol Extract	7/03/2005	0.5x	0%

C18 SPE is a methanol extract from the C18 column.

## **Tasmanian Investigation Test Number 10**

### **Sampling Details**

March 3, 2005. Sampled by Rick Krassoi, Ecotox Services Australia.

### **Location Sampled**

South George

### **Purpose**

Toxicity Identification and Evaluation. The purpose of the following series of tests is to identify and physically characterise the toxicant(s).

*Question 7: Is the toxicant(s) enhanced or inhibited by the addition of PBO?*

### **Investigating Laboratory(s)**

Ecotox Services Australia, Sydney

### **Tests Conducted by Ecotox and Advanced Analytical**

Cladoceran test

### **Sampling Method**

Only the skimmer box method was employed for this suite of tests.

### **Experiment**

Raw water samples were tested on the 4<sup>th</sup>, 7<sup>th</sup> and 9<sup>th</sup> of March 2005. These tests involve the addition of PBO to a variety of filtered samples. If a pyrethroid-like substance is present, PBO dramatically increases toxicity. If an Organo-phosphate is present, PBO removes or reduces toxicity.

### **Results**

Addition of PBO to a variety of samples enhanced toxicity (Table 12). In the case of C18 methanol extract, toxicity was enhanced by a factor of approximately 16 (Graph 6).

### **Conclusions**

The toxicant(s) is probably an organic pyrethroid-like substance.

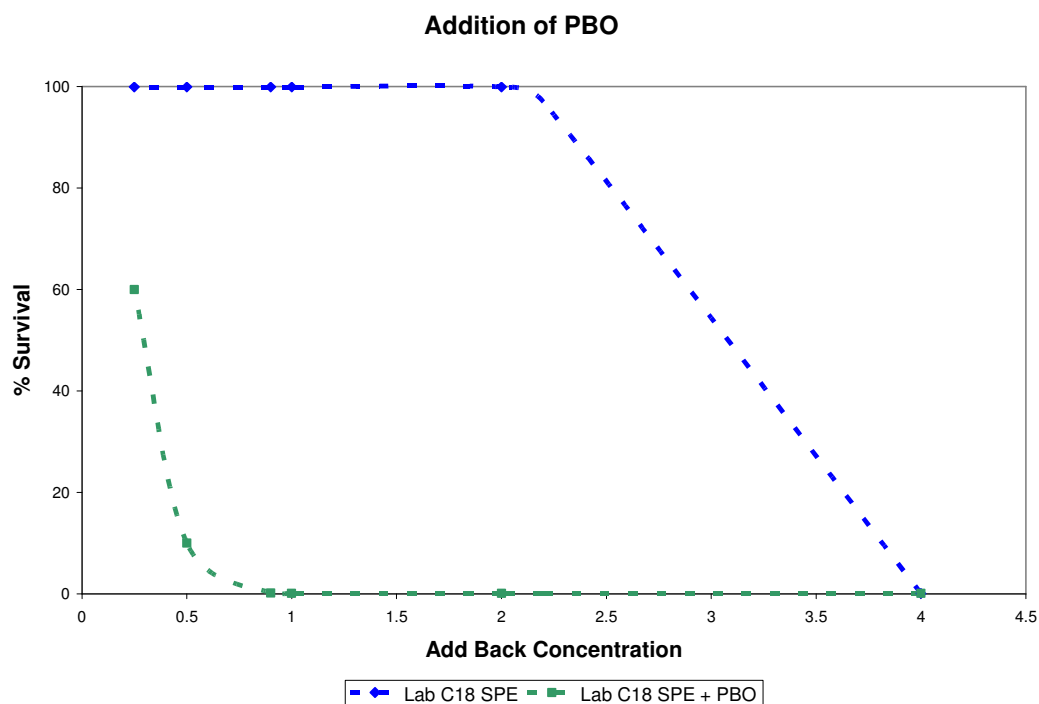
### **Status**

PBO synergism to be confirmed. PBO addition confirms that toxicant(s) is attached to fine particles by confirming which filtration methods remove toxicity.

**Table 12:** Cladoceran survival after the addition of PBO

<b>Treatment</b>	<b>Test Date</b>	<b>Concentration</b>	<b>% Survival</b>
Raw South George (SG)	9/03/2005	100	100%
Raw SG + PBO	9/03/2005	100	0%
		75	100%
<b>Treatment</b>	<b>Test Date</b>	<b>Concentration</b>	<b>% Survival</b>
Lab C18 SPE	9/03/2005	4x	0%
		2x	100%
		1x	100%
Lab C18 SPE + PBO	9/03/2005	4x	0%
		2x	0%
		1x	0%
		0.5x	10%
		0.25x	60%
Methanol Blank + PBO	9/03/2005	4.5x	100%
<b>Treatment</b>	<b>Test Date</b>	<b>Concentration</b>	<b>% Survival</b>
C18 Filtrate	9/03/2005	100	100%
C18 Filtrate + PBO	9/03/2005	100	100%
GFP Filtrate	4/03/2005	100	100%
GFP Filtrate + PBO	7/03/2005	100	0%
		75	0%
		50	40%
PBO Blank	7/03/2005	100	100%
<b>Treatment</b>	<b>Test Date</b>	<b>Concentration</b>	<b>% Survival</b>
Centrifuge	4/03/2005	100	0%
		50	100%
Centrifuge + PBO	4/03/2005	100	0%
		50	0%
PBO Blank	4/03/2005	100	100%
<b>Treatment</b>	<b>Test Date</b>	<b>Concentration</b>	<b>% Survival</b>
Centrifuge	7/03/2005	100	0%
		75	100%
		50	100%
Centrifuge + PBO	7/03/2005	100	0%
		75	30%
		50	100%
PBO Blank	7/03/2005	100	100%
Centrifuge Pellet	7/03/2005	100	100%
Centrifuge Pellet + PBO	7/03/2005	100	100%
<b>Treatment</b>	<b>Test Date</b>	<b>Concentration</b>	<b>% Survival</b>
Centrifuge	9/03/2005	100	100%
Centrifuge + PBO	9/03/2005	100	20%
		75	100%

**Graph 6:** Change in Cladoceran survival with the addition of PBO.



The methanol extraction of toxicant(s) from the C18 column concentrates the toxicant(s) from the original sample (usually 1 litre of water) into 2ml of methanol. This methanol is then added back to water allowing the concentration to increase. The add back concentration is the concentration of toxicant(s) compared with the amount present in the original sample.

## **Tasmanian Investigation Test Number 11**

### **Sampling Details**

March 24, 2005. Sampled by Rick Krassoi, Ecotox Services Australia.

### **Location Sampled**

South George

### **Purpose**

Toxicity Identification and Evaluation. The purpose of the following series of tests is to identify and physically characterise the toxicant(s).

*Question 8: Is the toxicant(s) an organic chemical?*

### **Investigating Laboratory(s)**

Ecotox Services Australia, Sydney  
Advanced Analytical Australia, Sydney

### **Tests Conducted by Ecotox and Advanced Analytical**

Cladoceran test

### **Sampling Method**

Only the skimmer box method was employed for this suite of tests.

### **Experiment**

Raw water samples were tested on the 24<sup>th</sup> of March 2005. These tests involve filtering a centrifuged sample through a C18 column and testing the material that is removed as well as the filtered water.

### **Results**

Toxicity was removed by filtration through the C18 column. Addition of PBO to the filtered water did not enhance toxicity. Filtration through glass fibre also reduced toxicity, but, addition of PBO revealed toxicant(s) had passed through the glass fibre filter (Table 13).

### **Conclusions**

The toxicant(s) is an organic chemical.

### **Status**

This test in conjunction with Test 6 confirms the toxicant(s) to be of an organic chemical nature. Chemical analysis did not identify the toxicant(s) at micrograms per litre. A combination of field pre-concentration and laboratory pre-concentration will be used on the next round of testing in order that chemical detection is enhanced.

**Table 13:** Cladoceran survival in filtrate (i.e. the water that has passed through a column or filter, testing whether toxicity was removed).

<b>Treatment</b>	<b>Test Date</b>	<b>Concentration</b>	<b>% Survival</b>
South George Raw (SG)	24/03/05	100	0%
		50	100%
Methanol Blank		0	100%
<b>Treatment</b>	<b>Test Date</b>	<b>Concentration</b>	<b>% Survival</b>
SG Raw + PBO	24/03/05	100	0%
		50	0%
		25	0%
		12.5	60%
		6.25	100%
Methanol Blank		0	100%
PBO Blank		0	100%
<b>Treatment</b>	<b>Test Date</b>	<b>Concentration</b>	<b>% Survival</b>
SG C18 Filtrate	24/03/05	100	100%
Methanol Blank		0	100%
<b>Treatment</b>	<b>Test Date</b>	<b>Concentration</b>	<b>% Survival</b>
SG C18 Filt. + PBO	24/03/05	100	100%
Methanol Blank		0	100%
<b>Treatment</b>	<b>Test Date</b>	<b>Concentration</b>	<b>% Survival</b>
GFB Filtrate	24/03/05	100	100%
Methanol Blank		0	100%
<b>Treatment</b>	<b>Test Date</b>	<b>Concentration</b>	<b>% Survival</b>
GFB Filtrate + PBO	24/03/05	100	50%
Methanol Blank		0	100%

## **Tasmanian Investigation Test Number 12**

### **Sampling Details**

March 24, 2005. Sampled by Rick Krassoi, Ecotox Services Australia.

### **Locations Sampled**

South George  
Pyengana  
Upstream of Town Water Intake Pipe (Water Intake)

### **Purpose**

Toxicity Identification and Evaluation. The purpose of the following series of tests is to identify and physically characterise the toxicant(s).

*Question 9: Is the toxicant(s) enhanced or inhibited by the addition of PBO?*

### **Investigating Laboratory(s)**

Ecotox Services Australia, Sydney  
Advanced Analytical Australia, Sydney

### **Tests Conducted by Ecotox**

Cladoceran test

### **Sampling Method**

Only the skimmer box method was employed for this suite of tests. Raw water samples were tested on the 24<sup>th</sup> of March 2005. These tests involve the addition of PBO to the three raw water samples. If a pyrethroid-like chemical is present, PBO dramatically increases toxicity. If an Organo-phosphate is present, PBO removes or reduces toxicity.

### **Results**

Addition of PBO to all samples enhanced toxicity. Toxicity is enhanced by a factor of approximately 6 (Table 14, Graphs 7, 8 & 9).

Advanced Analytical did not identify any pyrethroids at a detection limit of 1 microgram per litre.

### **Conclusions**

The toxicant(s) shares similar characteristics with pyrethroid-like substances. Chemical detection limits need to be improved.

### **Status**

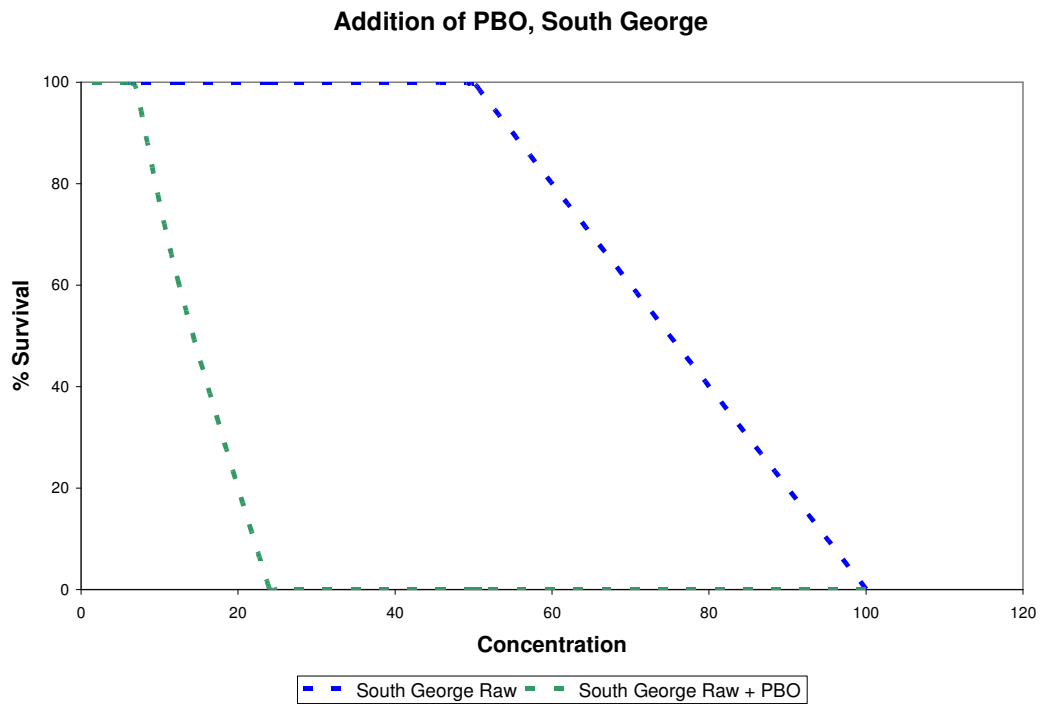
Combining this test result with Test 10, PBO synergism is confirmed.



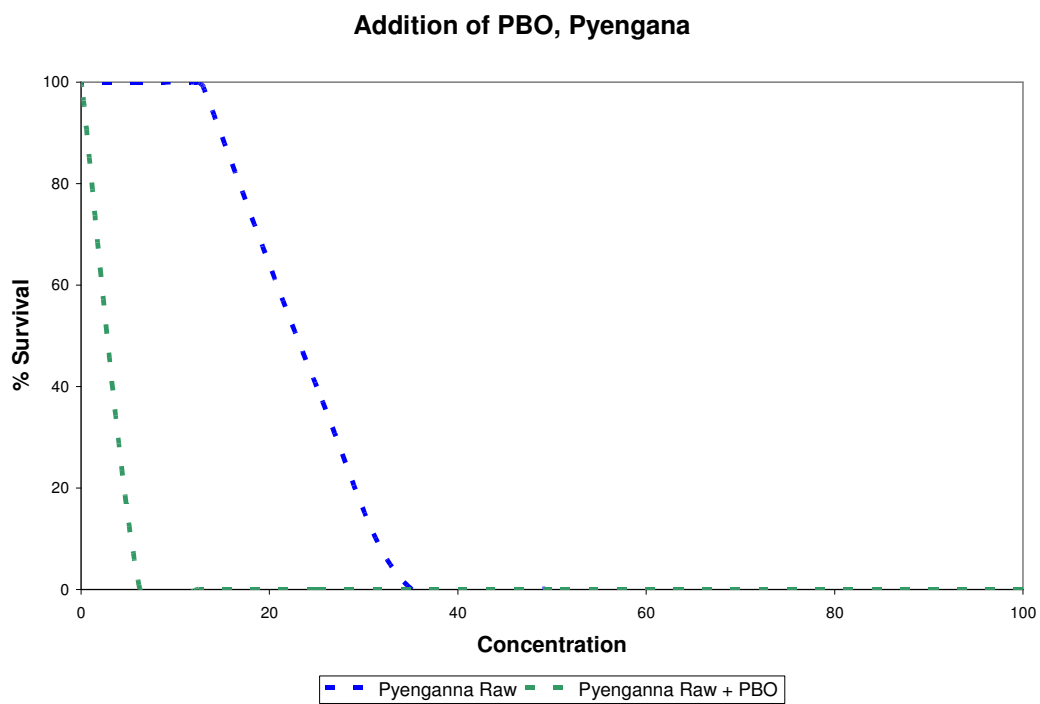
**Table 14:** Cladoceran survival before and after addition of PBO

<b>Treatment</b>	<b>Test Date</b>	<b>Concentration</b>	<b>% Survival</b>
South George Raw	24/03/05	100	0%
		50	100%
		25	100%
		12.5	100%
		6.25	100%
		0	100%
Methanol Blank		0	100%
SG Raw + PBO	24/03/05	100	0%
		50	0%
		25	0%
		12.5	60%
		6.25	100%
		0	100%
Methanol Blank		0	100%
PBO Blank		0	100%
<b>Treatment</b>	<b>Test Date</b>	<b>Concentration</b>	<b>% Survival</b>
Pyengana Raw	24/03/05	100	0%
		50	0%
		25	40%
		12.5	100%
		6.25	100%
		0	100%
Methanol Blank		0	100%
Pyengana + PBO	24/03/05	100	0%
		50	0%
		25	0%
		12.5	0%
		6.25	0%
		0	100%
Methanol Blank		0	100%
PBO Blank		0	100%
<b>Treatment</b>	<b>Test Date</b>	<b>Concentration</b>	<b>% Survival</b>
Water Intake Raw	24/03/05	100	0%
		50	0%
		25	80%
		12.5	100%
		6.25	100%
		0	100%
Methanol Blank		0	100%
WI + PBO	24/03/05	100	0%
		50	0%
		25	0%
		12.5	0%
		6.25	40%
		0	100%
Methanol Blank		0	100%
PBO Blank		0	100%

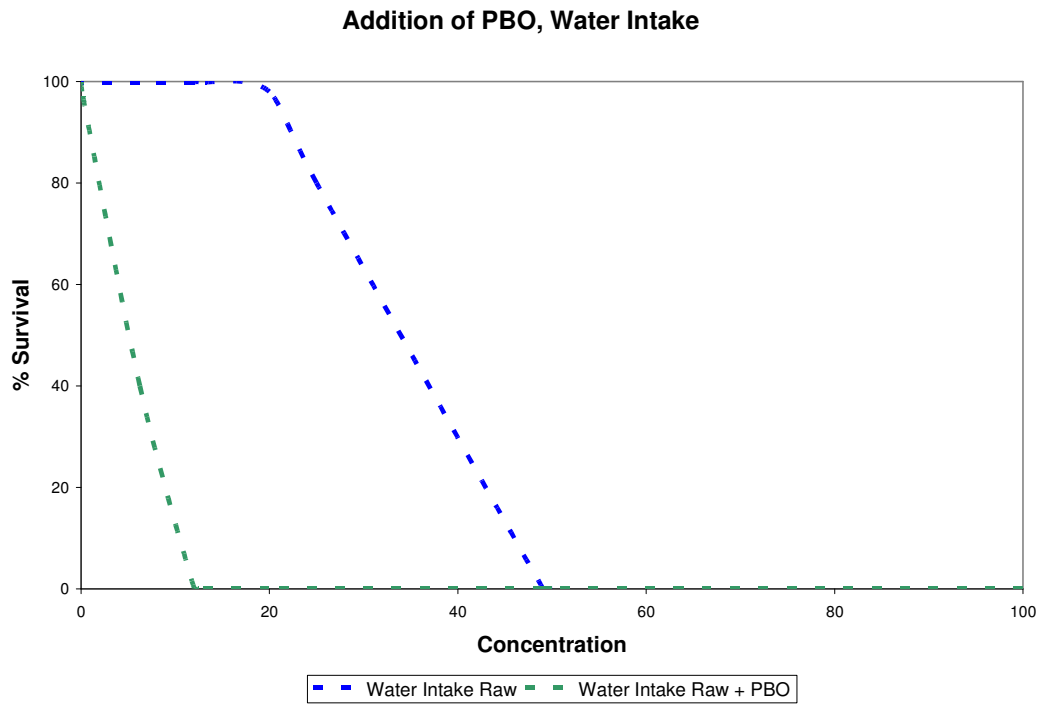
**Graph 7:** Change in Cladoceran survival with the addition of PBO, South George



**Graph 8:** Change in Cladoceran survival with the addition of PBO, Pyengana



**Graph 9:** Change in Cladoceran survival with the addition of PBO, Water Intake



## **Tasmanian Investigation Test Number 13**

### **Sampling Details**

April 19, 2005. Sampled by Rick Krassoi, Ecotox Services Australia.

### **Locations Sampled**

South George  
Upstream of Town Water Intake Pipe

### **Purpose**

Toxicity Identification and Evaluation. The purpose of the following series of tests is to identify and physically characterise the toxicant(s).

*Question 10: Is the toxicant(s) a pyrethroid?*

### **Investigating Laboratory(s)**

Ecotox Services Australia, Sydney  
Advanced Analytical Australia

### **Tests Conducted by Ecotox and Advanced Analytical**

Cladoceran test followed by general chemical screening in conjunction with specific screening for pyrethroids.

### **Method**

Only the skimmer box method was employed for this suite of tests.

### **Experiment**

Six litres of concentrated surface water were collected for immediate extraction onto C18 columns. A sub sample of this concentrated water was put to one side to be checked on arrival in Sydney. Tests on these sub samples indicated that a large amount of toxicant(s) was captured. The EC50 (i.e. the concentration at which 50% of test organisms die) was 9.4% for the South George sample and 5.7% for the Upstream of the Town Water Intake sample. This means the samples could be diluted by a factor of 11 and 17 respectively and still be toxic.

To isolate the toxicant(s), a technique called methanol fractionation was used. The toxicant(s) that was taken out of the water by passing it through C18 columns in the field (the toxicant(s) sticks to the carbon in the column) was then subjected to this isolation method. The toxicant(s) was removed from the C18 column by passing various dilutions of methanol through the column (organic chemicals are methanol soluble). Initially, 25% methanol mixed with clean water was passed through the columns. The total volume of each methanol dilution (fraction) was 2ml. Next, 50% methanol was passed through the columns; then 75% methanol; then 80%; 85%; 90%; 95% and finally 100%.

A toxicant will usually be isolated in one or two methanol fractions. This concentrates the toxicant by a factor of 250 (if isolated in two fractions) or 500 (if isolated in one fraction). Thus, by the time the toxicant is submitted to the laboratory, the concentration factor is several thousand times the concentration initially in the raw water column (the initial concentration factor, 11 to 17, multiplied by the concentration factor associated with methanol extraction, 250 to 500), which should make it very easy to identify.

## Results

Toxic methanol extracts were identified and will be discussed in the next section. The methanol extracts were submitted to Advanced Analytical Australia and screened. Advanced Analytical reported that no chemicals, either natural or man-made, were present in the methanol extract.

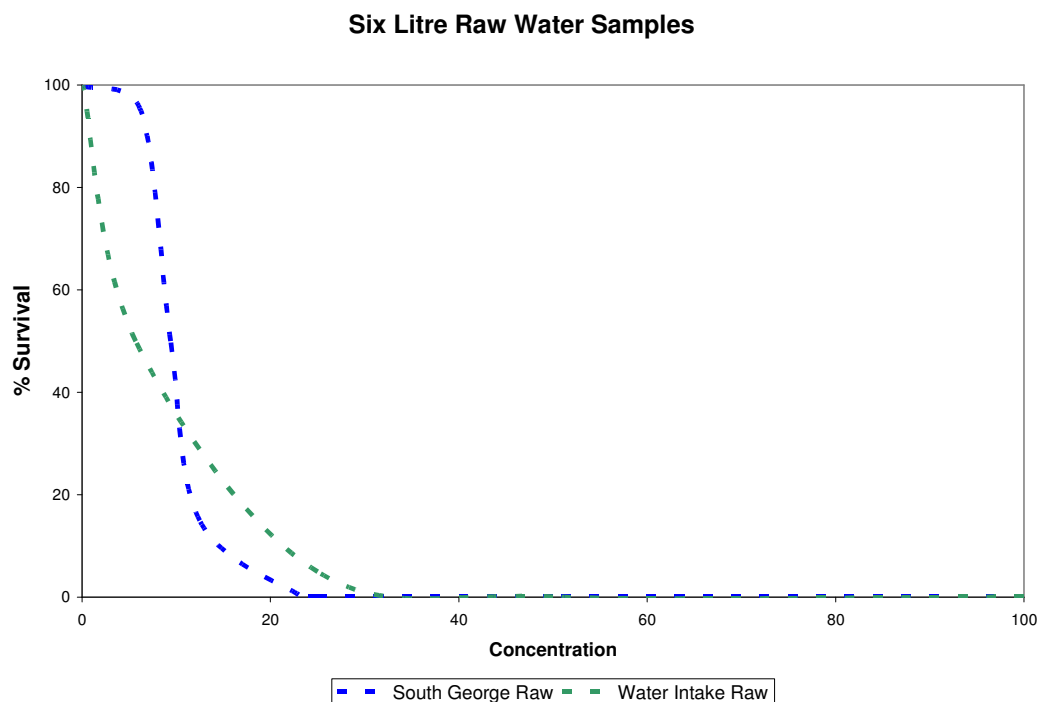
## Conclusions

A discussion was then held between Dr Scammell (one of the clients), Dr Krassoi (Ecotox Services), Dr Eckhard (Advanced Analytical Australia) and Dr Tottszer (Advanced Analytical Australia) to determine which types of chemicals could not be detected by their equipment. Dr Eckhard advised that non-polar chemicals like proteins, peptides and amino sugars would stick to the glass in part of the equipment and therefore be missed.

## Status

Methanol samples containing the concentrated toxicant(s) are to be submitted to a laboratory that can test for non-polar molecules.

**Graph 10:** Cladoceran survival in concentrated surface water



## **Tasmanian Investigation Test Number 14**

### **Sampling Details**

April 19, 2005. Sampled by Rick Krassoi, Ecotox Services Australia.

### **Locations Sampled**

South George.

Upstream of Town Water Intake Pipe (Water Intake)

### **Purpose**

Toxicity Identification and Evaluation. The purpose of the following series of tests is to identify and physically characterise the toxicant(s).

*Question 11: What methanol fraction can the toxicant(s) be isolated in?*

### **Investigating Laboratory(s)**

Ecotox Services Australia, Sydney

### **Tests Conducted by Ecotox and Advanced Analytical**

Methanol fractionation and add back to test using Cladocerans

### **Method**

Only the skimmer box method was employed for this suite of tests.

### **Experiment**

The toxicant(s) that was taken out of the water by passing it through C18 columns in the field (the toxicant(s) sticks to the carbon in the column) was then subjected to methanol fractionation. The toxicant(s) was removed from the C18 column by passing various dilutions of methanol through the column (organic chemicals are methanol soluble). Initially, 25% methanol in clean water was passed through the column. The total volume of each methanol dilution (fraction) was 2ml. Next, 50% methanol was passed through the column; then 75% methanol; then 80%; 85%; 90%; 95% and finally 100%.

These 2ml fractions were then added back to water to determine which fractions contained the toxicant(s) (Add Back).

### **Results**

Add Back revealed that toxicant(s) was not present in the 25% or 50% methanol fractions. Some toxicant(s) was present in the 75% fraction, while toxicant(s) was clearly present in the 80%; 85%; 90%; 95% and 100% fraction. Adding PBO did not result in enhanced toxicity for either site. These results suggest that multiple methanol soluble toxicants are present.

## Conclusions

The pyrethroid-like substance observed previously was now gone but a complex methanol soluble group of toxicants remain, as evident by toxicity being spread over six methanol fractions.

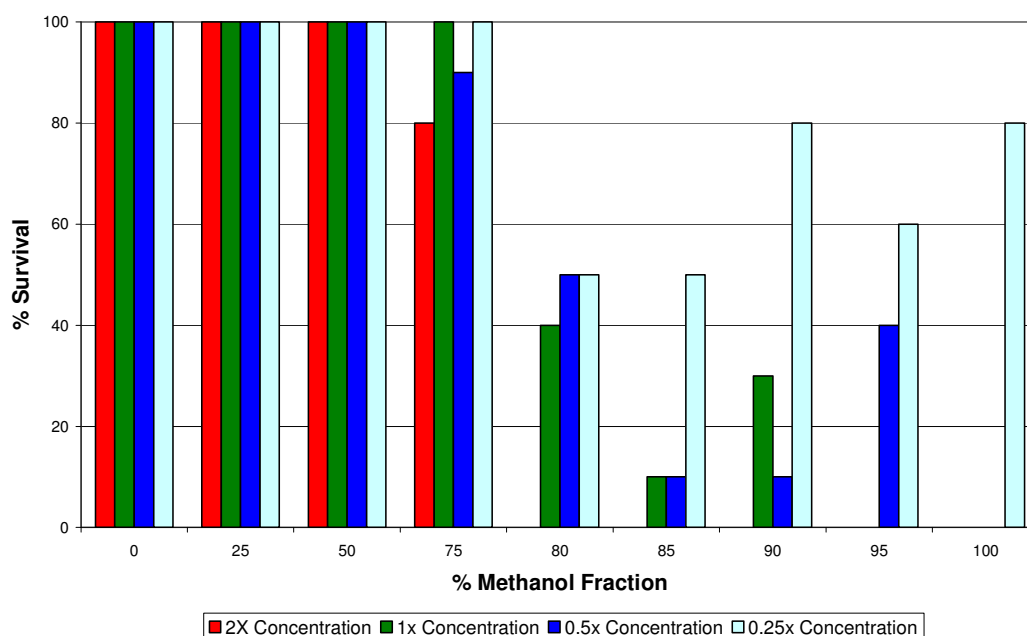
## Status

The methanol-soluble toxicant(s) (that seems to always be present) appears to be more than one toxicant.

(As a note, the addition of PBO decreased toxicity for the Water Intake sample.)

**Graph 11:** Cladoceran survival following Add Back of methanol fractions at a range of concentrations starting with twice the original concentration (2x) ranging down to one quarter the original concentration (0.25x).

**Toxin(s) Methanol Fractionation Profile**



## **Tasmanian Investigation Test Number 15**

### **Explanation**

The toxic methanol fraction previously identified as containing no polar molecules was submitted for Amino Acid analysis because peptides, proteins and potentially toxic amino sugars are all combinations of amino acids. The presence of proteins, peptides or toxic amino sugars should return positive amino acid detections. Alternative toxicants such as bacterial endotoxins would not be identified using this test.

### **Sampling Details**

April 19, 2005. Sampled by Rick Krassoi, Ecotox Services Australia. Methanol extract, tested by Advanced Analytical, submitted in June.

### **Locations Sampled**

South George.  
Upstream of Town Water Intake Pipe

### **Purpose**

Toxicity Identification and Evaluation. The purpose of the following series of tests is to identify and physically characterise the toxicants.

*Question 12: Are the toxicants non-polar molecules?*

### **Investigating Laboratory(s)**

Australian Proteome Analysis Facility, Macquarie University, Sydney

### **Sample Tested by Proteomics Laboratory**

Toxic Methanol fractionation SG A05/0594/4

### **Results**

Measurable quantities of many water soluble and water insoluble amino acids were found.

### **Conclusions**

The toxicants are potentially proteins, peptides or amino sugars. Many organisms produce toxic peptides and proteins including blue green algae, bacteria and fungi.

### **Status**

The methanol soluble toxicants that seems to always be present correlates with the presence of amino acids. Weight of evidence indicates the toxicants are biological in origin. This ends the TIE.

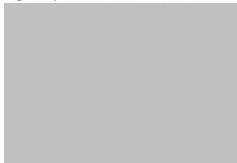
Although chemical identification may not be possible, the source of the biological toxicants may be able to be identified making management of the toxicants source possible.





## RESULTS OF AMINO ACID ANALYSIS

### Customer Details:



22 June 2005

### Sample Details:

Four environment samples were supplied for High Sensitivity amino acid analysis. An aliquot was taken out of the vial labelled SG A05/0594/4 for the analysis.

### Analysis Details:

Performed by Prithi Lopez

- Samples underwent 24hr gas phase hydrolysis at 110C.
- All analyses were performed using the Waters AccQTag chemistry. Cysteine and Tryptophan not analysed by this method.
- Samples were analysed in duplicate and results are expressed as an average.

### Results for Amino acid Analysis:

#### Sample Name : Environment Sample

Amino Acid	Amino Acid (µg/mL)	Mole %
Aspartic acid + Asparagine	0.29	5.49
Serine	0.40	9.94
Glutamic acid + Glutamine	0.47	7.93
Glycine	0.29	11.05
Histidine	Not detected	Not detected
Arginine	0.18	2.57
Threonine	0.21	4.57
Alanine	0.29	9.03
Proline	0.28	6.37
Tyrosine	0.14	1.91
Valine	0.44	9.58
Methionine	Not detected	Not detected
Lysine	0.06	1.10
Isoleucine	0.46	8.77
Leucine	0.90	17.27
Phenylalanine	0.30	4.41
<b>Total</b>	<b>4.71</b>	<b>100.00</b>

Australian Proteome Analysis Facility Ltd  
 Level 4, Building F7B, Macquarie University, Sydney, NSW, 2109, Australia  
 Ph: +61 2 9850 6201 Fax: +61 2 9850 6200  
[www.proteome.org.au](http://www.proteome.org.au) [apafinfo@proteome.org.au](mailto:apafinfo@proteome.org.au)