Chemical and Ecotoxicology Assessment of Discharge Waters from West Cliff Mine

For samples collected between 14 May and 25 June 2012 from Licensed Discharge Point 11, Brennans Creek Dam and Upper Georges River (upstream and downstream of the Brennans Creek confluence)

Report to the NSW Environment Protection Authority

August 2012

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Summary

Sampling of water from four sites at Brennans Creek and the Georges River was carried out on six occasions over May-June 2012. The water samples were analysed for an extensive suite of chemical water quality parameters along with ecotoxicological testing using a range of species with acute lethality and sublethal endpoints.

Bicarbonate was elevated in the licensed discharge (LDP10) as were a number of dissolved trace metals, aluminium(Al), nickel (Ni), zinc(Zn), cobalt (Co) and copper (Cu).

Ecotoxic effects in Georges River samples, taken immediately downstream of the Brennans Creek confluence, were primarily limited to acute lethality effects on larval fish and sublethal reproductive impairment effects on crustacean waterfleas. The larval fish effects generally occurred in the last day of the 4-day tests. Potential contributors to this toxicity include bicarbonate and trace metals, however the relative contribution of these factors is not currently clear.

Bicarbonate anion concentrations in the LDP10 water samples appears to be a likely significant driver in observed toxicity effects in this and previous PRP ecotoxicology laboratory and field studies.

Trace metals (Aluminium, Nickel, Zinc and Cobalt) may also have contributed to observed ecotoxicity effects but the complex chemical nature of the discharge (e.g. supersaturation of CO_2, unstable rising pH, high bicarbonate, low calcium, low hardness, and a number of concurrently elevated trace metals) make definitive interpretation difficult at this time. It is recommended that independent chemical modelling be carried out on current data to estimate concentrations of the various forms of the measured trace metals. This would assist in interpreting ecotoxicity effects and setting appropriate trace metal limits.

Recent published data on effects of bicarbonate to aquatic life have allowed calculation of bicarbonate trigger values, and these are presented along with previously calculated site-specific “salinity” trigger values.

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1. Sampling and Testing Summary

1.1 Sampling Dates

Water samples were collected by EPA officers on six occasions on 14, 21 and 28 May 2012 and 4, 16 and 25 June 2012.

1.2 Collection sites

On each date, samples were collected from four sites:

- Georges River - immediately upstream of confluence with Brennans Ck (LDP11)
- Georges River - immediately downstream of confluence with Brennans Ck (LDP12)
- Brennans Creek licensed discharge point – end of pipe (LDP10)
- Brennans Creek Dam (BCD) – surface sample

These sites are shown in Figure 1

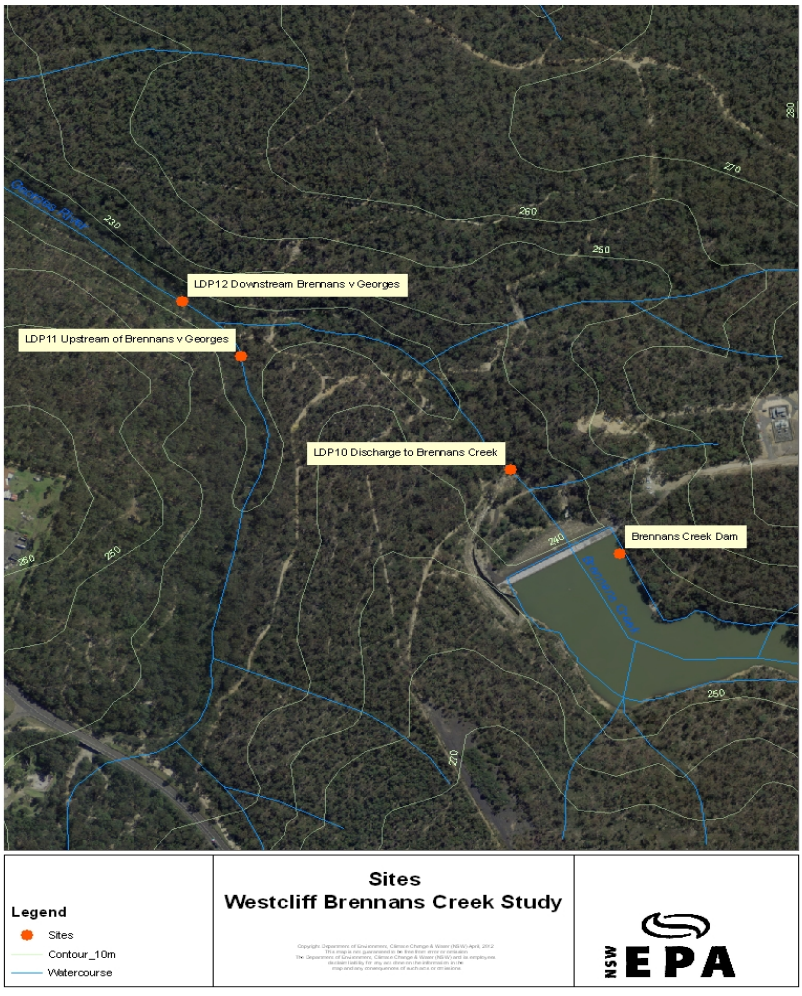

Figure 1. Collection Sites for the study (image courtesy of EPA)

1.3 Chemical analyses

Collected samples were analysed for:

- pH,
- sulphate,
- salinity,
- alkalinity (28/5/12 only),
- ammonia,
- NO_x-N,
- phosphorus (free reactive and total),
- TKN, and
- trace elements- in-situ 0.45µm-filtered and acid extractable (Al, As, Sb, Ba, Be, Bo, Ca, Cd, Cr, Co, Cu, Fe, Li, Mg, Mn, Mo, Ni, P, Si, Se, Ag, Na, Sr, S, Tl, Ti, Sn, V, Zn).

1.4 Ecotoxicity testing

Four test species were used during the study:

- *Melenotaenia duboulayi* (Crimsonspotted rainbowfish**)**
  96-hour larval imbalance test
- *Ceriodaphnia dubia* (waterflea)
  48-hour acute immobilisation test
  7-day chronic reproductive impairment test
- *Paratya austaliensis* (Glass shrimp)
  72-hour acute test
- Microtox® acute luminescence inhibition test

Test solutions were renewed only for the waterflea chronic test

Testing was carried out on samples as indicated in Table 1 below.

Table 1. Ecotoxicity test schedule

Sampling Date	Fish	Cladoceran (waterflea)		Glass shrimp	Bacteria
	96-h Larval *M. duboulayi*	48 hr *C. dubia* (acute)	7-d *C. dubia* reproductive impairment test	72-h *Paratya austaliensis* (acute)	Microtox® luminescence inhibition test
14/05/12	X	X	X[c]		
21/05/12	X	X			X
28/05/12	X		X[c]		
4/06/12	X[a]	X			
18/06/12	X		X[c]		
25/06/12	X[b]			X	

a: 48-hour test. b: 72-hour test.
c: test solution renewals utilized the following weeks samples.

2. Results

2.1 Analytical

Analytical test reports are contained in **Appendix 1.** Measured values for salinity (conductivity, µS/cm) for collected samples, as well as reported discharge rates for the LDP10 discharge are shown in Table 2.

Table 2. Sample salinity (µS/cm)/ discharge volume on collection days

Date	LDP11 Upstream of Brennans v Georges	LDP10 Discharge to Brennans Creek	LDP12 Downstream Brennans v Georges	Brennans Creek Dam	LPP10 Discharge (ML/day)
14-May-2012	161	1640	1140	1580	-
21-May-2012	220	1750	1300	1550	2.369
28-May-2012	150	1700	1200	1720	2.118
04-June-2012	184	1960	673	1720	1.005
18-June-2012	168	1910	988	-	7.865
25-June-2012	249	1780	1260	1620	7.922

Summarised analytical results including trace metals exceeding ANZECC/ARMCANZ (2000) trigger values are contained in Table 3.

Dissolved arsenic (As) and cadmium (Cd) were below ANZECC/ARMCANZ trigger values

Dissolved aluminium (**Al**) concentrations in the LDP10 discharge, ranging between **0.370** and **0.460** mg/L, were above the ANZECC/ARMCANZ (2000) trigger value to protect 95% of species of **0.055** mg/L.

Dissolved nickel **(Ni)** concentrations in the LDP10 discharge, ranging between **0.090** and **0.110** mg/L, were above the ANZECC/ARMCANZ (20000 trigger value to protect 95% of species of **0.011** mg/L.

Dissolved zinc (**Zn**) concentrations in the LDP10 discharge, ranging between **0.030** and **0.040** mg/L, were above the ANZECC/ARMCANZ (2000) trigger value to protect 95% of species of **0.008** mg/L.

Dissolved copper (**Cu**) concentrations in the LDP10 discharge, ranging between **0.0044** and **0.065** mg/L, were above the ANZECC/ARMCANZ (20000 trigger value to protect 95% of species of **0.0014** mg/L.

Dissolved cobalt (**Co**) concentrations in the LDP10 discharge, ranging between **0.008** and **0.009** mg/L, were above the ANZECC/ARMCANZ (20000 (low reliability) trigger value to protect 95% of species of **0.0014** mg/L.

Table 3. Selected averaged analytical results

Site	pH	Conductivity (µS/cm)	Alkalinity* mg/L $CaCO_3$		**Al** (diss.)	As (diss.)	Cd (diss.)	Ca (diss.)	**Co** (diss.)	**Cu** (diss.)	Mg (diss.)	**Ni** (diss.)	Na (diss.)	**Zn** (diss.)
			Bicarbonate	Carbonate										
U/S Georges River (LDP11)	7.6 (7.0-8.0)	194 (150-249)	6.2	<6	0.05 (<0.04-0.18)	0.003 (<0.001-0.009)	<0.0001	4.0 (1.9-10)	0.0006 (0.0004-0.0007)	0.0007 (<0.0004-0.0008)	3.1 (2.7-3.6)	0.002 (0.001-0.004)	21 (20-25)	0.007 (0.005-0.009)
LDP10	8.8 (8.6-9.3)	**1821** (1700-1960)	690	41	**0.42** (0.37-0.46)	0.007 (0.005-0.009)	0.0001 (<0.001-0.001)	6.7 (6.5-6.9)	**0.009** (0.008-0.009)	**0.0051** (0.0044-0.0065)	2.8 (2.6-3.0)	**0.095** (0.090-0.110)	430 (380-480)	**0.032** (0.030-0.040)
D/S Georges River (LDP12)	8.6 (8.4-9.0)	**1085** (673-1301)	460	26	**0.25** (0.08-0.35)	0.004 (<0.004-0.005)	<0.0001	5.5 (4.0-7.8)	**0.005** (0.001-0.008)	**0.0035** (0.009-0.0077)	3.0 (2.3-3.1)	**0.050** (0.008-0.060)	230 (220-290)	**0.018** (0.008-0.030)
BCD	8.8 (8.8-8.9)	1672 (1552-1800)	670	56	**0.48** (0.38-0.72)	0.007 (0.006-0.009)	0.0001 (<0.0001-0.0001)	6.4 (5.7-6.7)	**0.009** (0.006-0.012)	**0.0060** (0.0050-0.0083)	2.6 (2.3-2.7)	**0.083** (0.070-0.090)	380 (320-430)	**0.030** (0.020-0.040)
ANZECC/ARMCANZ 2000 Trigger Values (95%)		**350^**	**-**	**-**	**0.055**	0.013-0.024	0.0002	**-**	**0.0014#**	**0.0014**	**-**	**0.011**	**-**	**0.008**

All units are mg/L except pH and conductivity
Value ranges shown in brackets
Number of sampling = 6 unless otherwise indicated
Bold numbers indicate values above ANZECC/ARMCANZ 2000 trigger values
***** 28/5/12 sample only
# low reliability trigger value
^ANZECC/ARMCANZ 2000 default Trigger Value for slightly disturbed upland streams in south-east Australia (NSW)

2.1 Analytical (continued)

- **Bicarbonate *alkalinity* and bicarbonate *ion concentration***

Bicarbonate alkalinity in the LDP10 discharge was high at 690 mg/L (as $CaCO_3$) when measured in the 28 May sample. The reporting of alkalinity in terms of "mg/L $CaCO_3$" is standard practice, but conversion into bicarbonate *alkalinity* (mg/L HCO_3^-) requires multiplication by a factor of approximately 1.2 (at pH <9) (USGS 2001),

e.g. 690 mg/L bicarbonate alkalinity (as $CaCO_3$) X 1.22
= 828 mg/L bicarbonate alkalinity (as HCO_3^-)

The maximum value of the bicarbonate *ion* (HCO_3^-) from the above example is 828 mg/L, but the actual value will be lower due to the presence of other anions that contribute to "bicarbonate alkalinity", such as phosphates, borates, silicates, nitrates and dissolved ammonia. However, given the apparent relatively low value of other anions, then it is likely that the bicarbonate *ion* HCO_3^- is the major contributor to bicarbonate alkalinity.
A minimal decrease in bicarbonate concentration may also occur as the result of degassing of carbon dioxide leading to pH increase.

2.2 Ecotoxicity Tests

Ecotoxicity test reports are contained in Appendix 2.

2.2.1 Upstream Georges River (LDP11) Water Samples

The samples collected from the Georges River (LDP11) site, immediately upstream of the Brennans Creek confluence, did not cause observable effects in test animals in most tests.

The average number of young produced in undiluted (100%) samples collected on 14-21/5/12 was 21, a 30% reduction relative to the control group (30 young). No reproductive impairment occurred in a 30% concentration of the sample. There were no reproductive impairments noted in the two subsequent reproductive tests.

Table 4. Summary of Ecotoxicity test results for U/S Georges River (LDP11) water samples

Sample date	Fish	Cladoceran (waterflea)		Glass shrimp	Bacteria
	96-h Larval *M. duboulayi*	48-hr *C. dubia* (acute)	7-d *C. dubia* reproductive impairment test	72-h *Paratya austaliensis* (acute)	Microtox® Luminescence inhibition test
14/05/12	NT	NT	30% reduction in reproduction in 100% sample.		
21/05/12	NT	NT			NT
28/05/12	NT		NT		
4/06/12	NT	NT			
18/06/12	NT		NT		
25/06/12	NT			NT	

NT= no significant adverse effect relative to control group observed

2.2.2 Brennans Creek (LDP10) Water Samples

The LDP10 discharge samples did not cause observable acute effects in *Ceriodaphnia dubia*, or *Paratya australiensis* and caused only a minor effect in the Microtox® test.

Mortality was observed on larval rainbowfish in the first three weekly water samples at concentrations down to 30% sample. Most effects were noted to occur in the final day of the tests. Sample results for 4/06/12 and 25/06/12 are for 48 and 72 hours respectively, and no statistically significant toxic effects on fish were observed in these shorter duration tests. It is noted that PRP11 results for water samples collected on 5/06/12, resulted in fish mortalities at 25% sample concentration in a static 96-hour test.

The water samples collected on 18/06/12 did not exhibit an adverse effect on the fish, but for this test, larvae were 10 days old when tested, older than in the other 96-hour tests. The significance of this observation will be expanded upon in the discussion section.

Reproductive impairment in *C. dubia* occurred in LDP10 undiluted water samples, but reproduction was similar to controls in 30% sample concentration. There were no significant parental mortalities in the undiluted LDP10 water samples during the 7-day tests.

Table 5. Summary of Ecotoxicity test results for Brennans Ck. (LDP10) water samples

Sample date	Fish		Cladoceran (waterflea)		Glass shrimp	Bacteria
	Age of fish at start of test	96-h larval *M. duboulayi*	48-hr *C. dubia* (acute)	7-d *C. dubia* reproductive impairment test	72-h *Paratya austaliensis* (acute)	Microtox ® Lumines-cence inhibition test
14/05/12	6 days	90% mortality in 30% sample NOEC[§]= 10% sample	NT	82% reduction in reproduction in 100% sample. NOEC= 30%		
21/05/12	1 day	70% mortality in 30% sample NOEC= 10% sample	NT			14% reduction #
28/05/12	5 days	60% mortality in 30% sample NOEC= 30% sample		50% reduction in reproduction in 100% sample. NOEC= 30%		
4/06/12	14 days	NT (48 hour exposure) NOEC= 100% sample	NT			
18/06/12	10 days	NT NOEC= 100% sample		88% reduction in reproduction in 100% sample. NOEC= 30%		
25/06/12	5 days	NT (72 hour exposure) NOEC= 100% sample			NT	

NT= no significant adverse effect relative to control group observed
# maximum effect in luminescence inhibition observed in undiluted sample
§ NOEC = No Observed Effect Concentration (defined statistically)

2.2.3 Downstream Georges River (LDP12) Water Samples

The LDP12 Georges River downstream water samples did not cause observable acute effects in *Ceriodaphnia dubia*, *Paratya australiensis* or in the Microtox® test.

Mortality was observed in larval rainbowfish in the initial water samples of the study (collected weekly 14/5/12 and 21/5/12) at concentrations down to 30% sample. Most effects were noted to occur in the final day of the tests. Results for water samples collected on 4/06/12 and 25/06/12 are for 48 and 72 hours respectively, and no toxic affect was observed. The samples collected on 18/06/12 did not exhibit an adverse effect on the fish, but for this test, larvae were 10 days old when tested, older than in the other 96-hour tests. The significance of this observation will be expanded upon in the discussion section.

Reproductive impairment in *C. dubia* occurred in LDP12 undiluted and 30% concentration samples, collected on 14 & 21/5/12. Subsequent samples did not cause observable reproductive effects in undiluted samples. It is noted that sample conductivity varied lower in these latter tests, i.e. due to additional in-stream dilution effects due to varied LDP10 discharge rates and Georges River flows (see Table 2). There were no significant parental mortalities in the undiluted LDP10 samples during the 7-day tests.

Table 6. Summary of Ecotoxicity test results for D/S Georges R. (LDP12) water samples

Sample date	Fish	Cladoceran (waterflea)		Glass shrimp	Bacteria
	96 hour larval *M. duboulayi*	48-hour *C. dubia* (acute)	7-day *C. dubia* reproductive impairment test	72-hour *Paratya austaliensis* (acute)	Microtox® Luminescence inhibition test
14/05/12	100% mortality in 30% sample NOEC[§]= <30% sample[*]	NT	53% reduction in reproduction in 100% sample.		
21/05/12	70% mortality in 30% sample NOEC= <30% sample*	NT	28% reduction in 30% sample NOEC= <30%*		NT
28/05/12	100% mortality in 100% sample NOEC= 30% sample		NT 100% sample.		
4/06/12	NT (48-hour exposure) NOEC= 100% sample	NT	NOEC= 100% sample		
18/06/12	NT NOEC= 100% sample		NT 100% sample.		
25/06/12	NT (72-hour exposure) NOEC= 100% sample		NOEC= 100% sample	NT	

NT= no significant adverse effect relative to control group observed
§ NOEC = No Observed Effect Concentration (defined statistically)
* 30% was the lowest concentration tested for this sample.

2.2.4 Brennans Creek Dam Surface Water Samples

The Brennans Creek Dam (BCD) water samples did not cause observable acute effects in *Ceriodaphnia dubia*, *Paratya australiensis* or in the Microtox® test.

Mortality was observed in larval rainbowfish in the first three weekly samplings at concentrations down to 30% sample. The test on 28/05/12 resulted in 30% mortality at 100% samples (not statistically significant). The water samples collected on 18/06/12 did not exhibit an adverse effect on the fish, but it is noted that for this test, larvae were 10 days old when tested, older than in the other 96-hour tests. As mentioned in section 2.2.3, this point will be expanded upon in the discussion section. Results for 25/06/12 samples are for 72-hours exposure and no acute toxic affect was observed.

Reproductive impairment in *C. dubia* occurred in BCD undiluted and 30% concentration of water samples, collected on 14 & 21/5/12. There were no significant parental mortalities in the undiluted LDP10 water samples during the 7-day test. Subsequent water samples collected were not tested with the 7-day *C. dubia* test

Table 7. Summary of ecotoxicity test results for Brennans Creek Dam surface samples

Sample date	Fish	Cladoceran (waterflea)		Glass shrimp	Bacteria
	96-hour larval *M. duboulayi* (acute)	48-hour *C. dubia* (acute)	7-day *C. dubia* reproductive impairment test	72-hour *Paratya austaliensis* (acute)	Microtox® Luminescence inhibition test
14/05/12	90% mortality in 30% sample NOEC[§]= 30%[a] sample	NT	53% reduction in reproduction in 100% sample. NOEC= 30% sample		
21/05/12	70% mortality in 30% sample NOEC= 10% sample	NT			14% reduction#
28/05/12	30% mortality in 100% sample NOEC= 100% sample				
4/06/12	NT (48-hour exposure) NOEC= 100% sample	NT			
18/06/12					
25/06/12	NT (72-hour exposure) NOEC= 100% sample			NT	

NT= no significant adverse effect relative to control group observed
# maximum effect in luminescence inhibition observed in undiluted sample
§ NOEC = No Observed Effect Concentration (defined statistically)
"a" – 40% mortality in 30% concentration – not statistically different to control

3. Assessment

3.1 Overview

This study was implemented to provide an independent ecotoxicological assessment of the LDP10 discharge. It did not seek to reproduce the Pollution Reduction Program 11 study carried out by Ecoengineers P/L to assess the contribution of the coagulant Magnasol 572 on previously observed laboratory and field effects (but noting that that report concludes that the coagulant does not appear responsible). The PRP11 study report (Short 2012), does provide additional information which assists in interpretation of the results of this work.

- **Consistency in ecotoxicity results between different laboratories**

Ecotoxicity tests carried out at separate facilities on similar samples, returned similar results. For example PRP11 study samples collected from the LDP10 discharge on 5/6/12 were found to cause larval fish mortalities down to a 25% sample concentration (1:3 dilution), and these mortalities primarily occurred on the last day of the 96 hour test (noting test solutions were not renewed). Comparable toxicity effects were noted in similar LDP10 discharge samples in a number of larval fish tests carried out at the OEH facility. The absence of acute toxicity to *C. dubia* in the current (lower than average conductivity) LDP10 samples was the same between the two laboratories.

The presence or absence of observed toxicity in the PRP11 and current work are summarised in Table 8 below.

Table 8. Presence or absence of toxicity in PRP11 and current study

Site	Larval rainbowfish 96-h Acute immobilisation		*C. dubia* 48-h Acute immobilisation		*C. dubia* 7-d reproduction impairment	*P. australiensis* (glass shrimp) 96-h Acute immobilisation		Selenastrum (green algae) 72-h growth inhibition	Microtox® Bacterial luminescence inhibition
	PRP11 study	OEH study	PRP11 study	OEH study	OEH study	PRP11 study	OEH study	PRP 11 study	OEH study
	Toxicity effects generally evident?								
U/S GR LDP11		**No**		**No**	**No**		**No**		**No**
Untreated Process Water#			**Yes**			**Yes**			
Dosed inflow tanks#			**Yes**			**Yes**			
Clarified Process Water#			**Yes**			**Yes**			
BCD surface#		**Yes**	**No**	**No**	**Yes**	**Variable**	**No**		**No**
LDP10 discharge	**Yes** (static test) **No** (48-h renewed solution)*	**Yes** (static tests)		**No**	**Yes**	**No**	**No**	**No**	**No**
D/S GR LDP12		**Variable**		**No**	**Variable**		**No**		**No**

* 3 week delay between collection and testing may have influenced results.
# West Cliff internal process waters

- **Larval fish mortalities in West Cliff samples.**

The consistent larval rainbowfish mortalities observed in LDP10 and downstream water samples in non-renewed 96-hour exposures, and the absence of acute lethal effects after 96-hours in 48-hour renewed solutions (PRP11 report - Short, 2012), appear to be associated with pH elevation (due to CO_2 degassing).

The PRP11 study reported elimination in observed effects on larval fish in a subsequent test where solutions were renewed at 48 hours. This effect may require confirmation due to the near 3 week delay between sample collection (26/06/12) and testing (17/07/12). Also any future comparisons of static and renewal tests should be completed at the same time and on the same sample to avoid potentially confounded results.

At the time of writing, the likely major driver/s of toxicity in this species is not known. Bicarbonate concentrations in sample dilutions showing significant effects (i.e. 25-30%) are well below concentrations shown to cause acute effects on various other species of larval fish (Farag and Harper, 2012), and assessment of bicarbonate sensitivity of this life-stage is required. It is noted that newly hatched larval fish are not fed for the duration of the 96-hour test and though this is standard procedure for acute tests, it is possible that internal energy reserves became limiting under the stresses of maintaining internal ionic balance in the discharge samples. This however would not explain a difference in apparent toxicity between renewed and non-renewed solutions.

Typically when water is taken from depth and brought to the surface (as occurs with the LDP10 discharge), it is supersaturated with carbon dioxide (CO_2). This degassing of CO_2 results in pH increase, for example, pH increased from around 8.6 to 9.1 in LDP10 samples in non-renewed ecotoxicity tests. Changes in pH during the tests may have resulted in unusual metal speciation effects. Eliminating or confirming potential contributions to ecotoxic effects of various trace metals under varying pH would require further testing and sample manipulations.

It is noted that previous PRP studies on juvenile rainbowfish (i.e. less than approximately 6 month old) did not detect acute toxicity in the LDP10 discharge samples or in Georges River samples.

- **Stimulation of Algal growth rate in LDP10 water sample**

It was reported in the PRP11 study report (Short, 2012) that a sample collected on 5 June 2012 from the LDP10 discharge, resulted in biostimulation of algal growth relative to the control group, even at the lowest tested concentration of 6.3% sample. This was attributed to the nutrient content of the discharge. The biostimulation of the algal test species ("*Selenastrum capricornutum*"), indicates that the discharge may contribute to nuisance algal growth (eg filamentous algae) observed in the upper Georges River downstream the LDP10 inflow point.

3.2 Possible Toxic Constituents in the LDP10 discharge

A limitation of an "individualised" approach to assessing overall (whole effluent) ecotoxic acute and chronic effects on different species is that based on the current level of scientific knowledge, effects may well be difficult to totally disentangle. For example definitively

assigning or eliminating observed toxicity due to individual variables such as bicarbonate, variable and alkaline pH (8.6-9.3) and a number of trace metals, in waters of low calcium and magnesium concentrations may be difficult to accurately model and interpret.

That said, bicarbonate anion concentrations appear likely to be a major contributor to observed effects and this is discussed further below, followed by an assessment of relevant trace metals.

Methods are available which attempt to characterise ecotoxicity following sample manipulations, so called Toxicity Identification Evaluation (TIE) procedures. These procedures developed by the United States Environment Protection Agency (USEPA 1991) attempt to determine the primary driver/s for observed toxicity (e.g., trace metals, organics, ammonia), but procedures would need to be modified to allow assessment of the contribution of, for example, bicarbonate, which is not specifically targeted in the published TIE procedures. Assessment of bicarbonate toxicity could for example be achieved by acidification of the sample, followed by bubbling of nitrogen, then readjustment of the pH with a phosphate buffer.

3.2.1 Bicarbonate

- **Constituents making up salinity are important**

Salinity (or conductivity when reporting in µS/cm), is made up of a number of different ions present in waters. Bicarbonate (HCO_3^-) is one of the ions which contributes to salinity in the LDP10 discharge, and it is present in relatively high concentrations. Estimated maximum HCO_3^- in the 28/05/12 LDP10 sample was 828 mg/L, but it is noted that HCO_3^- has been reported at 1450 mg/L HCO_3^- (at approx 2750 µS/cm)(Section 9 Figures 4b and 4e, PRP10 report 2010).

Under low rainfall conditions it would be expected that the Brennans Creek Dam (and the LDP10 discharge), will effectively mirror the bicarbonate concentration of the clarified process waters. The current study was carried out at a time when rainfall had potentially reduced the LDP10 discharge to below average concentrations for salinity (2004 -2009 average approximately 2500 µS/cm – range 1000 to 3500 µS/cm (Short, 2012)).

Previous PRP studies identified HCO_3^- as one of the more likely contributors to observed field toxicity (PRP10 Report EL0809005, Executive Summary).

- **High bicarbonate concentrations can be toxic to aquatic life**

There have until recently been relatively few studies on the toxicity of the bicarbonate ion to aquatic organisms, probably based on the fact that the concentrations required to elicit a toxic response were considered to be too high to be of environmental concern given typical environmental concentrations. However of the various salts, HCO_3^- has been found to be one of the more toxic to aquatic organisms, with 48-hour LC50 to *C. dubia* of around 1000 mg/L (Mount *et al*, 1997). By way of comparison, salinity due to sodium chloride, NaCl, (i.e. "table salt") appears to be 2 to 2.5 times less acutely toxic to *C. dubia* than sodium bicarbonate ($NaHCO_3$) (Mount *et al*, 1997, USEPA 1991).

- **Narrow gap between no bicarbonate toxicity and high bicarbonate toxicity**

An important acute toxicity characteristic of HCO_3^- is the narrow range between non- or low toxicity and high toxicity. For example it was estimated that for *C. dubia* in 48-hour exposures, 20% mortality occurs at approximately 950 mg/L, while 80% mortality occurs at approximately 1250 mg/L (Mount *et al*, 1997). The use of average values with respect to salinity (bicarbonate) discharge concentrations would need to be carefully assessed so that they do not mask relevant high concentrations which may be of ecotoxic concern.

- **Bicarbonate - mode of toxicity action**

An extensive body of scientific work investigating the potential laboratory and field effects of $NaHCO_3$ on aquatic life has recently been published (Farag and Harper 2012) providing insight to this "all or nothing" acute effect. In this work, detailed biochemical studies on exposed fish concluded that one mechanism of toxicity, even in chronic tests, is due to the acute overwhelming of the animal's ability to effectively maintain internal ion regulation by interfering with sodium and potassium "pumps" (chloride cells) located on gill and epithelial surfaces. Thus while HCO_3^- aqueous concentrations remain below a critical threshold, toxicity is not evident, but once the threshold is past, acute lethal effects occur due to the rapid "shut down" of the chloride cell "pumps". An additional mode of toxic action is suggested due to HCO_3^- involvement in respiration. Carbon dioxide (CO_2), produced during respiration, is converted to HCO_3^- in the blood. It is suggested that (chloride cell mediated) HCO_3^- excretion across gill membranes, is inhibited by elevated water concentrations of HCO_3^-, leading to an acute and lethal inability to maintain blood acid/base balance.

Whilst critical acute thresholds for different taxa are likely to differ considerably (as they do for most toxicants), it is likely that the relatively sudden onset of toxicity will hold for many species, given the likely mode of toxic action.

- **Long-term (chronic) exposure to lower concentrations lead to toxic effects**

Although the lethal toxicity of HCO_3^- appears to be acutely mediated, other adverse effects appear to occur at lower HCO_3^- concentrations, when exposure continues for longer periods. Long term (60 day) exposure to elevated HCO_3^- concentrations found histopathological lesions and diseased organs including gills, kidneys and livers in exposed fish (Farag and Harper 2012). These studies found repeatable adverse effects on multiple species of both invertebrates and fish at concentrations above 360-730 mg/L HCO_3^-. An acute to chronic ratio (ACR) of 2.41 was calculated using data from three taxa (fish, cladoceran and a mussel).

- **Effects more severe on early life stages**

Another important effect noted in the Farag and Harper studies was a significant difference in larval fish sensitivity depending on the age of the minnow larvae used to start the test. Two day old fathead minnow (FHM) larvae were found to be considerably more sensitive to HCO_3^- than 4-6 day old FHM larvae. This may provide some explanation of the absence of effect on larval rainbowfish for the 18/6/12 LDP10 sample as 10-day old larvae were used, compared to one- to five-day old fish used in earlier 96-hour tests.

- **Comparison of observed *C. dubia* acute toxicity in West Cliff waters matches known sensitivity to bicarbonate**

The North American clone of *C. dubia* has a reported 48-hour LC50 of approximately 1000 mg/L for bicarbonate (Mount *et al*, 1997). The *C. dubia* clone used in the current tests may differ slightly in sensitivity to bicarbonate but it is considered likely to fall close to the value indicated above.

The results in Table 9 below show that when estimated HCO_3^- concentrations exceeded the known acute toxicity value for *C. dubia*, toxicity was observed. However if it was below the toxicity threshold, acute toxicity was not observed. This would indicate that bicarbonate is potentially a major factor in observed acute toxicity effects.

Table 9. Estimated concentrations of HCO_3^- at which acute toxicity to *C. dubia* occurs

	Untreated Process Water	Clarified Process Water	BCD surface	LDP10	D/S Georges R. LDP12
Concentration of HCO_3^- (mg/L)* at which 48-h acute toxic effects observed	1660*	1460*	-	-	-
Concentration of HCO_3^- (mg/L)* at which 48-h acute toxic effects NOT observed	830*	730*	804#	828#	551#

*Estimation based on 1450 mg/L HCO_3^- = 2750 µS/cm = 0.53 conversion factor (Section 9 Figures 4b and 4e, PRP10 report 2010). Toxicity and conductivity values taken from PRP11 report Tables 2 and 3
#Estimated maximum values calculated from bicarbonate alkalinity values 28/05/12 samples this study (see Section 2.1)

Chronic reproductive impairment in the North American clone of *C. dubia* has been reported to occur at bicarbonate concentrations greater than 500mg/L (Farag and Harper 2012). In the current tests, reproductive impairment in *C. dubia* occurred at estimated (maximum) bicarbonate concentrations of 828 mg/L (100% LDP10 28/05/12), while estimated 280mg/L (30% LDP10 28/05/12) bicarbonate did not cause observable adverse effects.

3.2.2 Trace metals

It should be noted that the ANZECC/ARMCANZ (2000) trigger values (TVs) apply to receiving environment concentrations, not necessarily "end of pipe" concentrations, so immediate dilution effects (e.g. by Georges River flows) need to be taken into account.

Given that the LDP10 discharge can, under low rainfall conditions, become more concentrated (than those evident in this study), and concurrently environmental flows in the Upper Georges River may fall, then the potential "end of pipe" concentrations of the LDP10 Brennans Creek discharge can be expected to linger for variable distances downstream in Brennans Creek and the Georges River depending on environmental flows and LDP10 discharge rates.

A number of metals (e.g. nickel and zinc) known to be toxic in "soft" waters are known to become significantly less toxic to aquatic life when carbonate (e.g. $CaCO_3$) hardness increases, and algorithms are available in the ANZECC/ARMCANZ (2000) guidelines which can be used to adjust TVs according to water hardness concentrations.

The LDP10 discharge can be considered a "soft" water with respect to hardness, but sodium bicarbonate ($NaHCO_3$) alkalinity is high. The ameliorating effects of bicarbonate (HCO_3^-) on trace metal bioavailability is less well studied (empirically) or has been confounded with carbonate, so whilst it will be expected that some reduction in toxicity is likely with increasing HCO_3^- concentrations for some trace metals, this does not appear to be the case for all metals and species e.g. zinc and *C. dubia* (Hyne et al, 2005). Additionally the relatively low levels of calcium and magnesium in the LDP10 waters (and in the Georges River), may result in relatively greater toxic effects (Zalizniak *et al*, 2006).

Recommendation: Independent chemical modelling using a range of current models and utilizing present measured water quality variables may assist in interpreting ecotoxicity test data and in setting appropriate discharge limits. The modelling would allow estimation of the various speciation "forms" of the dissolved trace metals and this would allow more specific assessment as to whether the exceedance of the various ANZECC/ARMCANZ 2000 trace metal trigger values are of ecotoxicological concern. It is noted that for some trace metals "simple" speciation estimates may not adequately assess ecotoxic potential, particularly when the interaction of the large number of ionic constituents present in the LDP10 discharge waters are considered.

Specific trace metals that were detected at concentrations above ANZECC/ARMCANZ (2000) trigger values are considered separately below.

- **Aluminium**

Dissolved aluminium (Al) concentrations in the LDP10 discharge ranged up to 8 times the ANZECC/ARMCANZ (2000) 95% species protection trigger value of 0.055 mg/L.
The toxicity of Aluminium in alkaline waters (as occurs in the LDP10 discharge) has had limited attention in the literature, possibly due to the historical focus on Al toxicity under acidic conditions. For example the bioavailability of $Al(OH)_4^-$, which predominates in water with pH >7, is poorly understood (Gensemer and Playle, 1999).

It is not known if the elevated aluminium concentrations in the LDP10 samples may have contributed to the observed ecotoxic effects. Based on published data, it would seem less likely that the measured Al concentrations in the LDP10 samples would have caused acute toxic effects in current tests (i.e. including PRP11 tests in Short, 2012) with cladocerans (and likely shrimp), but potential contribution to effects observed in larval fish or sublethal effects on *Ceriodaphnia dubia* is not currently known.

- **Nickel**

Dissolved nickel (Ni) concentrations in the LDP10 discharge ranged up to 10 times the ANZECC/ARMCANZ (2000) 95% species protection trigger value of 0.011 mg/L. Annual return data for LDP10 indicates Ni has on occasion reached 0.235 mg/L, 20 times higher than the trigger value (Appendix 3) .

Acute nickel toxicity to aquatic organisms is considered to be moderate (often in the tens of mg/L) and based on published data it is considered unlikely that acute effects observed in current samples were due to nickel. Fish appear to be relatively less (acutely) sensitive than crustaceans to nickel. Chronic effects however have been reported at significantly lower concentrations, and similar to those in the LPD10 discharge, but it is not currently possible to quantify the possible ameliorating effects of elevated bicarbonate. Nickel is also expected to co-precipitate with iron hydroxides at pH > 6.5 (ANZECC/ARMCANZ 2000), but this is somewhat counteracted by observations that nickel toxicity (in high carbonate waters) was highest at pH 8.5, similar to that initially in the LDP10 discharge (pH≈8.5). It therefore

remains possible that nickel in the LDP10 discharge may have contributed to observed sublethal ecotoxic effects in *Ceriodaphnia dubia*.

- **Zinc**

Dissolved Zinc (Zn) concentrations in the LDP10 discharge, ranged up to 5 times the ANZECC/ARMCANZ (2000) 95% species protection trigger value of 0.008 mg/L. Annual return data for LDP10 indicates zinc has on occasion been measured at 0.245 mg/L, 30 times higher than the trigger value (Appendix 3).

Acute zinc toxicity varies with carbonate hardness and pH, with increasing hardness and lowering pH typically reducing toxicity. However published data on toxicity trends for Zn beyond pH 8, are not consistent. Complicating interpretation Hyne *et a*l (2005) found increasing bicarbonate alkalinity did not provide protective effects to *Ceriodaphnia dubia* in acute tests at pH 8.4.

At the concentrations of dissolved zinc measured in this study, and at the measured pH >8, it is possible that zinc concentrations contributed to observed sublethal (reproductive) effects in *Ceriodaphnia dubia* and may have contributed to acute toxicity observed in the West Cliff internal process waters (contained in the PRP11 study report - Short, 2012).

- **Copper**

Dissolved copper (Cu) concentrations in the LDP10 discharge, ranged up to 5 times the ANZECC/ARMCANZZ (2000), 95% species protection trigger value of 0.0014 mg/L.

Available information on (lack of) effects of carbonate or bicarbonate concentration on chronic copper toxicity (De Schamphelaere and Janssen, 2004) indicates relationships may not follow simple speciation models. But at the concentration of dissolved organic carbon reported for LDP10 samples in the PRP11 report (≈5mg/L) it is unlikely that copper is involved in observed ecotoxic effects (Hyne *et al* 2005, Erickson *et al* 1996).

- **Cobalt**

Dissolved cobalt (Co) concentrations in the LDP10 discharge, ranged up to 6 times the ANZECC/ARMCANZZ (2000), 95% species protection trigger value of 0.0014 mg/L.

Cladoceran species such as *C. dubia* appear particularly sensitive (chronic) to cobalt, with measured concentrations in LDP10 in the range of published effects (ANZECC/ARMCANZ 2000, Diamond *et al*, 1992). However the potential affects of bicarbonate and pH on reducing toxicity are not well studied. Therefore, it is not clear to what extent reproductive impairment effects *C. dubia* in LDP10 may have been affected by cobalt concentrations.

4. Calculations of Receiving Waters Trigger Values for Bicarbonate (using ANZECC/ARMCANZ (2000) procedures) and comparison with USEPA generated criteria

4.1 ANZECC/ARMCANZ procedure generated trigger values for bicarbonate

Using bicarbonate toxicity test data contained in Farag and Harper (2012), various trigger values (TVs) were calculated following the ANZECC/ARMCANZ (2000) procedure. Acute No Observed Effect Concentrations (NOECs) were estimated (i.e. based on what appeared to be likely significant adverse effect concentrations and noting many tests produced all or nothing data). Measured bicarbonate concentrations for these NOECs were used to generate the trigger values. The data set is included in Table 10 below, to allow validation of data used and calculated trigger values. An Acute to Chronic Ratio (ACR) of 2.4 was used which was also sourced from Farag and Harper (2012).

The calculated trigger values for bicarbonate were:

- **95 % species protection level = 225 mg/L HCO_3^-**
- **90 % species protection level = 261 mg/L HCO_3^-**
- **80 % species protection level = 319 mg/L HCO_3^-**

Although the above TVs are calculated exclusively using North American species, there is no information available that would suggest endemic south eastern Australian flora and fauna are likely to be inherently more resistant to bicarbonate. Additionally compared to waters used in the Farag and Harper (2012) studies, LDP10 waters contain much lower levels of calcium which has been suggested as being more toxic than waters high in calcium (Zalizniak, 2006).

4.2 USEPA procedure generated water-quality criteria for bicarbonate

By way of comparison, the United States Environment Protection Agency (USEPA) uses a different procedure (to ANZECC/ARMCANZ 2000), to calculate water-quality criteria for the protection of aquatic life. Following the USEPA procedure, Farag and Harper (2012) calculated the following bicarbonate criteria values;

Criterion Continuous Concentration (CCC) "Chronic criteria" = 290 mg/L HCO_3^-

Criterion Maximum Concentration (CMC) "Acute criteria" = 317 mg/L HCO_3^-

CCC values are defined as the maximum continuous concentration of HCO_3^- that would be allowed in an effort to protect aquatic life. This is further defined in terms of duration and frequency, and the USEPA default recommendation is that the average concentration in a 24-hour period should not exceed the CCC more than once every three years (though the applicability of this may be chemical specific).

CMC values define the concentration allowed in an effort to protect aquatic life. This concentration also needs to be defined in terms of duration and frequency and the USEPA

default recommendation is that the average in a 1-hour period should not exceed the CMC more than once every three years (though the applicability of this may be chemical specific).

It is noted in the Farag and Harper (2012) report that the CCC and CMC values may need to be further refined as the experimental data only included macroinvertebrates known to be generally pollution tolerant (i.e. the tubifex and chironomid worms).

Table 10 Acute No Observed Effect Concentrations (NOEC) values for bicarbonate to a range of North American species

Species	Estimated HCO_3^- Acute NOEC* (mg/L)
Pallid sturgeon	**844**
Fathead minnow	**1020**
White sucker	**1300**
African clawed frog	**900**
Freshwater mussel	**623**
Rainbow trout	**4280**
Ceriodaphnia dubia	**727**
Chironomid	**4320**
Tubifex	**2136**
Amphipod	**900**
Shovelnose sturgeon	**565**
Walleye	**1880**
Northern pike	**5000 (est)**

*All values sourced from Farag and Harper (2012) and based on visual estimation of NOECs

4.3 Previously calculated "salinity" trigger values for the West Cliff LDP10 - Brennans Creek/Upper Georges River system

Based on a range of industry sponsored laboratory and field studies on the LDP10 discharge, site-specific trigger values were calculated using ANZECC/ARMCANZ 2000 procedures (Lincoln-Smith 2010).

The "Lab and field" and "Field only" values were generated using presence data for macroinvertebrates, and the validity of this approach was questioned in an industry commissioned peer review of the work (PRP10 2010).

It appears some of the macroinvertebrate field data were calculated based on presence in tributary streams (i.e. out of the influence of the LDP10 discharge), which may be more relevant to pure "salinity/conductivity" values rather the LDP10 discharge.

Given that "salinity" as expressed as electrical conductance (µS/cm) appears to be only a proxy for the possible primary toxicant driver/s, (e.g. bicarbonate, and perhaps some trace metals), then the values below should be treated with caution. It may be possible to recalculate the trigger values based on for example, bicarbonate, if this data exists (or can confidently be generated) for the tests/taxa used to generate the trigger values.

The generated "salinity" species protection values for Brennans Creek/Georges River (Lincoln-Smith 2010) were:

Laboratory ecotoxicity data only used
PC_{95} = 495 µS/cm (i.e. the value to afford protection to 95% of aquatic species)
PC_{90} = 534 µS/cm
PC_{80} = 605 µS/cm

Laboratory and Field (presence data)
PC_{95} = 585 µS/cm
PC_{90} = 695µS/cm
PC_{80} = 921µS/cm

Field (presence data) only used
PC_{95} = 876 µS/cm
PC_{90} = 1263 µS/cm
PC_{80} = 1992 µS/cm

5. References

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6. Appendices