Appendix D – Ecotoxicology

RP1 Ecotoxicology – February 2013

Vista Gold Australia Pty Ltd

Mt Todd Discharge Surface Water Report

March 2014

WATER | ENERGY & RESOURCES | ENVIRONMENT | PROPERTY & BUILDINGS | TRANSPORTATION

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1. Introduction

1.1 Project Background

Vista Gold Australia Pty Ltd (Vista Gold) received a Waste Discharge Licence (WDL 178-3) on 26 November 2013 from the Northern Territory Environment Protection Authority (NT EPA). The WDL outlines environmental requirements for protection of the Edith River from mine wastewater discharges. Prior to discharge from RP1, RP3 or RP7 dilution factors must be calculated, based on ecotoxicological assessment, to meet 80% species protection at SW4.

The use of dilution factors derived from direct toxicity assessment (DTA) for three water bodies at the Mt Todd mine site has been selected as the most appropriate method for deriving safe concentrations of metals for environmental protection of the Edith River to meet the requirements of WDL 178-3. This method is recommended by ANZECC & ARMCANZ (2000) where chemicals are present in a complex mixture. DTA allows the determination of direct biological effects on appropriate species to be assessed and also enables metal concentrations to be established that would not cause adverse environmental effects.

1.2 Objective

WDL 178-3 requires that ecotoxicological assessment be conducted for a wastewater source. This retention pond (RP) 1 Ecotox DTA Report has been developed to address the relevant sections of the WDL 178-3 to derive a dilution factor for RP1 prior to discharging into the Edith River.

1.3 Scope of Work

This Report addresses the following aspects of the WDL 178-3:

- The methodology for calculating the 80% species protection ANZECC & ARMCANZ (2000) dilution factor for ecosystem protection applied at SW4 for mine discharges from RP1.
- Deriving Monitoring Values for the RP1 mine water to be met at SW4 to check that the dilution factor for the treated mine water is being met.

1.4 Limitations

This Vista Gold Australia Discharge Plan Revision 1 ("Report"):

1. *has been prepared by GHD Pty Ltd ("GHD") for Vista Gold Australia Pty Ltd (Vista Gold) and the NT EPA;*
2. *may only be used and relied on by Vista Gold and the NT EPA;*
3. *must not be copied to, used by, or relied on by any person other than Vista Gold without the prior written consent of GHD;*
4. *may only be used for the purpose of addressing WDL 178-2 requirements (and must not be used for any other purpose).*

GHD and its servants, employees and officers otherwise expressly disclaim responsibility to any person other than Vista Gold arising from or in connection with this Report.

To the maximum extent permitted by law, all implied warranties and conditions in relation to the services provided by GHD and the Report are excluded unless they are expressly stated to apply in this Report.

The services undertaken by GHD in connection with preparing this Report were limited to those specifically detailed in section 1.2 of this Report.

The opinions, conclusions and any recommendations in this Report are based on assumptions made by GHD when undertaking services and preparing the Report ("Assumptions"), including (but not limited to): Current accepted practices.

GHD expressly disclaims responsibility for any error in, or omission from, this Report arising from or in connection with any of the Assumptions being incorrect.

Subject to the paragraphs in this section of the Report, the opinions, conclusions and any recommendations in this Report are based on conditions encountered and information reviewed at the time of preparation and may be relied on until 6 months, after which time, GHD expressly disclaims responsibility for any error in, or omission from, this Report arising from or in connection with those opinions, conclusions and any recommendations.

1.5 Assumptions

The assumptions upon which this report is based are:

- The mine water tested was representative of the RP1 water at the time of testing; and
- SW2 water used in the DTA was representative of wet season Edith River water.

2. Direct Toxicity Methodology

2.1 Direct Toxicity Assessment (DTA)

DTA provides an integrated measure of the toxicity of chemicals within a complex mixture, and accounts for interactions between compounds which may be additive, synergistic or ameliorative. DTA provides a better representation of natural environmental conditions than single chemical testing, and is therefore recommended by ANZECC & ARMCANZ (2000) for assessing the environmental impact from complex effluents.

The Mt Todd DTA program was designed in line with the recommendations contained in ANZECC & ARMCANZ (2000). ANZECC & ARMCANZ (2000) states that a DTA program should address the following design considerations:

- Test species selection
- Receiving water selection
- Nature of contaminant(s)
- Test methods
- Statistical considerations.

2.1.1 Selected Species

ANZECC & ARMCANZ (2000) "Minimum requirements for DTA" (section 8.3.6.8) recommend that toxicity data from between three and five species representing at least four different taxonomic groups (where five species are used) is required for effluent DTA. Further information on species used in the DTAs conducted for Mt Todd discharge to the Edith River is shown in Table 1 below.

DTAs conducted on RP1, RP7 and RP3 previously used a maximum of seven species to provide confidence in the species sensitivity distribution calculation. Often, due to laboratory issues, not all tests listed in Table 1 are available at the time of testing, however, all bioassays were available to assess the toxicity of RP1 in this study.

Tests were selected adhering to the following criteria:

- a minimum of five bioassays covering at least four taxa
- preferably chronic endpoints to avoid the used of application factors
- locally occurring species or species representative of the ecosystem
- dilution water sampled from SW2
- sufficient test dilutions to obtain a statistically valid EC10 (i.e. tight 95% confidence limits)
- bioassay suite sensitive to a broad range of contaminants

In the past, the bioassays were conducted in two laboratories because not all bioassays are available from one laboratory. The cladoceran (*Moinodaphnia macleayi*) is the most sensitive bioassay species and is only able to be conducted by the ERISS Laboratory in Darwin, however, the ERISS laboratory is no longer conducting bioassays on a commercial basis. Currently all bioassays, as shown in Table 1, are conducted by Ecotox Services Australasia located in Sydney.

Table 1 Species used in the Ecotoxicological Assessment of RP1 Discharge

Test Organism	Test Duration	Test Endpoint	Key Reference
Selenastrum capricornutum (green alga)	72 hour (chronic)	Growth inhibition	USEPA Method 1003.0 (2002)
Lemna aequinoctialis (duckweed)	96 hour (chronic)	Growth (frond number)	OECD Method 221 (2006)
Ceriodaphnia dubia (water flea)	3 brood (chronic)	Reproduction	USEPA (2002)
Hydra viridissima Pallas (green hydra)	96 hour (chronic)	Population growth	Riethmuller *et al.* (2003)
Chironomus tepperi (chironomid)	48 hour (acute)	Survival	USEPA (2002), OECD (2011)
Paratya australiensis (shrimp)	96 hour (acute)	Survival	ESA SOP 123 (ESA 2012)
Melanotaenia splendida (rainbowfish)	10 day (chronic)	Embryonic development and post hatch survival	USEPA (2002)

2.1.2 Concentrations Tested

Edith River water sampled from the upstream site SW2 was used as the diluent to assess the toxicity of the RP1 mine water. A laboratory control was run concurrently with all tests to confirm that the natural diluent water was not confounding the bioassays (as can happen in many cases). All samples were serially diluted with the SW2 river water to achieve the required test concentrations. Concentrations differed between bioassays due to methodologies used and sensitivity of the species tested as determined by previous bioassays, as shown in Table 2.

Table 2 Concentrations of RP1 used in Bioassays

Microalga % RP1	Duckweed % RP1	Cladoceran % RP1	Hydra % RP1	Chironomid % RP1	Shrimp %RP1	Fish % RP1
0	0	0	0	0	0	0
0.031	0.031	0.031	0.031	0.3	0.031	0.016
0.063	0.063	0.063	0.063	0.6	0.063	0.031
0.125	0.125	0.125	0.125	1.3	0.125	0.063
0.25	0.25	0.25	0.25	2.5	0.25	0.125
0.5	0.5	0.5	0.5	5.0	0.5	0.25
1.0	1.0	1.0	1.0	10	1.0	0.5

2.1.3 Dilution Factors

Dilution factors are derived from results of DTA using bioassays representative of the receiving ecosystem which incorporates all actions of the constituents of the effluent. These results are

placed in the BurrliOZ (Campbell *et al.* 2000) statistics program for calculation of a species sensitivity distribution and the concentration of effluent is determined to protect 80% of the populations in the receiving water from a 10% decrease in growth and / or reproduction.

Concentrations of individual chemicals cannot be extrapolated from DTAs for use as trigger values. However, individual chemical concentrations can be used for monitoring purposes to ensure that the dilution factors are met at the appropriate monitoring site. If the dilution factors are not met, the investigation procedure shown in Figure 1 (GHD 2013) will be followed.

2.2 Surface Water Chemistry

Samples from SW2 (diluent) and RP1 water were analysed for the suite of analytes shown in Table 3.

Table 3 Analytes for SW2 and RP1

	Analytes
In-Situ	EC, pH
Metals (dissolved i.e. 0.45 µm)	Al, Cd, Co, Cu, Cr, Fe, Pb, Mg, Mn, Hg, Ni, Zn
Others	SO4, Ca

2.3 Sample Sites

RP1 and SW2 sampling locations are shown in Table 4.

Table 4 Sampling Sites

Site Name	Site Description	Easting (UTM)	Northing	Latitude (degree, decimal)	Longitude
SW2	Edith River at bridge on Edith Falls Road	0189088	8431347	-14.1718	132.12
RP1	Waste rock wastewater source	0187843	8432432	-14.1620	132.1085

3. Results

3.1 Ecotoxicology Testing of RP1 Water

The species used were appropriate for the site and the EC10 and LC50 results were of sufficient quality to be used in the BurrliOZ (Campbell *et al.* 2000) program for species sensitivity distribution (SSD) calculations.

A copy of the Ecotox Report by Ecotox Services, "*Toxicity Assessment of a Treated Water Sample, February 2014*" is located in Appendix A. It must be noted that the title of the report is incorrect as the sample was not treated.

A summary of the results is provided in Table 5. The results of the site specific DTA testing performed by Ecotox Services used in this assessment do meet the laboratory NATA requirements for quality assurance/quality control (QA/QC) parameters. It must be noted however, that the majority of the reference toxicant results are in the lower 10% of the Cusum chart limits, indicating that the organisms used were more sensitive to the toxicants than is usual (Appendix A). This sensitivity means that the results from this RP1 DTA are very conservative.

Table 5 Summary of RP1 Ecotox Results

Test	EC/IC10 and LC10 (95% confidence limits) % RP1	EC/IC50 and LC50 (95% confidence limits) % RP1
Microalgal 72-hour growth inhibition	0.23	0.41 (0.31-0.44)
Duckweed 96-hour growth inhibition	0.58 (0.35-0.63)	0.95**
Cladoceran 8-day reproduction	0.03*	0.06 (0.05-0.08)
Hydra 96-hour growth	0.14 (0.08-0.22)	0.35 (0.33-0.36)
Chironomid 48-hour survival	>10*	>10
Shrimp 96-hour survival	1.0*	>1.0
Fish 10-day post hatch survival	0.05**	0.20 (0.13-0.30)

**No observable effect concentration (NOEC) ** 95% confidence limits not reliable*

The results show that the cladoceran is the most sensitive species to the RP1 water. Algae and duckweed usually show similar sensitivities to most toxicants and Gausman (2006) suggests that the two tests can be used interchangeably. Gausman (2006) also cites literature that shows that unicellular alga is more sensitive than duckweed to some metals as is shown in this suite of bioassay results.

In this case, aluminium, copper and zinc are present in elevated concentrations (Table 8) in the RP1 mine water and may be causing the toxicity observed in all the bioassays. Cobalt, nickel, lead and cadmium are also present in concentrations that have potential to cause toxicity to the test species at low dilutions (Table 8). No toxicity was attributed to pH.

3.2 Acute to Chronic Ratios (ACR)

Two of the bioassays used in this study were acute bioassays where survival was the end point. The chironomid and shrimp are acute bioassays and the results need to be adjusted so that

they can be incorporated into the statistics to calculate the SSD. ANZECC & ARMCANZ (2000) recommend an acute to chronic factor of 10 to go from an acute LC50 to a chronic NOEC or EC10. Unfortunately, the results as presented are not appropriate for this operation. Therefore, an acute to chronic ratio of 2.5 was applied to the NOEC values. This methodology was discussed in GHD (2013). This acute to chronic ratio (ACR) was applied to the chironomid and shrimp NOEC data to obtain a chronic EC10.

3.3 Concentrations used to calculate the SSD

The acute bioassays required an ACR adjustment, applied to the NOEC values for the chironomid and shrimp as discussed in Section 3.2, this method provides a conservative estimate of the chironomid EC10. The values used to calculate the SSD and dilution factors are shown in Table 6.

Table 6 Values used to Calculate SSD

Bioassay	EC10/NOEC % RP1
Microalgal 72-hour growth inhibition	0.23
Duckweed 96-hour growth inhibition	0.58
Cladoceran 6-day reproduction	0.031
Hydra 96-hour growth	0.14
Chironomid 48-hour survival	4.0
Shrimp 96-hour survival	0.4
Fish 10-day post hatch survival	0.05

3.4 Species Protection Values

The EC10 and NOEC/2.5 values from Table 6 were input into the BurrliOZ (Campbell *et al*. 2000) statistics program for calculation of the 80% species protection value using the SSD. The calculated value and the dilution factor required to reach the concentration are provided in Table 7.

Table 7 Species Protection Values

Species Protection Level	Concentration of RP1 (%)	Dilution Factor
80%	0.06	1,666

3.5 Dilution Factor

The 80% species protection was selected as appropriate to provide a level of protection for the Edith River from the discharge of RP1 water to meet the requirements of the WDL. A dilution factor of 1:1,667 for the RP1 water discharged into the Edith River will provide 80% of the species with protection from a 10% decrease in reproduction or growth (Table 7). The concentrations of chemicals at this dilution are shown in Table 8. The level of copper has been selected as the monitoring value to be met to ensure that the dilution of the RP1 water is at 1:1,667 to meet the requirements of WDL 178-3.

3.6 80 Percent Species Protection Monitoring Values

The chemistry of the RP1 water at the 1:1,667 dilution is shown in Table 8. The complete chemistry results are located in Appendix B. The concentration of copper at 3.4 µg/L should be met at SW4 to ensure the correct dilution is being achieved. If this dilution is not met then action is triggered as shown in Figure 1 of the Discharge Plan (GHD 2013).

Table 8 Dilution Rates of RP1 Water to Meet 80 percent SSD Dilution (1:1,667)

Analytes (metals 0.45 µm)	SW2 02/02/14	RP1 Water 02/02/14	Conc. in RP1 water at 1:1,667 to be met at SW4	ANZECC 80% species protection TVs/ISSTV	ANZECC 95% species protection TVs/ISSTV
pH	6.2	3.8	-	6-8	6-8
Conductivity (µS/cm)	10	1,600	-	20-250	20-250
Ca (mg/L)	<0.5	66	-	-	-
Mg (mg/L)	<0.5	160	0.10	2.5	2.5
SO_4 (mg/L)	<1.0	830	0.50	129	129
Al (µg/L)	90	23,000	13.8	150	149
Cd (µg/L)	<0.1	73	0.04	0.8	0.2
Co (µg/L)	<1.0	870	0.52	90	90
Cr (µg/L)	<1.0	<1.0	<1.0	40	1.0
Cu (µg/L)	<1.0	5,600	3.4	2.5	1.4
Fe (µg/L)	130	210	0.12	300	300
Mn (µg/L)	<0.5	10,000	6.0	3,600	1,700
Ni (µg/L)	<1.0	900	0.54	17	11
Pb (µg/L)	<1.0	47	0.03	9.4	3.4
Hg (µg/L)	<0.05	<0.05	<0.05	5.4	0.6
Zn (µg/L)	2.0	20,000	12.0	31	8.0

Note. Cells in green are below the ISSTVs and ANZECC & ARMCANZ (2000) default 95% trigger values

Table 9 shows the Monitoring Value for RP1.

A revised dilution factor and revised Monitoring Values will be calculated for the 2014/2015 wet season using data extrapolated from the last 3 years of DTA.

Table 9 Monitoring Value for SW4 (February 2013)

Discharge	Chemical	Concentration (µg/L) 0.45 µm filtered
RP1 Untreated Water	Cu	3.4

4. Conclusions and Recommendations

4.1 Conclusions

Copper and zinc are the only metals present in the treated RP1 mine water above the 95% species protection ISSTVs at the 1:1,667 dilution factor. The copper concentration is above the ANZECC & ARMCANZ 80% species protection trigger value.

4.2 Recommendations

To meet the 80% species protection level required by WDL 178-3, based on ecotoxicological testing, it is recommended that a dilution factor of 1:1,667 be applied to the discharge of RP1 mine water, with the following Monitoring Value applied at SW4:

- Cu 3.4 µg/L

This monitoring value will apply for the wet season 2013/2014. A dilution factor for 2014/2015 wet season will be calculated from DTAs conducted to date.

5. References

ANZECC & ARMCANZ (2000) Australian and New Zealand Environment and Conservation Council and Agriculture and Resource Management Council of Australia and New Zealand. National water quality management strategy, Australian and New Zealand guidelines for fresh and marine water quality. ANZECC and ARMCANZ, Canberra, Australia.

Campbell E., Palmer M.J., Shao Q., Warne M.StJ. and Wilson D. (2000) BurrliOZ: A computer program for calculating toxicant trigger values for the ANZECC and ARMCANZ water quality guidelines. Perth, Western Australia.

GHD (2013). Vista Gold Australia Pty. Ltd. Discharge Plan. Revision 1. February 2013.

Gausman M. 2006. A comparison of duckweed and standard algal phytotoxicity tests as indicators of aquatic toxicology. Masters Thesis. Miami University, Oxford, Ohio, USA.

OECD (2006) *Lemna sp*. Growth inhibition test. Method 221. OECD guideline for the testing of chemicals. Organisation for Economic Cooperation and Development. Paris.

OECD (2011) OECD guideline for the testing of chemicals. Test Guideline 235: *Chironomus sp.* Acute immobilisation test. Organisation for Economic Cooperation and Development. Paris.

Riethmuller N., Camilleri C., Franklin N., Hogan A., King A., Markich S.J., Turley C. and van Dam R. (2003) Green hydra population growth test. In: Ecotoxicological Testing Protocols for Australian Tropical Freshwater Ecosystems. Supervising Scientist Report 173. ERISS, Darwin, NT.

USEPA (2002) Short-term methods for measuring the chronic toxicity of effluents and receiving waters to freshwater organisms. 4th Edition. United States Environmental Protection Agency, Office of Water, Washington DC.

Appendices

Appendix A – Ecotox Report

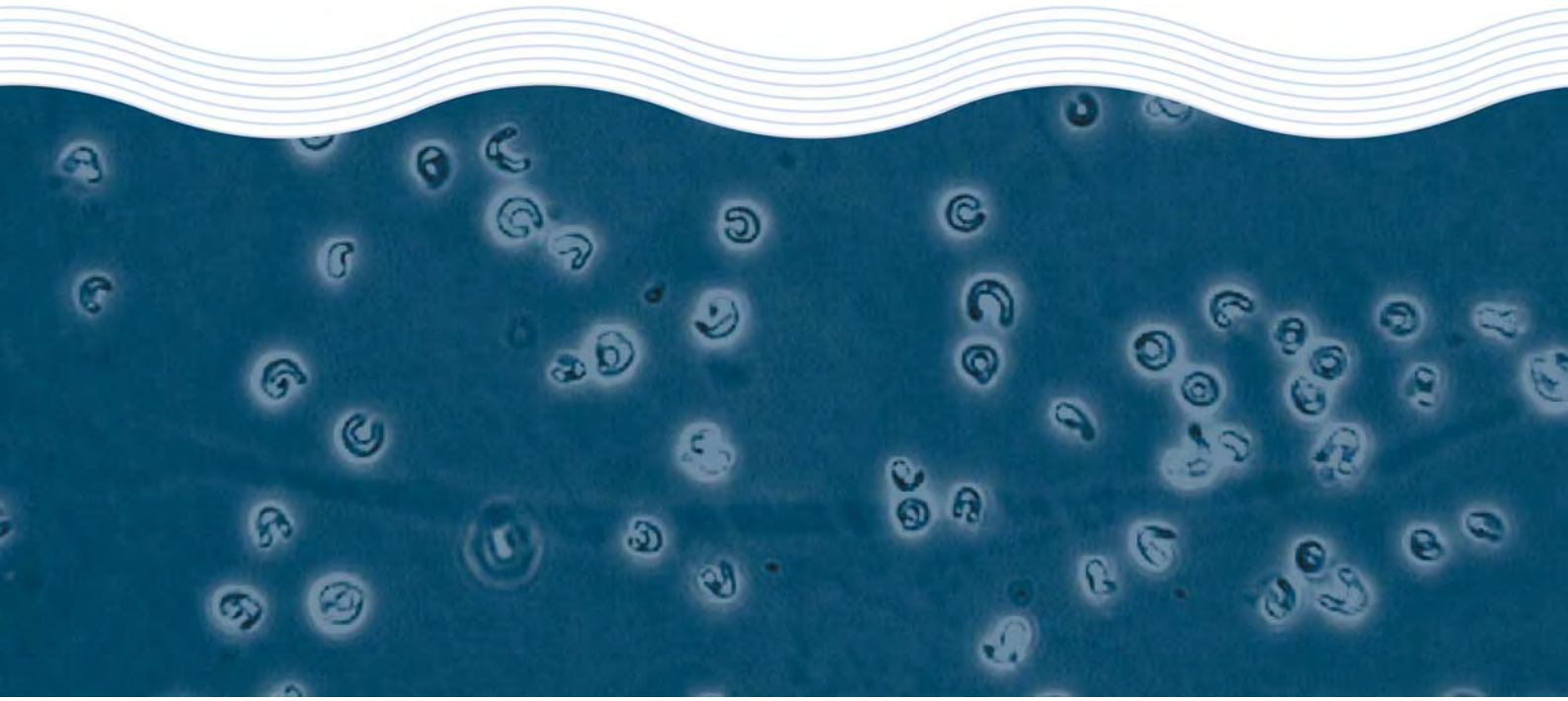
ecotox
SERVICES AUSTRALASIA

Toxicity Assessment of a Treated Water Sample from Mt Todd

Vista Gold Australia Pty Ltd

Test Report

February 2014



Toxicity Assessment of a Treated Water Sample from Mt Todd

Vista Gold Australia Pty Ltd

Test Report

February 2014

ECOTOX Services Australasia Pty Ltd ABN>45 094 714 904
unit 27/2 chaplin drive lane cove nsw 2066 T>61 2 9420 9481 F>61 2 9420 9484 W>www.ecotox.com.au

Toxicity Test Report: TR1108/8

This document is issued in accordance with NATA's accreditation requirements

Client:	Vista Gold Pty Ltd Level 3, 43 Cavenagh Street Darwin NT 0801	**ESA Job #:** **Date Sampled:** **Date Received:**	PR1108 2 February 2014 3 February 2014
Attention:	Austin Brandis	**Sampled By:**	Client
Client Ref:	Not applicable	**ESA Quote #:**	PL1108_q03

Lab ID No.:	Sample Name:	Sample Description:
6461	SW2	Aqueous sample, pH 6.1, conductivity 10.6µS/cm. Total ammonia <2.0mg/L*. Sample received at room temperature in apparent good condition
6462	RP1	Aqueous sample, pH 3.8, conductivity 1622µS/cm. Total ammonia <2.0mg/L*. Sample received at room temperature in apparent good condition

*Ammonia analysis is not covered by Ecotox Services Australasia's scope of accreditation

Test Performed:	72-hr microalgal growth inhibition test using the green alga *Selenastrum capricornutum*
Test Protocol:	ESA SOP 103 (ESA 2013), based on USEPA (2002)
Test Temperature:	The test was performed at 25±1°C.
Deviations from Protocol:	Nil
Comments on Solution Preparation:	The samples were filtered to 0.45µm prior to testing. The highest concentration was prepared by diluting sample 6462 'RP1' with sample 6461 'SW2'. Sample 6462 'RP1' was then serially diluted with sample 6461 'SW2' to achieve the test concentrations. A USEPA control and a diluent control (SW2) were tested concurrently with the sample.
Source of Test Organisms:	ESA Laboratory culture, originally sourced from CSIRO Microalgal Supply Service, TAS
Test Initiated:	4 February 2014 at 1630h

Sample 6462: *RP1* **Concentration (%)**	**Cell Yield x10^4 cells/mL (Mean ± SD)**	*Vacant*	*Vacant*
USEPA Control	90.3 ± 16.0		
Diluent Control	153.2 ± 51.3		
0.031	148.6 ± 25.3		
0.063	155.3 ± 30.2		
0.125	140.7 ± 18.0		
0.25	137.2 ± 7.5		
0.5	43.7 ± 0.7 *		
1	5.1 ± 0.5 *		
72-hr IC10 = 0.226%**			
72-hr IC50 = 0.412 (0.309-0.442)%			
NOEC = 0.25%			
LOEC = 0.5%			

*Significantly lower cell yield compared with the Diluent Control (Steel's Many-One Rank Test, 1-tailed, P=0.05)
**95% confidence limits are not available

Toxicity Test Report: TR1108/8

QA/QC Parameter	Criterion	This Test	Criterion met?
Control mean cell density	≥16.0x10^4 cells/mL	91.3x10^4 cells/mL	Yes
Control coefficient of variation	<20%	17.7%	Yes
Reference Toxicant within cusum chart limits	1.3-5.8g KCl/L	3.7g KCl/L	Yes

Test Report Authorised by: Dr Rick Krassoi, Director on 28 February 2014

Results are based on the samples in the condition as received by ESA.

NATA Accredited Laboratory Number: 14709

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Citations:

ESA (2013) *ESA SOP 103 – Green Alga, Selenastrum capricornutum, Growth Test*. Issue No 10. Ecotox Services Australasia, Sydney, NSW.

USEPA (2002) *Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms*. Fourth Edition. EPA-821-R-02-013. United States Environmental Protection Agency, Office of Research and Development, Washington DC, USA,

Toxicity Test Report: TR1108/9

This document is issued in accordance with NATA's accreditation requirements

Client:	Vista Gold Pty Ltd Level 3, 43 Cavenagh Street Darwin NT 0801	**ESA Job #:** **Date Sampled:** **Date Received:**	PR1108 2 February 2014 3 February 2014
Attention:	Austin Brandis	**Sampled By:**	Client
Client Ref:	Not applicable	**ESA Quote #:**	PL1108_q03

Lab ID No.:	Sample Name:	Sample Description:
6461	SW2	Aqueous sample, pH 6.1, conductivity 10.6µS/cm. Total ammonia <2.0mg/L*. Sample received at room temperature in apparent good condition
6462	RP1	Aqueous sample, pH 3.8, conductivity 1622µS/cm. Total ammonia <2.0mg/L*. Sample received at room temperature in apparent good condition

*Ammonia analysis is not covered by Ecotox Services Australasia's scope of accreditation

Test Performed:	96-hr Growth inhibition of the freshwater aquatic duckweed *Lemna aequinoctialis*
Test Protocol:	ESA SOP 112 (ESA 2012), based on OECD method 221 (2006)
Test Temperature:	The test was performed at 29±2°C.
Deviations from Protocol:	Nil
Comments on Solution Preparation:	The highest concentration was prepared by diluting sample 6462 'RP1' with sample 6461 'SW2'. Sample 6462 'RP1' was then serially diluted with sample 6461 'SW2' to achieve the test concentrations. A CAAC control and a diluent control (SW2) were tested concurrently with the sample.
Source of Test Organisms:	ESA Laboratory culture
Test Initiated:	3 February 2014 at 1500h

Sample 6462: *RP1*		*Vacant*	*Vacant*
Concentration (%)	**Specific Growth Rate (Mean ± SD)**		
CAAC Control	0.35 ± 0.03		
Diluent Control	0.34 ± 0.03		
0.031	0.37 ± 0.03		
0.063	0.37 ± 0.01		
0.125	0.36 ± 0.04		
0.25	0.36 ± 0.02		
0.5	0.36 ± 0.05		
1	0.16 ± 0.07 *		
96-hr IC10 = 0.583 (0.347-0.628)% **96-hr IC50 = 0.952%**** **NOEC = 0.5%** **LOEC = 1%**			

*Significantly lower specific growth rate compared with the Diluent Control (Dunnett's Test, 1-tailed, P=0.05)
**95% confidence limits are not reliable

Toxicity Test Report: TR1108/9

QA/QC Parameter	Criterion	This Test	Criterion met?
Control frond doubling time	<3.0 days	2.0days	Yes
Reference Toxicant within cusum chart limits	6.9-56.7mg Mg/L	16.1mg Mg/L	Yes

Test Report Authorised by: Dr Rick Krassoi, Director on 28 February 2014

Results are based on the samples in the condition as received by ESA.

NATA Accredited Laboratory Number: 14709

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Citations:

ESA (2012) *SOP 112 – Duckweed Growth Inhibition Test.* Issue No. 5. Ecotox Services Australasia, Sydney NSW

OECD (2006) *Lemna sp*. Growth Inhibition Test. Method 221. OECD Guideline for the Testing of Chemicals. Organisation for Economic Cooperation and Development, Paris

Toxicity Test Report: TR1108/10

This document is issued in accordance with NATA's accreditation requirements

Client:	Vista Gold Pty Ltd Level 3, 43 Cavenagh Street Darwin NT 0801	**ESA Job #:** **Date Sampled:** **Date Received:**	PR1108 2 February 2014 3 February 2014
Attention:	Austin Brandis	**Sampled By:**	Client
Client Ref:	Not applicable	**ESA Quote #:**	PL1108_q03

Lab ID No.:	Sample Name:	Sample Description:
6461	SW2	Aqueous sample, pH 6.1, conductivity 10.6µS/cm. Total ammonia <2.0mg/L*. Sample received at room temperature in apparent good condition
6462	RP1	Aqueous sample, pH 3.8, conductivity 1622µS/cm. Total ammonia <2.0mg/L*. Sample received at room temperature in apparent good condition

*Ammonia analysis is not covered by Ecotox Services Australasia's scope of accreditation

Test Performed:	48-hr acute (survival) toxicity test using the freshwater chironomid *Chironomus tepperi*
Test Protocol:	ESA SOP 121 (ESA 2012), based on OECD (2011) USEPA (2002) and Bailey *et al*. (2000)
Test Temperature:	The test was performed at 25±1°C.
Deviations from Protocol:	Nil
Comments on Solution Preparation:	The highest concentration was prepared by diluting sample 6462 'RP1' with sample 6461 'SW2'. Sample 6462 'RP1' was then serially diluted with sample 6461 'SW2' to achieve the test concentrations. A DMW control and a diluent control (SW2) were tested concurrently with the sample.
Source of Test Organisms:	ESA Laboratory culture
Age of Test Organisms:	11 days old
Test Initiated:	4 February 2014 at 1400h

Sample 6462: *RP1*		*Vacant*	*Vacant*
Concentration (%)	**% Survival (Mean ± SD)**		
DMW Control	90.0 ± 20.0		
Diluent Control	100 ± 0.0		
0.3	85.0 ± 10.0		
0.6	85.0 ± 10.0		
1.3	90.0 ± 11.6		
2.5	85.0 ± 19.2		
5	100 ± 0.0		
10	100 ± 0.0		
48-hr EC10 = >10% **48-hr EC50 = >10%** **NOEC = 10%** **LOEC = >10%**			

QA/QC Parameter	Criterion	This Test	Criterion met?
Control mean % survival	≥85.0%	90.0%	Yes
Reference Toxicant within cusum chart limits	29.9-1292.1µg Cu/L	162.7µg Cu/L	Yes

Test Report Authorised by: Dr Rick Krassoi, Director on 28 February 2014

Results are based on the samples in the condition as received by ESA.

NATA Accredited Laboratory Number: 14709

This document is issued in accordance with NATA's accreditation requirements. Accredited for compliance with ISO/IEC 17025. NATA is a signatory to the APLAC mutual recognition arrangement for the mutual recognition of the equivalence of testing, calibration and inspection reports. This document shall not be reproduced except in full.

Citations:

Bailey, H.C., Krassoi, R., Elphick, J.R., Mulhall, A., Hunt, 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* 19:88-93.

ESA (2012) *SOP 121 – Acute toxicity test using Chironomus tepperi*. Issue No. 1. Ecotox Services Australasia, Sydney, New South Wales.

OECD (2011) OECD Guideline for the Testing of Chemicals. Test Guideline 235: *Chironomus sp*, Acute Immobilisation Test.

USEPA (2002) *Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms. 4th Ed.* United States Environmental Protection Agency, Office of Water, Washington DC.

Toxicity Test Report: TR1108/11

This document is issued in accordance with NATA's accreditation requirements

Client:	Vista Gold Pty Ltd Level 3, 43 Cavenagh Street Darwin NT 0801	**ESA Job #:** **Date Sampled:** **Date Received:**	PR1108 2 February 2014 3 February 2014
Attention:	Austin Brandis	**Sampled By:**	Client
Client Ref:	Not applicable	**ESA Quote #:**	PL1108_q03

Lab ID No.:	Sample Name:	Sample Description:
6461	SW2	Aqueous sample, pH 6.1, conductivity 10.6µS/cm. Total ammonia <2.0mg/L*. Sample received at room temperature in apparent good condition.
6462	RP1	Aqueous sample, pH 3.8, conductivity 1622µS/cm. Total ammonia <2.0mg/L*. Sample received at room temperature in apparent good condition.

*Ammonia analysis is not covered by Ecotox Services Australasia's scope of accreditation

Test Performed:	96-hr acute toxicity test using the freshwater hydra *hydra viridissima*
Test Protocol:	ESA SOP 125 (2012), based on Riethmuller et al. (2003)
Test Temperature:	The test was performed at 27±1°C.
Deviations from Protocol:	Nil
Comments on Solution Preparation:	The highest concentration was prepared by diluting sample 6462 'RP1' with sample 6461 'SW2'. Sample 6462 'RP1' was then serially diluted with sample 6461 'SW2' to achieve the test concentrations. A Laboratory Water control and a diluent control (SW2) were tested concurrently with the sample.
Source of Test Organisms:	ESA Laboratory culture
Test Initiated:	4 February 2014 at 1530h

Sample 6462: *RP1* Concentration (%)	Population Growth Rate (Mean ± SD)	*Vacant*	*Vacant*
Lab Control	0.32 ± 0.01		
Diluent Control	0.36 ± 0.02		
0.031	0.38 ± 0.01		
0.063	0.36 ± 0.01		
0.125	0.34 ± 0.02		
0.25	0.30 ± 0.02 *		
0.5	0.00 ± 0.00		
1	0.00 ± 0.00		
96-hr IC10 = 0.14 (0.08-0.22)% **96-hr IC50 = 0.35 (0.33-0.36)%** **NOEC = 0.125%** **LOEC = 0.25%**			

*Significantly lower population growth rate compared with the Diluent Control (Dunnett's Test, 1-tailed, P=0.05)

QA/QC Parameter	Criterion	This Test	Criterion met?
Control mean population growth rate	≥0.25	0.32	Yes
Reference Toxicant within cusum chart limits	0.9-16.4µg Cu/L	3.4µg Cu/L	Yes

Test Report Authorised by: Dr Rick Krassoi, Director on 28 February 2014

Results are based on the samples in the condition as received by ESA.

NATA Accredited Laboratory Number: 14709

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Citations:

ESA (2012) SOP 125 –*Hydra Population Growth Test.* Issue No 1. Ecotox Services Australasia, Sydney, NSW

Riethmuller N, Camilleri C, Franklin N, Hogan A, King A, Koch A, Markich SJ, Turley C and van Dam R (2003). Green Hydra Population Growth Test. In: *Ecotoxicological testing protocols for Australian tropical freshwater ecosystems*. Supervising Scientist Report 173, Supervising Scientist, Darwin NT.

Toxicity Test Report: TR1108/12

This document is issued in accordance with NATA's accreditation requirements

Client:	Vista Gold Pty Ltd Level 3, 43 Cavenagh Street Darwin NT 0801	**ESA Job #:** **Date Sampled:** **Date Received:**	PR1108 2 February 2014 3 February 2014
Attention:	Austin Brandis	**Sampled By:**	Client
Client Ref:	Not applicable	**ESA Quote #:**	PL1108_q03

Lab ID No.:	Sample Name:	Sample Description:
6461	SW2	Aqueous sample, pH 6.1, conductivity 10.6µS/cm. Total ammonia <2.0mg/L*. Sample received at room temperature in apparent good condition
6462	RP1	Aqueous sample, pH 3.8, conductivity 1622µS/cm. Total ammonia <2.0mg/L*. Sample received at room temperature in apparent good condition

*Ammonia analysis is not covered by Ecotox Services Australasia's scope of accreditation

Test Performed:	Partial life-cycle toxicity test using the freshwater cladoceran *Ceriodaphnia cf dubia*
Test Protocol:	ESA SOP 102 (ESA 2011), based on USEPA (2002) and Bailey *et al*. (2000)
Test Temperature:	The test was performed at 25±1°C.
Deviations from Protocol:	The test was extended to 8 days.
Comments on Solution Preparation:	The highest concentration was prepared by diluting sample 6462 'RP1' with sample 6461 'SW2'. Sample 6462 'RP1' was then serially diluted with sample 6461 'SW2' to achieve the test concentrations. A DMW control and a diluent control (SW2) were tested concurrently with the sample.
Source of Test Organisms:	ESA Laboratory culture
Test Initiated:	6 February 2014 at 1630h

Sample 6462: *RP1*

Concentration (%)	% Survival at 8 days (Mean ± SD)	
DMW Control	100 ± 0.0	
Diluent Control	100 ± 0.0	
0.031	90.0 ± 31.6	
0.063	100 ± 0.0	
0.125	50.0 ± 52.7	*
0.25	10.0 ± 31.6	*
0.5	0.0 ± 0.0	
1	0.0 ± 0.0	

8 day EC10 (survival) = 0.053%***
8 day EC50 (survival) = 0.122 (0.086-0.171)%
NOEC = 0.063%
LOEC = 0.125%

Sample 6462: *RP1*

Concentration (%)	Number of Young (Mean ± SD)
DMW Control	16.0 ± 4.1
Diluent Control	21.8 ± 9.0
0.031	20.2 ± 8.7
0.063	12.2 ± 5.5 **
0.125	4.4 ± 5.5 **
0.25	0.0 ± 0.0
0.5	0.0 ± 0.0
1	0.0 ± 0.0

8 day IC10 (reproduction) = <0.031%
8 day IC50 (reproduction) = 0.064 (0.048-0.083)%
NOEC = 0.031%
LOEC = 0.063%

*Significantly lower survival compared with the Diluent Control (Fisher's Exact Test, 1-tailed, P=0.05)
**Significantly lower number of young compared with the Diluent Control (Bonferroni t Test, 1-tailed, P=0.05)
***95% confidence limits not reliable

Toxicity Test Report: TR1108/12

QA/QC Parameter	Criterion	This Test	Criterion met?
Control mean % survival	≥80.0%	100%	Yes
Control mean number of young	≥15.0	16.0	Yes
Reference Toxicant within cusum chart limits	177.6-326.7mgKCl/L	227.6mgKCl/L	Yes

Test Report Authorised by: Dr Rick Krassoi, Director on 28 February 2014

Results are based on the samples in the condition as received by ESA.

NATA Accredited Laboratory Number: 14709

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Citations:

Bailey, H.C., Krassoi, R., Elphick, J.R., Mulhall, A., Hunt, 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* 19:88-93.

ESA (2011) ESA SOP 102 – *Acute Toxicity Test Using Ceriodaphnia dubia.* Issue No 8. Ecotox Services Australasia, Sydney, NSW.

USEPA (2002) *Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms.4th Ed.* United States Environmental Protection Agency, Office of Water, Washington DC.

Toxicity Test Report: TR1108/13

This document is issued in accordance with NATA's accreditation requirements

Client:	Vista Gold Pty Ltd Level 3, 43 Cavenagh Street Darwin NT 0801	**ESA Job #:** **Date Sampled:** **Date Received:**	PR1108 2 February 2014 3 February 2014
Attention:	Austin Brandis	**Sampled By:**	Client
Client Ref:	Not applicable	**ESA Quote #:**	PL1108_q03

Lab ID No.:	Sample Name:	Sample Description:
6461	SW2	Aqueous sample, pH 6.1, conductivity 10.6µS/cm. Total ammonia <2.0mg/L*. Sample received at room temperature in apparent good condition.
6462	RP1	Aqueous sample, pH 3.8, conductivity 1622µS/cm. Total ammonia <2.0mg/L*. Sample received at room temperature in apparent good condition.

*Ammonia analysis is not covered by Ecotox Services Australasia's scope of accreditation

Test Performed:	Rainbowfish embryo hatching test using *Melanotaenia splendida splendida*
Test Protocol:	ESA SOP 126 (2013), based on USEPA (2002), but adapted for use with native rainbowfish
Test Temperature:	The test was performed at 25±1°C.
Deviations from Protocol:	Solutions were renewed every 48 hours instead of every 24 hours. Three replicates were used for the 0.016% concentration, instead of four, due to an outlier.
Comments on Solution Preparation:	The highest concentration was prepared by diluting sample 6462 'RP1' with sample 6461 'SW2'. Sample 6462 'RP1' was then serially diluted with sample 6461 'SW2' to achieve the test concentrations. A DMW control and a diluent control (SW2) were tested concurrently with the sample.
Source of Test Organisms:	ESA Laboratory culture
Test Initiated:	5 February 2014 at 1400

Sample 6462: *RP1* **Concentration (%)**	**% Survival (Mean ± SD)**	*Vacant*	*Vacant*
DMW Control	90.0 ± 20.0		
Diluent Control	95.0 ± 10.0		
0.016	86.7 ± 11.6		
0.031	100 ± 0.0		
0.063	80.0 ± 16.3		
0.125	53.3 ± 35.3 *		
0.25	45.0 ± 30.0 *		
0.5	20.0 ± 16.3 *		
13-d EC10 = 0.049%**			
13-d EC50 = 0.200 (0.125-0.303)%			
NOEC = 0.063%			
LOEC = 0.125%			

*Significant lower percent survival compared with the Diluent Control (Bonferroni t Test, 1-tailed, P=0.05)
**95% confidence limits are not available

Toxicity Test Report: TR1108/13

QA/QC Parameter	Criterion	This Test	Criterion met?
Control mean % survival	≥80.0%	90.0%	Yes
Reference Toxicant within cusum chart limit	9.3-467.6µg Cu/L	21.2µg Cu/L	Yes

Test Report Authorised by: Dr Rick Krassoi, Director on 28 February 2014

Results are based on the samples in the condition as received by ESA.

NATA Accredited Laboratory Number: 14709

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Citations:

ESA (2013) *SOP 126- Rainbowfish Embryo Hatching Test*. Issue N°3. Ecotox Services Australasia, Sydney NSW

USEPA (2002) *Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms.4th Ed.* United States Environmental Protection Agency, Office of Water, Washington DC.

Toxicity Test Report: TR1108/14

This document is issued in accordance with NATA's accreditation requirements

Client:	Vista Gold Pty Ltd Level 3, 43 Cavenagh Street Darwin NT 0801	**ESA Job #:** **Date Sampled:** **Date Received:**	PR1108 2 February 2014 3 February 2014
Attention:	Austin Brandis	**Sampled By:**	Client
Client Ref:	Not applicable	**ESA Quote #:**	PL1108_q03

Lab ID No.:	Sample Name:	Sample Description:
6461	SW2	Aqueous sample, pH 6.1, conductivity 10.6µS/cm. Total ammonia <2.0mg/L*. Sample received at room temperature in apparent good condition
6462	RP1	Aqueous sample, pH 3.8, conductivity 1622µS/cm. Total ammonia <2.0mg/L*. Sample received at room temperature in apparent good condition

*Ammonia analysis is not covered by Ecotox Services Australasia's scope of accreditation

Test Performed:	96-hr acute survival test using the freshwater shrimp *Paratya australiensis*
Test Protocol:	ESA SOP 123 (ESA 2012), based on USEPA (1996)
Test Temperature:	The test was performed at 25±1°C.
Deviations from Protocol:	Nil
Comments on Solution Preparation:	The highest concentration was prepared by diluting sample 6462 'RP1' with sample 6461 'SW2'. Sample 6462 'RP1' was then serially diluted with sample 6461 'SW2' to achieve the test concentrations. A DMW control and a diluent control (SW2) were tested concurrently with the sample.
Source of Test Organisms:	Hatchery reared, QLD
Test Initiated:	7 February 2014 at 1530h

Sample 6462: *RP1*		*Vacant*	*Vacant*
Concentration (%)	**% Unaffected (Mean ± SD)**		
DMW Control	100 ± 0.0		
Diluent Control	90.0 ± 11.6		
0.031	80.0 ± 16.3		
0.063	85.0 ± 10.0		
0.125	100 ± 0.0		
0.25	95.0 ± 10.0		
0.5	90.0 ± 11.6		
1	55.0 ± 25.2		
96-hr EC10 = 0.8%*			
96-hr EC50 = >1%			
NOEC = 1%			
LOEC = >1%			

*95% confidence limits are not available

QA/QC Parameter	Criterion	This Test	Criterion met?
Control mean % unaffected	≥90.0%	100%	Yes
Reference Toxicant within cusum chart limits	61.7-629.4µg Cu/L	74.5µg Cu/L	Yes

Test Report Authorised by: Dr Rick Krassoi, Director on 28 February 2014

Results are based on the samples in the condition as received by ESA.

NATA Accredited Laboratory Number: 14709

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Citations:

ESA (2012) SOP 123 –*Acute Toxicity Test Using Freshwater Shrimp.* Issue No 1. Ecotox Services Australasia, Sydney, NSW

USEPA (1996) Ecological Effects Test Guidelines: OPPTS 850.1035 Mysid Acute Toxicity Test. Public Draft. United States Environmental Protection Agency, Washington DC, USA.

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SERVICES AUSTRALASIA

Report of Chemical Analyses

Envirolab Services Pty Ltd
ABN 37 112 535 645
12 Ashley St Chatswood NSW 2067
ph 02 9910 6200 fax 02 9910 6201
enquiries@envirolabservices.com.au
www.envirolabservices.com.au

CERTIFICATE OF ANALYSIS 104464

Client:
Vista Gold Australia Pty Ltd
Mt Todd Mine Site
Edith Falls Rd
NT 2067

Attention: Austin Brandis

Sample log in details:

Your Reference:	**Ecotox-Vista Gold**		
No. of samples:	2 Waters		
Date samples received / completed instructions received	04/02/2014	/	04/02/2014

Analysis Details:
Please refer to the following pages for results, methodology summary and quality control data.
Samples were analysed as received from the client. Results relate specifically to the samples as received.
Results are reported on a dry weight basis for solids and on an as received basis for other matrices.
Please refer to the last page of this report for any comments relating to the results.

Report Details:

Date results requested by: / Issue Date:	6/02/14	/	6/02/14
Date of Preliminary Report:	Not issued		

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Accredited for compliance with ISO/IEC 17025. **Tests not covered by NATA are denoted with *.**

Results Approved By:

Jacinta Hurst
Laboratory Manager

HM in water - dissolved Our Reference: Your Reference Type of sample	UNITS ---------- ----------	104464-1 SW2 Water	104464-2 RP1 Water
Date prepared	-	05/02/2014	05/02/2014
Date analysed	-	05/02/2014	05/02/2014
Aluminium-Dissolved	µg/L	90	23,000
Cadmium-Dissolved	µg/L	<0.1	73
Chromium-Dissolved	µg/L	<1	<1
Copper-Dissolved	µg/L	<1	5,600
Cobalt-Dissolved	µg/L	<1	870
Iron-Dissolved	µg/L	130	210
Manganese-Dissolved	µg/L	<5	10,000
Nickel-Dissolved	µg/L	<1	900
Lead-Dissolved	µg/L	<1	47
Mercury-Dissolved	µg/L	<0.05	<0.05
Zinc-Dissolved	µg/L	2	20,000

Cations in water Dissolved Our Reference: Your Reference Type of sample	UNITS ---------- ----------	104464-1 SW2 Water	104464-2 RP1 Water
Date digested	-	05/02/2014	05/02/2014
Date analysed	-	05/02/2014	05/02/2014
Calcium - Dissolved	mg/L	<0.5	66
Magnesium - Dissolved	mg/L	<0.5	160

Miscellaneous Inorganics Our Reference: Your Reference Type of sample	UNITS ---------- ----------	104464-1 SW2 Water	104464-2 RP1 Water
Date prepared	-	04/02/2014	04/02/2014
Date analysed	-	04/02/2014	04/02/2014
pH	pH Units	6.2	3.8
Electrical Conductivity	µS/cm	10	1,600
Sulphate, SO4	mg/L	<1	830

Method ID	Methodology Summary
Metals-022 ICP-MS	Determination of various metals by ICP-MS.
Metals-021 CV-AAS	Determination of Mercury by Cold Vapour AAS.
Metals-020 ICP-AES	Determination of various metals by ICP-AES.
Inorg-001	pH - Measured using pH meter and electrode in accordance with APHA 22nd ED, 4500-H+.
Inorg-112	Dissolved Oxygen using membrane electrode. The method is based upon APHA 4500-O G. Note this analysis should ideally be carried out immediately after sampling.
Inorg-002	Conductivity and Salinity - measured using a conductivity cell and dedicated meter, in accordance with APHA 22nd ED 2510 and Rayment & Lyons.
Inorg-081	Anions - a range of Anions are determined by Ion Chromatography, in accordance with APHA 22nd ED, 4110 -B.

Client Reference: Ecotox-Vista Gold

QUALITY CONTROL HM in water - dissolved	UNITS	PQL	METHOD	Blank	Duplicate Sm#	Duplicate results Base II Duplicate II %RPD	Spike Sm#	Spike % Recovery
Date prepared	-			05/02/2014	104464-1	05/02/2014 || 05/02/2014	LCS-W1	05/02/2014
Date analysed	-			05/02/2014	104464-1	05/02/2014 || 05/02/2014	LCS-W1	05/02/2014
Aluminium-Dissolved	µg/L	10	Metals-022 ICP-MS	<10	104464-1	90 || 90 || RPD: 0	LCS-W1	92%
Cadmium-Dissolved	µg/L	0.1	Metals-022 ICP-MS	<0.1	104464-1	<0.1 || <0.1	LCS-W1	87%
Chromium-Dissolved	µg/L	1	Metals-022 ICP-MS	<1	104464-1	<1 || <1	LCS-W1	87%
Copper-Dissolved	µg/L	1	Metals-022 ICP-MS	<1	104464-1	<1 || <1	LCS-W1	88%
Cobalt-Dissolved	µg/L	1	Metals-022 ICP-MS	<1	104464-1	<1 || <1	LCS-W1	92%
Iron-Dissolved	µg/L	10	Metals-022 ICP-MS	<10	104464-1	130 || 130 || RPD: 0	LCS-W1	100%
Manganese-Dissolved	µg/L	5	Metals-022 ICP-MS	<5	104464-1	<5 || <5	LCS-W1	92%
Nickel-Dissolved	µg/L	1	Metals-022 ICP-MS	<1	104464-1	<1 || <1	LCS-W1	89%
Lead-Dissolved	µg/L	1	Metals-022 ICP-MS	<1	104464-1	<1 || <1	LCS-W1	86%
Mercury-Dissolved	µg/L	0.05	Metals-021 CV-AAS	<0.05	104464-1	<0.05 || [N/T]	LCS-W1	100%
Zinc-Dissolved	µg/L	1	Metals-022 ICP-MS	<1	104464-1	2 || 2 || RPD: 0	LCS-W1	90%

Client Reference: Ecotox-Vista Gold

QUALITY CONTROL Cations in water Dissolved	UNITS	PQL	METHOD	Blank	Duplicate Sm#	Duplicate results Base II Duplicate II %RPD	Spike Sm#	Spike % Recovery
Date digested	-			05/02/2014	[NT]	[NT]	LCS-W1	05/02/2014
Date analysed	-			05/02/2014	[NT]	[NT]	LCS-W1	05/02/2014
Calcium - Dissolved	mg/L	0.5	Metals-020 ICP-AES	<0.5	[NT]	[NT]	LCS-W1	108%
Magnesium - Dissolved	mg/L	0.5	Metals-020 ICP-AES	<0.5	[NT]	[NT]	LCS-W1	110%

QUALITY CONTROL Miscellaneous Inorganics	UNITS	PQL	METHOD	Blank	Duplicate Sm#	Duplicate results Base II Duplicate II %RPD	Spike Sm#	Spike % Recovery
Date prepared	-			04/02/2014	[NT]	[NT]	LCS-W1	04/02/2014
Date analysed	-			04/02/2014	[NT]	[NT]	LCS-W1	04/02/2014
pH	pH Units		Inorg-001	[NT]	[NT]	[NT]	LCS-W1	101%
Dissolved Oxygen*	mg/L	0.1	Inorg-112	<0.1	[NT]	[NT]	[NR]	[NR]
Electrical Conductivity	µS/cm	1	Inorg-002	<1	[NT]	[NT]	LCS-W1	101%
Sulphate, SO4	mg/L	1	Inorg-081	<1	[NT]	[NT]	LCS-W1	87%

QUALITY CONTROL HM in water - dissolved	UNITS	Dup. Sm#	Duplicate Base + Duplicate + %RPD	Spike Sm#	Spike % Recovery
Date prepared	-	[NT]	[NT]	104464-2	05/02/2014
Date analysed	-	[NT]	[NT]	104464-2	05/02/2014
Aluminium-Dissolved	µg/L	[NT]	[NT]	104464-2	#
Cadmium-Dissolved	µg/L	[NT]	[NT]	104464-2	99%
Chromium-Dissolved	µg/L	[NT]	[NT]	104464-2	91%
Copper-Dissolved	µg/L	[NT]	[NT]	104464-2	#
Cobalt-Dissolved	µg/L	[NT]	[NT]	104464-2	100%
Iron-Dissolved	µg/L	[NT]	[NT]	104464-2	83%
Manganese-Dissolved	µg/L	[NT]	[NT]	104464-2	#
Nickel-Dissolved	µg/L	[NT]	[NT]	104464-2	110%
Lead-Dissolved	µg/L	[NT]	[NT]	104464-2	95%
Mercury-Dissolved	µg/L	[NT]	[NT]	[NR]	[NR]
Zinc-Dissolved	µg/L	[NT]	[NT]	104464-2	#

Report Comments:

Trace metals: # Percent recovery is not possible to report due to the high concentration of the element/s in the sample/s. However an acceptable recovery was obtained for the LCS.

Asbestos ID was analysed by Approved Identifier:	Not applicable for this job
Asbestos ID was authorised by Approved Signatory:	Not applicable for this job

INS: Insufficient sample for this test	PQL: Practical Quantitation Limit	NT: Not tested
NA: Test not required	RPD: Relative Percent Difference	NA: Test not required
<: Less than	>: Greater than	LCS: Laboratory Control Sample

Quality Control Definitions

Blank: This is the component of the analytical signal which is not derived from the sample but from reagents, glassware etc, can be determined by processing solvents and reagents in exactly the same manner as for samples.

Duplicate: This is the complete duplicate analysis of a sample from the process batch. If possible, the sample selected should be one where the analyte concentration is easily measurable.

Matrix Spike : A portion of the sample is spiked with a known concentration of target analyte. The purpose of the matrix spike is to monitor the performance of the analytical method used and to determine whether matrix interferences exist.

LCS (Laboratory Control Sample) : This comprises either a standard reference material or a control matrix (such as a blank sand or water) fortified with analytes representative of the analyte class. It is simply a check sample.

Surrogate Spike: Surrogates are known additions to each sample, blank, matrix spike and LCS in a batch, of compounds which are similar to the analyte of interest, however are not expected to be found in real samples.

Laboratory Acceptance Criteria

Duplicate sample and matrix spike recoveries may not be reported on smaller jobs, however, were analysed at a frequency to meet or exceed NEPM requirements. All samples are tested in batches of 20. The duplicate sample RPD and matrix spike recoveries for the batch were within the laboratory acceptance criteria.
Filters, swabs, wipes, tubes and badges will not have duplicate data as the whole sample is generally extracted during sample extraction.
Spikes for Physical and Aggregate Tests are not applicable.
For VOCs in water samples, three vials are required for duplicate or spike analysis.

Duplicates: <5xPQL - any RPD is acceptable; >5xPQL - 0-50% RPD is acceptable.
Matrix Spikes, LCS and Surrogate recoveries: Generally 70-130% for inorganics/metals; 60-140% for organics and 10-140% for SVOC and speciated phenols is acceptable.

In circumstances where no duplicate and/or sample spike has been reported at 1 in 10 and/or 1 in 20 samples respectively, the sample volume submitted was insufficient in order to satisfy laboratory QA/QC protocols.

When samples are received where certain analytes are outside of recommended technical holding times (THTs), the analysis has proceeded. Where analytes are on the verge of breaching THTs, every effort will be made to analyse within the THT or as soon as practicable.

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Statistical Printouts for the *Selenastrum* Growth Inhibition Tests

Microalgal Cell Yield-Cell Yield

Start Date:	4/02/2014 16:30	Test ID:	PR1108/02	Sample ID:	RP1
End Date:	7/02/2014 15:30	Lab ID:	6462	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 103	Test Species:	SC-Selenastrum capricornutum
Comments:					

Conc-%	1	2	3	4	5	6	7	8
USEPA Control	78.35	82.75	89.15	78.35	84.35	117.95	78.75	112.75
Diluent Control	229.95	122.35	129.95	130.35				
0.031	143.15	127.55	185.15	138.35				
0.063	139.55	134.75	146.75	199.95				
0.125	160.35	148.35	118.35	135.55				
0.25	140.75	129.95	131.95	145.95				
0.5	43.55	42.75	44.35	43.95				
1	5.55	4.35	5.15	5.15				

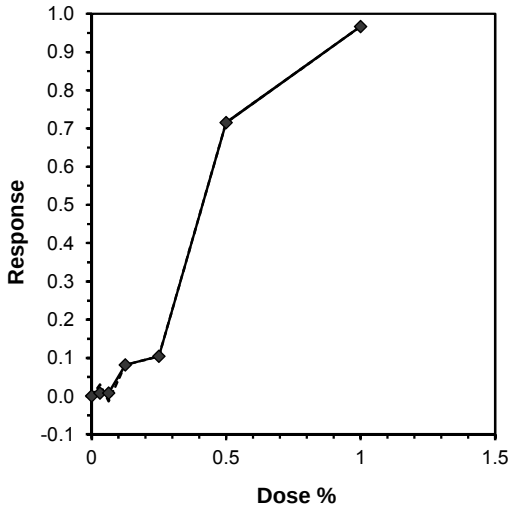
			Transform: Untransformed					Rank	1-Tailed	Isotonic	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Mean	N-Mean
USEPA Control	90.30	0.5896	90.30	78.35	117.95	17.666	8				
Diluent Control	153.15	1.0000	153.15	122.35	229.95	33.518	4	*		153.15	1.0000
0.031	148.55	0.9700	148.55	127.55	185.15	17.003	4	20.00	10.00	151.90	0.9918
0.063	155.25	1.0137	155.25	134.75	199.95	19.456	4	22.00	10.00	151.90	0.9918
0.125	140.65	0.9184	140.65	118.35	160.35	12.789	4	19.00	10.00	140.65	0.9184
0.25	137.15	0.8955	137.15	129.95	145.95	5.477	4	20.50	10.00	137.15	0.8955
*0.5	43.65	0.2850	43.65	42.75	44.35	1.565	4	10.00	10.00	43.65	0.2850
*1	5.05	0.0330	5.05	4.35	5.55	9.972	4	10.00	10.00	5.05	0.0330

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.05)	0.834658	0.924	1.784625	4.346498
Bartlett's Test indicates unequal variances (p = 7.34E-08)	44.01656	16.81189		
The control means are significantly different (p = 8.04E-03)	3.29771	2.228139		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	0.25	0.5	0.353553	400
Treatments vs Diluent Control				

Linear Interpolation (200 Resamples)

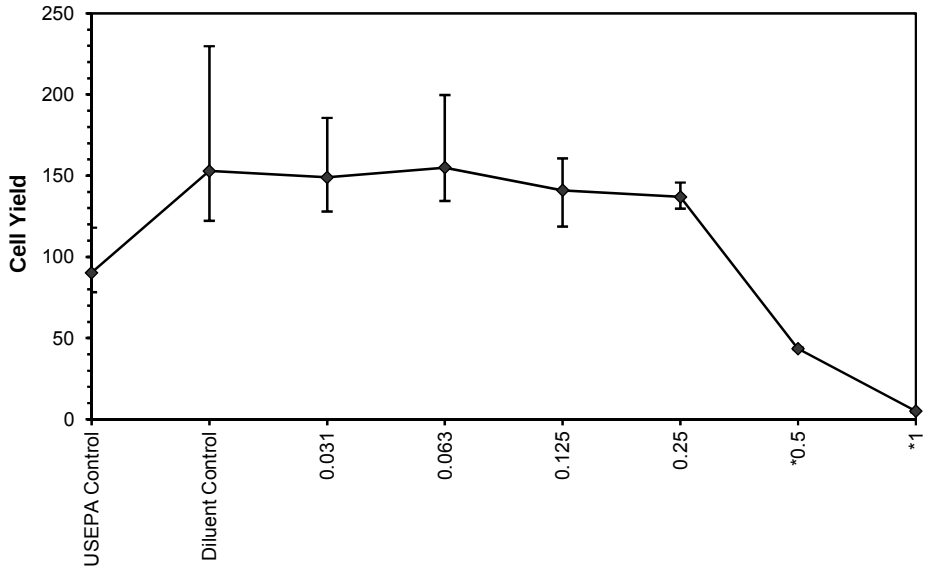
Point	%	SD	95% CL(Exp)		Skew
IC05	0.0983	0.0918	0.0000	0.3531	0.6167
IC10	0.2255	0.1033	0.0000	0.3168	-0.1834
IC15	0.2686	0.1026	0.0000	0.3225	-0.9866
IC20	0.2891	0.0940	0.0000	0.3395	-1.2579
IC25	0.3096	0.0705	0.0000	0.3566	-2.2214
IC40	0.3710	0.0285	0.2477	0.4077	-1.2897
IC50	0.4120	0.0231	0.3090	0.4418	-1.1222



Microalgal Cell Yield-Cell Yield

Start Date:	4/02/2014 16:30	Test ID:	PR1108/02	Sample ID:	RP1
End Date:	7/02/2014 15:30	Lab ID:	6462	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 103	Test Species:	SC-Selenastrum capricornutum
Comments:					

Dose-Response Plot



Microalgal Cell Yield-Cell Yield

Start Date:	4/02/2014 16:30	Test ID:	PR1108/02	Sample ID:	RP1
End Date:	7/02/2014 15:30	Lab ID:	6462	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 103	Test Species:	SC-Selenastrum capricornutum
Comments:					

Auxiliary Data Summary

Conc-%	Parameter	Mean	Min	Max	SD	CV%	N
USEPA Control	Cell Yield	90.30	78.35	117.95	15.95	4.42	8
Diluent Control		153.15	122.35	229.95	51.33	4.68	4
0.031		148.55	127.55	185.15	25.26	3.38	4
0.063		155.25	134.75	199.95	30.21	3.54	4
0.125		140.65	118.35	160.35	17.99	3.02	4
0.25		137.15	129.95	145.95	7.51	2.00	4
0.5		43.65	42.75	44.35	0.68	1.89	4
1		5.05	4.35	5.55	0.50	14.06	4
USEPA Control	pH	7.30	7.30	7.30	0.00	0.00	1
Diluent Control		7.30	7.30	7.30	0.00	0.00	1
0.031		7.30	7.30	7.30	0.00	0.00	1
0.063		7.20	7.20	7.20	0.00	0.00	1
0.125		7.20	7.20	7.20	0.00	0.00	1
0.25		7.20	7.20	7.20	0.00	0.00	1
0.5		7.20	7.20	7.20	0.00	0.00	1
1		7.20	7.20	7.20	0.00	0.00	1
USEPA Control	Conductivity uS/cm	93.70	93.70	93.70	0.00	0.00	1
Diluent Control		103.40	103.40	103.40	0.00	0.00	1
0.031		104.00	104.00	104.00	0.00	0.00	1
0.063		105.10	105.10	105.10	0.00	0.00	1
0.125		106.70	106.70	106.70	0.00	0.00	1
0.25		108.80	108.80	108.80	0.00	0.00	1
0.5		113.90	113.90	113.90	0.00	0.00	1
1		124.30	124.30	124.30	0.00	0.00	1

Statistical Printouts for the Duckweed Growth Inhibition Tests

Duckweed Growth Inhibtion Test-Specific Growth Rate

Start Date:	3/02/2014 15:00	Test ID:	PR1108/02	Sample ID:	RP1
End Date:	7/02/2014 14:30	Lab ID:	6462	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 112	Test Species:	LA-Lemna aequinoctialis
Comments:					

Conc-%	1	2	3	4
CAAC Control	0.3638	0.3638	0.3080	0.3466
Diluent Control	0.3638	0.3375	0.2974	0.3638
0.031	0.3466	0.3553	0.4094	0.3638
0.063	0.3720	0.3638	0.3800	0.3638
0.125	0.3466	0.4094	0.3182	0.3638
0.25	0.3638	0.3800	0.3375	0.3638
0.5	0.3720	0.3182	0.3182	0.4163
1	0.2218	0.2218	0.1130	0.0892

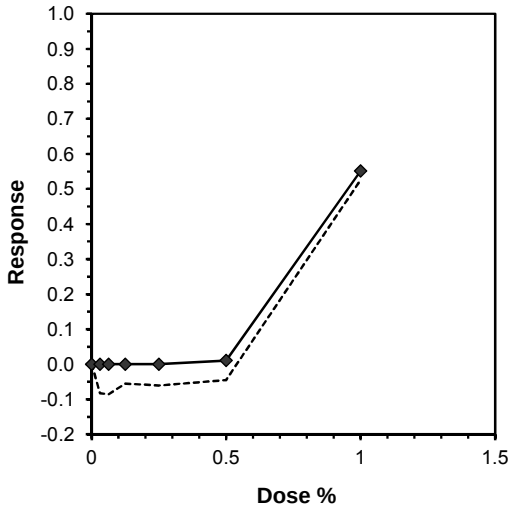
			Transform: Untransformed						1-Tailed		Isotonic	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
CAAC Control	0.3456	1.0145	0.3456	0.3080	0.3638	7.613	4					
Diluent Control	0.3406	1.0000	0.3406	0.2974	0.3638	9.213	4	*			0.3600	1.0000
0.031	0.3688	1.0827	0.3688	0.3466	0.4094	7.587	4	-1.014	2.451	0.0681	0.3600	1.0000
0.063	0.3699	1.0859	0.3699	0.3638	0.3800	2.091	4	-1.054	2.451	0.0681	0.3600	1.0000
0.125	0.3595	1.0554	0.3595	0.3182	0.4094	10.626	4	-0.680	2.451	0.0681	0.3600	1.0000
0.25	0.3613	1.0606	0.3613	0.3375	0.3800	4.869	4	-0.743	2.451	0.0681	0.3600	1.0000
0.5	0.3562	1.0457	0.3562	0.3182	0.4163	13.305	4	-0.560	2.451	0.0681	0.3562	0.9894
*1	0.1615	0.4740	0.1615	0.0892	0.2218	43.595	4	6.452	2.451	0.0681	0.1615	0.4485

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.05)	0.966399	0.924	0.09892	-0.36627
Bartlett's Test indicates equal variances (p = 0.06)	11.96629	16.81189		
The control means are not significantly different (p = 0.82)	0.240922	2.446912		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	0.5	1	0.707107	200	0.068077	0.199856	0.022762	0.001542	1.4E-06	6, 21
Treatments vs Diluent Control										

Linear Interpolation (200 Resamples)

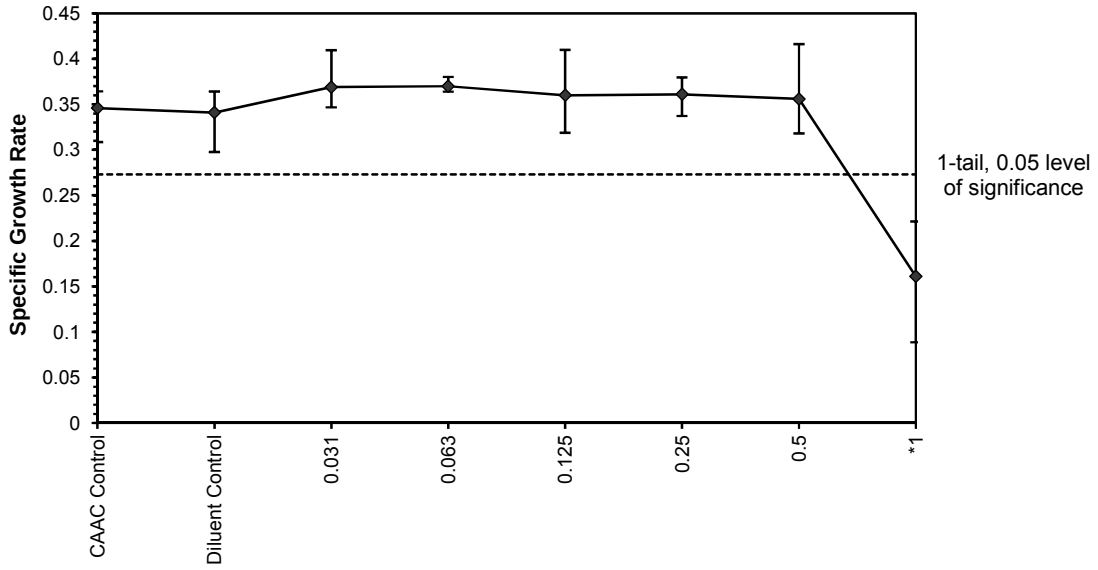
Point	%	SD	95% CL(Exp)		Skew
IC05	0.5364	0.1019	0.0000	0.5668	-1.9584
IC10	0.5826	0.0449	0.3470	0.6278	-1.4872
IC15	0.6288	0.0384	0.4550	0.6952	-0.6316
IC20	0.6750	0.0398	0.5141	0.7770	-0.1878
IC25	0.7213	0.0430	0.5774	0.8588	0.1364
IC40	0.8599				
IC50	0.9524				



Duckweed Growth Inhibtion Test-Specific Growth Rate

Start Date:	3/02/2014 15:00	Test ID:	PR1108/02	Sample ID:	RP1
End Date:	7/02/2014 14:30	Lab ID:	6462	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 112	Test Species:	LA-Lemna aequinoctialis
Comments:					

Dose-Response Plot



Duckweed Growth Inhibtion Test-Specific Growth Rate

Start Date:	3/02/2014 15:00	Test ID:	PR1108/02	Sample ID:	RP1
End Date:	7/02/2014 14:30	Lab ID:	6462	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 112	Test Species:	LA-Lemna aequinoctialis
Comments:					

		Auxiliary Data Summary					
Conc-%	**Parameter**	**Mean**	**Min**	**Max**	**SD**	**CV%**	**N**
CAAC Control	Specific Growth Rate	0.35	0.31	0.36	0.03	46.94	4
Diluent Control		0.34	0.30	0.36	0.03	52.01	4
0.031		0.37	0.35	0.41	0.03	45.36	4
0.063		0.37	0.36	0.38	0.01	23.78	4
0.125		0.36	0.32	0.41	0.04	54.37	4
0.25		0.36	0.34	0.38	0.02	36.71	4
0.5		0.36	0.32	0.42	0.05	61.12	4
1		0.16	0.09	0.22	0.07	164.32	4
CAAC Control	pH	6.10	6.10	6.10	0.00	0.00	1
Diluent Control		6.30	6.30	6.30	0.00	0.00	1
0.031		6.30	6.30	6.30	0.00	0.00	1
0.063		6.30	6.30	6.30	0.00	0.00	1
0.125		6.30	6.30	6.30	0.00	0.00	1
0.25		6.20	6.20	6.20	0.00	0.00	1
0.5		6.20	6.20	6.20	0.00	0.00	1
1		6.00	6.00	6.00	0.00	0.00	1
CAAC Control	Cond uS/cm	43.40	43.40	43.40	0.00	0.00	1
Diluent Control		42.40	42.40	42.40	0.00	0.00	1
0.031		42.10	42.10	42.10	0.00	0.00	1
0.063		42.10	42.10	42.10	0.00	0.00	1
0.125		45.00	45.00	45.00	0.00	0.00	1
0.25		48.10	48.10	48.10	0.00	0.00	1
0.5		51.80	51.80	51.80	0.00	0.00	1
1		61.80	61.80	61.80	0.00	0.00	1

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Statistical Printouts for the Acute Test with *Chironomus tepperi*

Chironomid Acute Toxicity Test-48hr Survival

Start Date:	4/02/2014 14:00	Test ID:	PR1108/02	Sample ID:	RP1
End Date:	6/02/2014 14:20	Lab ID:	6462	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 121	Test Species:	CT-Chironomus tepperi
Comments:					

Conc-%	1	2	3	4
DMW Control	1.0000	0.6000	1.0000	1.0000
Diluent Control	1.0000	1.0000	1.0000	1.0000
0.3	1.0000	0.8000	0.8000	0.8000
0.6	0.8000	0.8000	0.8000	1.0000
1.3	0.8000	1.0000	0.8000	1.0000
2.5	0.6000	0.8000	1.0000	1.0000
5	1.0000	1.0000	1.0000	1.0000
10	1.0000	1.0000	1.0000	1.0000

			Transform: Arcsin Square Root					Rank	1-Tailed	Isotonic	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Mean	N-Mean
DMW Control	0.9000	0.9000	1.2305	0.8861	1.3453	18.660	4				
Diluent Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	*		1.0000	1.0000
0.3	0.8500	0.8500	1.1667	1.1071	1.3453	10.206	4	12.00	10.00	0.9083	0.9083
0.6	0.8500	0.8500	1.1667	1.1071	1.3453	10.206	4	12.00	10.00	0.9083	0.9083
1.3	0.9000	0.9000	1.2262	1.1071	1.3453	11.212	4	14.00	10.00	0.9083	0.9083
2.5	0.8500	0.8500	1.1709	0.8861	1.3453	18.840	4	14.00	10.00	0.9083	0.9083
5	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0.9083	0.9083
10	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0.9083	0.9083

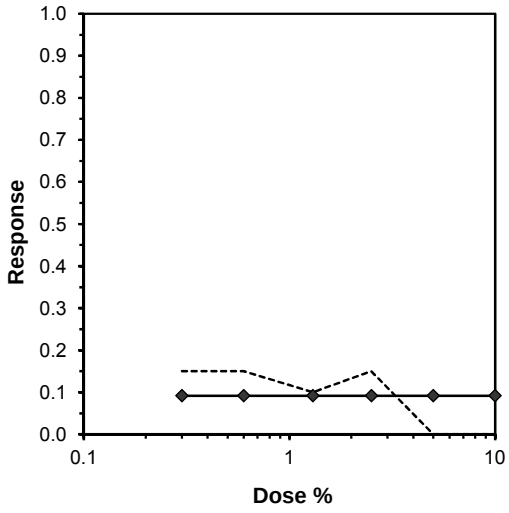
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.05)	0.874792	0.924	-0.09614	1.263671
Equality of variance cannot be confirmed				
The control means are not significantly different (p = 0.36)	1	2.446912		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	10	>10		10
Treatments vs Diluent Control				

Log-Logit Interpolation (200 Resamples)

Point	%	SD	95% CL(Exp)	Skew
IC05*	0.2683			
IC10	>10			
IC15	>10			
IC20	>10			
IC25	>10			
IC40	>10			
IC50	>10			

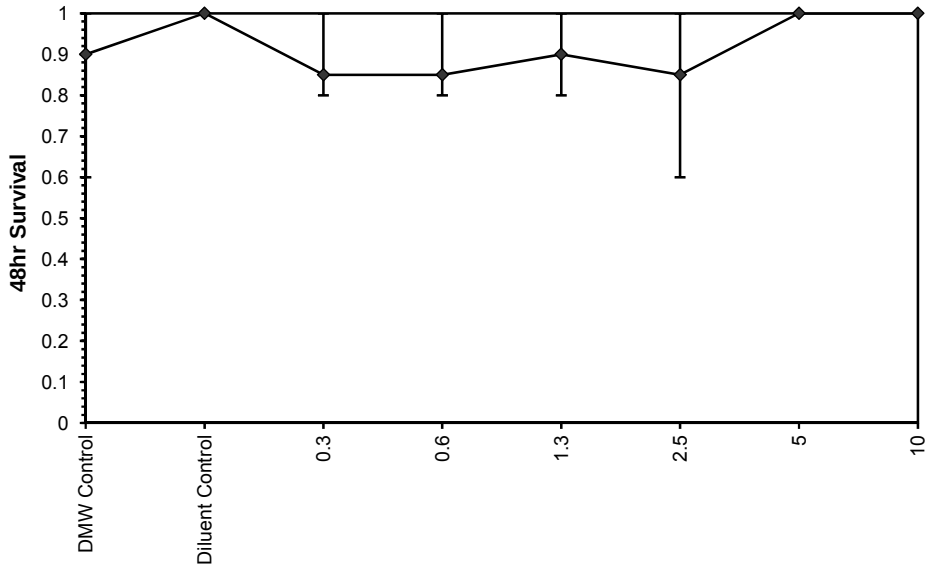
* indicates IC estimate less than the lowest concentration



Chironomid Acute Toxicity Test-48hr Survival

Start Date:	4/02/2014 14:00	Test ID:	PR1108/02	Sample ID:	RP1
End Date:	6/02/2014 14:20	Lab ID:	6462	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 121	Test Species:	CT-Chironomus tepperi
Comments:					

Dose-Response Plot



Chironomid Acute Toxicity Test-48hr Survival

Start Date:	4/02/2014 14:00	Test ID:	PR1108/02	Sample ID:	RP1
End Date:	6/02/2014 14:20	Lab ID:	6462	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 121	Test Species:	CT-Chironomus tepperi
Comments:					

Auxiliary Data Summary

Conc-%	Parameter	Mean	Min	Max	SD	CV%	N
DMW Control	% Survival	90.00	60.00	100.00	20.00	4.97	4
Diluent Control		100.00	100.00	100.00	0.00	0.00	4
0.3		85.00	80.00	100.00	10.00	3.72	4
0.6		85.00	80.00	100.00	10.00	3.72	4
1.3		90.00	80.00	100.00	11.55	3.78	4
2.5		85.00	60.00	100.00	19.15	5.15	4
5		100.00	100.00	100.00	0.00	0.00	4
10		100.00	100.00	100.00	0.00	0.00	4
DMW Control	pH	8.10	8.10	8.10	0.00	0.00	1
Diluent Control		8.00	8.00	8.00	0.00	0.00	1
0.3		7.30	7.30	7.30	0.00	0.00	1
0.6		7.10	7.10	7.10	0.00	0.00	1
1.3		6.60	6.60	6.60	0.00	0.00	1
2.5		5.60	5.60	5.60	0.00	0.00	1
5		4.90	4.90	4.90	0.00	0.00	1
10		4.60	4.60	4.60	0.00	0.00	1
DMW Control	DO (%)	98.90	98.90	98.90	0.00	0.00	1
Diluent Control		96.40	96.40	96.40	0.00	0.00	1
0.3		99.50	99.50	99.50	0.00	0.00	1
0.6		99.10	99.10	99.10	0.00	0.00	1
1.3		99.30	99.30	99.30	0.00	0.00	1
2.5		99.60	99.60	99.60	0.00	0.00	1
5		99.20	99.20	99.20	0.00	0.00	1
10		99.60	99.60	99.60	0.00	0.00	1
DMW Control	Conductivity (uS/cm)	169.60	169.60	169.60	0.00	0.00	1
Diluent Control		11.70	11.70	11.70	0.00	0.00	1
0.3		17.90	17.90	17.90	0.00	0.00	1
0.6		25.20	25.20	25.20	0.00	0.00	1
1.3		39.90	39.90	39.90	0.00	0.00	1
2.5		69.10	69.10	69.10	0.00	0.00	1
5		127.40	127.40	127.40	0.00	0.00	1
10		232.00	232.00	232.00	0.00	0.00	1

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Statistical Printouts for *Hydra* Population Growth Tests

Hydra Population Growth Test-Growth Rate

Start Date:	4/02/2014 15:30	Test ID:	PR1108/02	Sample ID:	RP1
End Date:	8/02/2014 13:30	Lab ID:	6462	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 125	Test Species:	HV-Hydra viridissima
Comments:					

Conc-%	1	2	3	4
Lab Control	0.3099	0.3227	0.3289	0.3099
Diluent Control	0.3577	0.3785	0.3577	0.3408
0.031	0.3735	0.3683	0.3785	0.3883
0.063	0.3631	0.3522	0.3577	0.3522
0.125	0.3099	0.3577	0.3466	0.3289
0.25	0.3099	0.3227	0.2894	0.2670
0.5	0.0000	0.0000	0.0000	0.0000
1	0.0000	0.0000	0.0000	0.0000

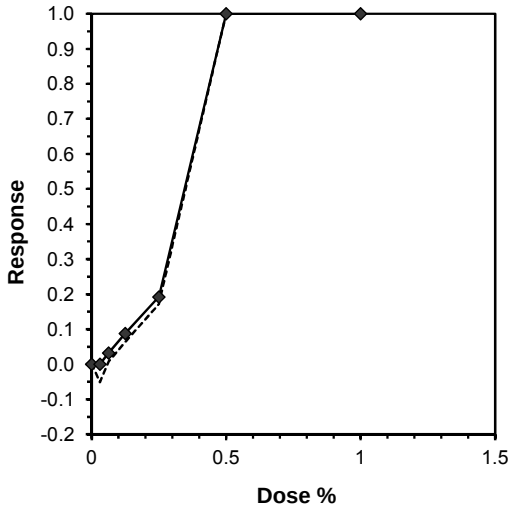
			Transform: Untransformed						1-Tailed		Isotonic	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
Lab Control	0.3179	0.8862	0.3179	0.3099	0.3289	2.996	4					
Diluent Control	0.3587	1.0000	0.3587	0.3408	0.3785	4.304	4	*			0.3679	1.0000
0.031	0.3772	1.0515	0.3772	0.3683	0.3883	2.262	4	-1.578	2.360	0.0276	0.3679	1.0000
0.063	0.3563	0.9933	0.3563	0.3522	0.3631	1.462	4	0.205	2.360	0.0276	0.3563	0.9684
0.125	0.3358	0.9361	0.3358	0.3099	0.3577	6.228	4	1.955	2.360	0.0276	0.3358	0.9126
*0.25	0.2972	0.8287	0.2972	0.2670	0.3227	8.218	4	5.244	2.360	0.0276	0.2972	0.8079
0.5	0.0000	0.0000	0.0000	0.0000	0.0000	0.000	4				0.0000	0.0000
1	0.0000	0.0000	0.0000	0.0000	0.0000	0.000	4				0.0000	0.0000

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.05)	0.969454	0.905	-0.2297	-0.0492
Bartlett's Test indicates equal variances (p = 0.15)	6.723245	13.2767		
The control means are significantly different (p = 4.11E-03)	4.499297	2.446912		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	0.125	0.25	0.176777	800	0.027647	0.077078	0.003715	0.000274	7.2E-05	4, 15
Treatments vs Diluent Control										

Linear Interpolation (200 Resamples)

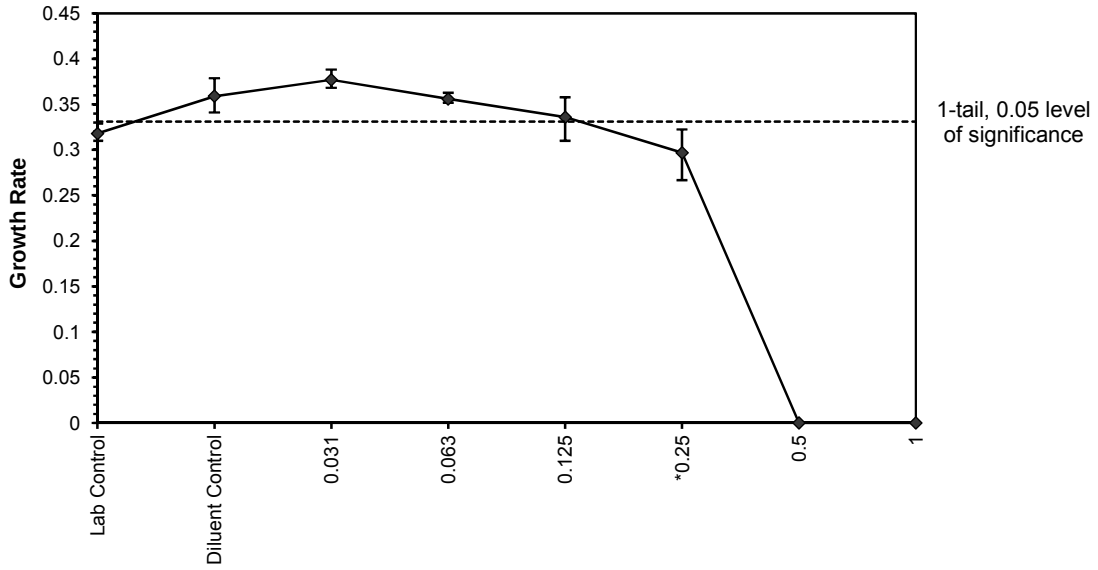
Point	%	SD	95% CL(Exp)		Skew
IC05	0.0834	0.0172	0.0488	0.1644	1.3126
IC10	0.1401	0.0232	0.0795	0.2191	0.3554
IC15	0.1997	0.0276	0.1071	0.2874	-0.1083
IC20	0.2524	0.0158	0.1933	0.2789	-0.9997
IC25	0.2679	0.0084	0.2439	0.2927	-0.3816
IC40	0.3143	0.0066	0.2951	0.3342	-0.0959
IC50	0.3453	0.0055	0.3293	0.3618	-0.0959



Hydra Population Growth Test-Growth Rate

Start Date:	4/02/2014 15:30	Test ID:	PR1108/02	Sample ID:	RP1
End Date:	8/02/2014 13:30	Lab ID:	6462	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 125	Test Species:	HV-Hydra viridissima
Comments:					

Dose-Response Plot



Hydra Population Growth Test-Growth Rate

Start Date:	4/02/2014 15:30	Test ID:	PR1108/02	Sample ID:	RP1
End Date:	8/02/2014 13:30	Lab ID:	6462	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 125	Test Species:	HV-Hydra viridissima
Comments:					

		Auxiliary Data Summary					
Conc-%	**Parameter**	**Mean**	**Min**	**Max**	**SD**	**CV%**	**N**
Lab Control	Growth Rate	0.32	0.31	0.33	0.01	30.70	4
Diluent Control		0.36	0.34	0.38	0.02	34.64	4
0.031		0.38	0.37	0.39	0.01	24.49	4
0.063		0.36	0.35	0.36	0.01	20.26	4
0.125		0.34	0.31	0.36	0.02	43.07	4
0.25		0.30	0.27	0.32	0.02	52.58	4
0.5		0.00	0.00	0.00	0.00		4
1		0.00	0.00	0.00	0.00		4
Lab Control	Conductivity	26.00	26.00	26.00	0.00	0.00	1
Diluent Control		18.20	18.20	18.20	0.00	0.00	1
0.031		11.40	11.40	11.40	0.00	0.00	1
0.063		11.60	11.60	11.60	0.00	0.00	1
0.125		13.00	13.00	13.00	0.00	0.00	1
0.25		16.20	16.20	16.20	0.00	0.00	1
0.5		21.80	21.80	21.80	0.00	0.00	1
1		33.40	33.40	33.40	0.00	0.00	1
Lab Control	pH	6.90	6.90	6.90	0.00	0.00	1
Diluent Control		6.90	6.90	6.90	0.00	0.00	1
0.031		6.90	6.90	6.90	0.00	0.00	1
0.063		6.80	6.80	6.80	0.00	0.00	1
0.125		6.80	6.80	6.80	0.00	0.00	1
0.25		6.70	6.70	6.70	0.00	0.00	1
0.5		6.60	6.60	6.60	0.00	0.00	1
1		6.40	6.40	6.40	0.00	0.00	1
Lab Control	DO, % sat	98.70	98.70	98.70	0.00	0.00	1
Diluent Control		99.60	99.60	99.60	0.00	0.00	1
0.031		98.00	98.00	98.00	0.00	0.00	1
0.063		97.70	97.70	97.70	0.00	0.00	1
0.125		97.60	97.60	97.60	0.00	0.00	1
0.25		98.00	98.00	98.00	0.00	0.00	1
0.5		97.90	97.90	97.90	0.00	0.00	1
1		97.60	97.60	97.60	0.00	0.00	1

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Statistical Printouts for the 7-d Chronic Test with *Ceriodaphnia dubia*

Ceriodaphnia Partial Life-Cycle Test-Reproduction

Start Date:	6/02/2014 16:30	Test ID:	PR1108/02	Sample ID:	RP1
End Date:	14/02/2014 09:25	Lab ID:	6462	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 102	Test Species:	CD-Ceriodaphnia dubia
Comments:					

Conc-%	1	2	3	4	5	6	7	8	9	10
DMW Control	22.000	12.000	19.000	15.000	19.000	12.000	21.000	14.000	16.000	10.000
Diluent Control	21.000	26.000	24.000	17.000	31.000	24.000	18.000	31.000	26.000	
0.031	21.000	27.000	22.000	0.000	18.000	22.000	30.000	24.000	26.000	12.000
0.063	9.000	11.000	16.000	7.000	17.000	23.000	14.000	11.000	4.000	10.000
0.125	10.000	6.000	17.000	0.000	0.000	2.000	4.000	5.000	0.000	0.000
0.25	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.5	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

			Transform: Untransformed					1-Tailed			Isotonic	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
DMW Control	16.000	0.6606	16.000	10.000	22.000	25.685	10					
Diluent Control	24.222	1.0000	24.222	17.000	31.000	20.619	9	*			24.222	1.0000
0.031	20.200	0.8339	20.200	0.000	30.000	42.955	10	1.373	2.215	6.491	20.200	0.8339
*0.063	12.200	0.5037	12.200	4.000	23.000	44.862	10	4.103	2.215	6.491	12.200	0.5037
*0.125	4.400	0.1817	4.400	0.000	17.000	125.949	10	6.766	2.215	6.491	4.400	0.1817
0.25	0.000	0.0000	0.000	0.000	0.000	0.000	10				0.000	0.0000
0.5	0.000	0.0000	0.000	0.000	0.000	0.000	10				0.000	0.0000
1	0.000	0.0000	0.000	0.000	0.000	0.000	10				0.000	0.0000

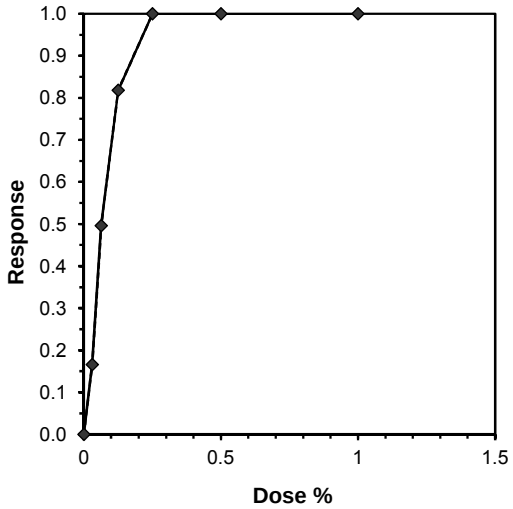
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.05)	0.965226	0.939	-0.57922	2.016985
Bartlett's Test indicates equal variances (p = 0.32)	3.474755	11.34487		
The control means are significantly different (p = 1.07E-03)	3.935149	2.109816		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test	0.031	0.063	0.044193	3225.806	6.490821	0.26797	745.9396	40.66159	2.5E-07	3, 35
Treatments vs Diluent Control										

Linear Interpolation (200 Resamples)

Point	%	SD	95% CL		Skew
IC05*	0.0093	0.0103	0.0035	0.0345	1.0097
IC10*	0.0187	0.0103	0.0070	0.0385	0.2580
IC15*	0.0280	0.0097	0.0106	0.0425	-0.1716
IC20	0.0343	0.0091	0.0141	0.0463	-0.4371
IC25	0.0391	0.0088	0.0176	0.0504	-0.6040
IC40	0.0537	0.0085	0.0282	0.0691	-0.6820
IC50	0.0637	0.0090	0.0488	0.0827	0.3758

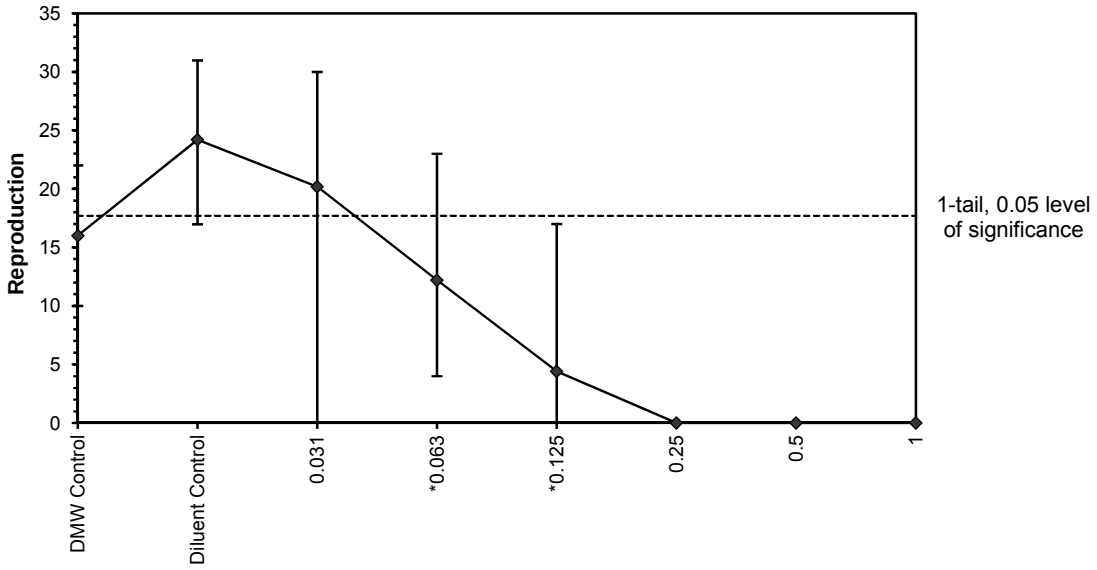
* indicates IC estimate less than the lowest concentration



Ceriodaphnia Partial Life-Cycle Test-Reproduction

Start Date:	6/02/2014 16:30	Test ID:	PR1108/02	Sample ID:	RP1
End Date:	14/02/2014 09:25	Lab ID:	6462	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 102	Test Species:	CD-Ceriodaphnia dubia
Comments:					

Dose-Response Plot



 ToxCalc v5.0.23 Reviewed by:_____

Ceriodaphnia Partial Life-Cycle Test-Reproduction

Start Date:	6/02/2014 16:30	Test ID:	PR1108/02	Sample ID:	RP1
End Date:	14/02/2014 09:25	Lab ID:	6462	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 102	Test Species:	CD-Ceriodaphnia dubia
Comments:					

		Auxiliary Data Summary					
Conc-%	**Parameter**	**Mean**	**Min**	**Max**	**SD**	**CV%**	**N**
DMW Control	No of Young	16.00	10.00	22.00	4.11	12.67	10
Diluent Control		24.22	17.00	31.00	4.99	9.23	9
0.031		20.20	0.00	30.00	8.68	14.58	10
0.063		12.20	4.00	23.00	5.47	19.18	10
0.125		4.40	0.00	17.00	5.54	53.50	10
0.25		0.00	0.00	0.00	0.00		10
0.5		0.00	0.00	0.00	0.00		10
1		0.00	0.00	0.00	0.00		10
DMW Control	% survival	100.00	100.00	100.00	0.00	0.00	10
Diluent Control		100.00	100.00	100.00	0.00	0.00	10
0.031		90.00	0.00	100.00	31.62	6.25	10
0.063		100.00	100.00	100.00	0.00	0.00	10
0.125		50.00	0.00	100.00	52.70	14.52	10
0.25		10.00	0.00	100.00	31.62	56.23	10
0.5		0.00	0.00	0.00	0.00		10
1		0.00	0.00	0.00	0.00		10
DMW Control	pH	8.10	8.10	8.10	0.00	0.00	1
Diluent Control		8.20	8.20	8.20	0.00	0.00	1
0.031		7.90	7.90	7.90	0.00	0.00	1
0.063		7.80	7.80	7.80	0.00	0.00	1
0.125		7.60	7.60	7.60	0.00	0.00	1
0.25		7.40	7.40	7.40	0.00	0.00	1
0.5		7.30	7.30	7.30	0.00	0.00	1
1		7.20	7.20	7.20	0.00	0.00	1
DMW Control	DO %	102.20	102.20	102.20	0.00	0.00	1
Diluent Control		100.20	100.20	100.20	0.00	0.00	1
0.031		99.20	99.20	99.20	0.00	0.00	1
0.063		99.80	99.80	99.80	0.00	0.00	1
0.125		99.90	99.90	99.90	0.00	0.00	1
0.25		100.10	100.10	100.10	0.00	0.00	1
0.5		99.90	99.90	99.90	0.00	0.00	1
1		99.90	99.90	99.90	0.00	0.00	1
DMW Control	Cond uS/cm	181.80	181.80	181.80	0.00	0.00	1
Diluent Control		21.80	21.80	21.80	0.00	0.00	1
0.031		20.60	20.60	20.60	0.00	0.00	1
0.063		21.40	21.40	21.40	0.00	0.00	1
0.125		23.20	23.20	23.20	0.00	0.00	1
0.25		25.50	25.50	25.50	0.00	0.00	1
0.5		31.40	31.40	31.40	0.00	0.00	1
1		43.70	43.70	43.70	0.00	0.00	1

Ceriodaphnia Partial Life-Cycle Test-8 day survival

Start Date:	6/02/2014 16:30	Test ID:	PR1108/02	Sample ID:	RP1
End Date:	14/02/2014 09:25	Lab ID:	6462	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 102	Test Species:	CD-Ceriodaphnia dubia
Comments:					

Conc-%	1	2	3	4	5	6	7	8	9	10
DMW Control	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
Diluent Control	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
0.031	1.0000	1.0000	1.0000	0.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
0.063	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
0.125	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.0000	1.0000	0.0000	0.0000
0.25	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	1.0000	0.0000	0.0000
0.5	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
1	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

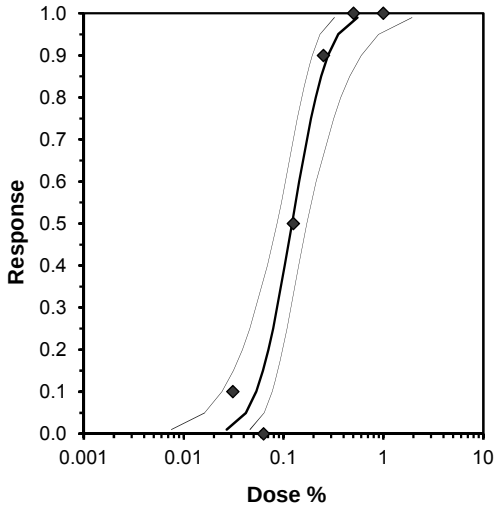
Conc-%	Mean	N-Mean	Resp	Not Resp	Total	N	Fisher's Exact P	1-Tailed Critical	Number Resp	Total Number
DMW Control	1.0000	1.0000	0	10	10	10	0.6238			
Diluent Control	1.0000	1.0000	0	10	10	10	*		0	10
0.031	0.9000	0.9000	1	9	10	10	0.5000	0.0500	1	10
0.063	1.0000	1.0000	0	10	10	10	1.0000	0.0500	0	10
*0.125	0.5000	0.5000	5	5	10	10	0.0163	0.0500	5	10
*0.25	0.1000	0.1000	9	1	10	10	0.0001	0.0500	9	10
0.5	0.0000	0.0000	10	0	10	10			10	10
1	0.0000	0.0000	10	0	10	10			10	10

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Fisher's Exact Test	0.063	0.125	0.088741	1587.302
Treatments vs Diluent Control				

Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	3.560348	0.805868	1.980847	5.139849	0	6.122434	9.487729	0.19	-0.91496	0.280871	3
Intercept	8.257571	0.772135	6.744187	9.770955							
TSCR											

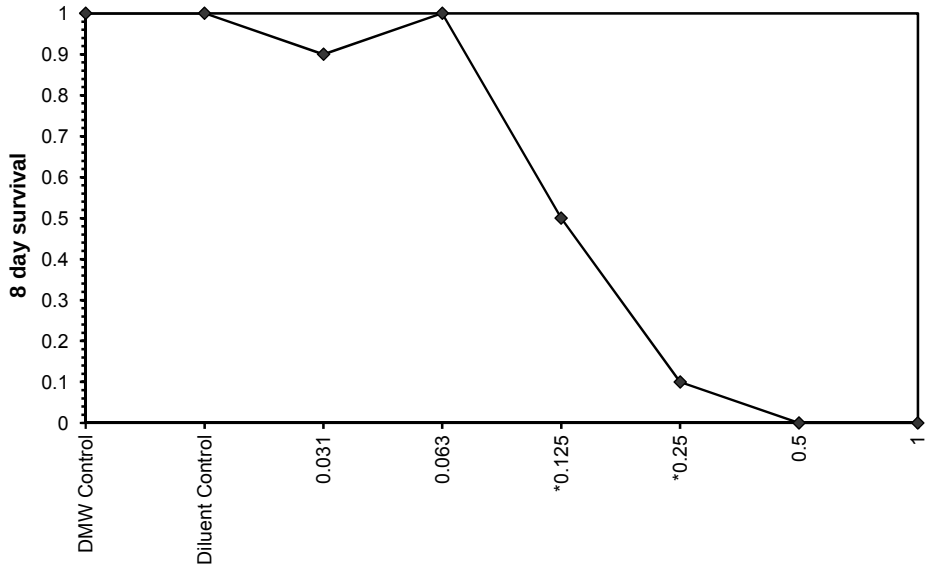
Point	Probits	%	95% Fiducial Limits	
EC01	2.674	0.027017	0.00752	0.046123
EC05	3.355	0.041981	0.016202	0.064149
EC10	3.718	0.053099	0.024198	0.077104
EC15	3.964	0.062221	0.031541	0.087783
EC20	4.158	0.070575	0.038751	0.097783
EC25	4.326	0.078631	0.046023	0.10776
EC40	4.747	0.103248	0.069043	0.141532
EC50	5.000	0.12163	0.085824	0.17122
EC60	5.253	0.143285	0.104226	0.21202
EC75	5.674	0.188142	0.137554	0.316538
EC80	5.842	0.209618	0.151782	0.375464
EC85	6.036	0.237764	0.169263	0.460762
EC90	6.282	0.278607	0.192902	0.599978
EC95	6.645	0.352398	0.232083	0.895199
EC99	7.326	0.547578	0.323089	1.926969



Ceriodaphnia Partial Life-Cycle Test-8 day survival

Start Date:	6/02/2014 16:30	Test ID:	PR1108/02	Sample ID:	RP1
End Date:	14/02/2014 09:25	Lab ID:	6462	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 102	Test Species:	CD-Ceriodaphnia dubia
Comments:					

Dose-Response Plot



Ceriodaphnia Partial Life-Cycle Test-8 day survival

Start Date:	6/02/2014 16:30	Test ID:	PR1108/02	Sample ID:	RP1
End Date:	14/02/2014 09:25	Lab ID:	6462	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 102	Test Species:	CD-Ceriodaphnia dubia
Comments:					

		Auxiliary Data Summary					
Conc-%	**Parameter**	**Mean**	**Min**	**Max**	**SD**	**CV%**	**N**
DMW Control	No of Young	16.00	10.00	22.00	4.11	12.67	10
Diluent Control		24.22	17.00	31.00	4.99	9.23	9
0.031		20.20	0.00	30.00	8.68	14.58	10
0.063		12.20	4.00	23.00	5.47	19.18	10
0.125		4.40	0.00	17.00	5.54	53.50	10
0.25		0.00	0.00	0.00	0.00		10
0.5		0.00	0.00	0.00	0.00		10
1		0.00	0.00	0.00	0.00		10
DMW Control	% survival	100.00	100.00	100.00	0.00	0.00	10
Diluent Control		100.00	100.00	100.00	0.00	0.00	10
0.031		90.00	0.00	100.00	31.62	6.25	10
0.063		100.00	100.00	100.00	0.00	0.00	10
0.125		50.00	0.00	100.00	52.70	14.52	10
0.25		10.00	0.00	100.00	31.62	56.23	10
0.5		0.00	0.00	0.00	0.00		10
1		0.00	0.00	0.00	0.00		10
DMW Control	pH	8.10	8.10	8.10	0.00	0.00	1
Diluent Control		8.20	8.20	8.20	0.00	0.00	1
0.031		7.90	7.90	7.90	0.00	0.00	1
0.063		7.80	7.80	7.80	0.00	0.00	1
0.125		7.60	7.60	7.60	0.00	0.00	1
0.25		7.40	7.40	7.40	0.00	0.00	1
0.5		7.30	7.30	7.30	0.00	0.00	1
1		7.20	7.20	7.20	0.00	0.00	1
DMW Control	DO %	102.20	102.20	102.20	0.00	0.00	1
Diluent Control		100.20	100.20	100.20	0.00	0.00	1
0.031		99.20	99.20	99.20	0.00	0.00	1
0.063		99.80	99.80	99.80	0.00	0.00	1
0.125		99.90	99.90	99.90	0.00	0.00	1
0.25		100.10	100.10	100.10	0.00	0.00	1
0.5		99.90	99.90	99.90	0.00	0.00	1
1		99.90	99.90	99.90	0.00	0.00	1
DMW Control	Cond uS/cm	181.80	181.80	181.80	0.00	0.00	1
Diluent Control		21.80	21.80	21.80	0.00	0.00	1
0.031		20.60	20.60	20.60	0.00	0.00	1
0.063		21.40	21.40	21.40	0.00	0.00	1
0.125		23.20	23.20	23.20	0.00	0.00	1
0.25		25.50	25.50	25.50	0.00	0.00	1
0.5		31.40	31.40	31.40	0.00	0.00	1
1		43.70	43.70	43.70	0.00	0.00	1

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Statistical Printouts for the Fish Embryonic Development and Post-hatch Survival Tests

Fish Embryonic Development and Post-Hatch Survival Test-Survival

Start Date:	5/02/2014 14:00	Test ID:	PR1108/02	Sample ID:	RP1
End Date:	18/02/2014 12:10	Lab ID:	6462	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 126	Test Species:	MS-Melanotaenia splendida
Comments:					

Conc-%	1	2	3	4
DMW Control	1.0000	0.6000	1.0000	1.0000
Diluent Control	1.0000	0.8000	1.0000	1.0000
0.016	0.8000	1.0000	0.8000	
0.031	1.0000	1.0000	1.0000	1.0000
0.063	1.0000	0.6000	0.8000	0.8000
0.125	0.6000	0.2000	0.3333	1.0000
0.25	0.2000	0.8000	0.6000	0.2000
0.5	0.0000	0.4000	0.2000	0.2000

			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
DMW Control	0.9000	0.9474	1.2305	0.8861	1.3453	18.660	4					
Diluent Control	0.9500	1.0000	1.2857	1.1071	1.3453	9.261	4	*			1	20
0.016	0.8667	0.9123	1.1865	1.1071	1.3453	11.587	3	0.567	2.613	0.4569	2	15
0.031	1.0000	1.0526	1.3453	1.3453	1.3453	0.000	4	-0.368	2.613	0.4230	0	20
0.063	0.8000	0.8421	1.1114	0.8861	1.3453	16.874	4	1.077	2.613	0.4230	4	20
*0.125	0.5333	0.5614	0.8276	0.4636	1.3453	46.738	4	2.829	2.613	0.4230	10	21
*0.25	0.4500	0.4737	0.7301	0.4636	1.1071	43.920	4	3.431	2.613	0.4230	11	20
*0.5	0.2000	0.2105	0.4594	0.2255	0.6847	40.823	4	5.104	2.613	0.4230	16	20

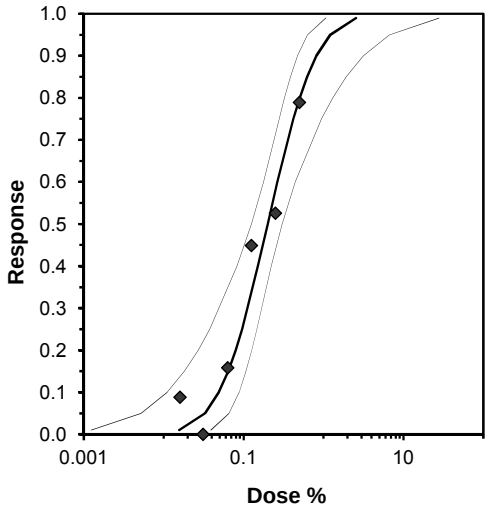
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.05)	0.951929	0.923	0.505728	0.646888
Equality of variance cannot be confirmed				
The control means are not significantly different (p = 0.68)	0.427374	2.446912		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test	0.063	0.125	0.088741	1587.302	0.343909	0.373439	0.421797	0.052436	1.7E-04	6, 20
Treatments vs Diluent Control										

Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	2.102632	0.520809	1.081847	3.123418	0.05	4.635377	9.487729	0.33	-0.69994	0.475594	4
Intercept	6.471727	0.434117	5.620857	7.322597							
TSCR	0.053532	0.04059	-0.02603	0.133089							

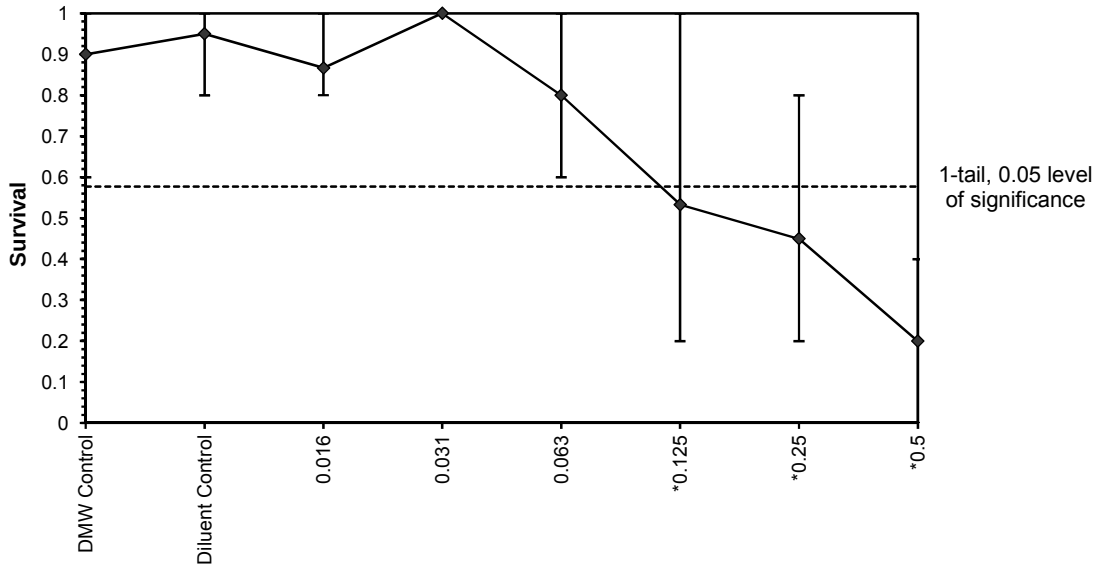
Point	Probits	%	95% Fiducial Limits	
EC01	2.674	0.015619	0.00124	0.038987
EC05	3.355	0.032944	0.005176	0.065826
EC10	3.718	0.049041	0.011001	0.087713
EC15	3.964	0.064141	0.018187	0.107095
EC20	4.158	0.079393	0.026968	0.126224
EC25	4.326	0.095339	0.03758	0.146226
EC40	4.747	0.151204	0.082733	0.222036
EC50	5.000	0.199552	0.12525	0.303119
EC60	5.253	0.263357	0.177333	0.442482
EC75	5.674	0.417675	0.27892	0.940416
EC80	5.842	0.501563	0.325357	1.301474
EC85	6.036	0.620836	0.385523	1.91951
EC90	6.282	0.811996	0.472718	3.160022
EC95	6.645	1.208754	0.632168	6.692555
EC99	7.326	2.549489	1.070666	27.85452



Fish Embryonic Development and Post-Hatch Survival Test-Survival

Start Date:	5/02/2014 14:00	Test ID:	PR1108/02	Sample ID:	RP1
End Date:	18/02/2014 12:10	Lab ID:	6462	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 126	Test Species:	MS-Melanotaenia splendida
Comments:					

Dose-Response Plot



Fish Embryonic Development and Post-Hatch Survival Test-Survival

Start Date:	5/02/2014 14:00	Test ID:	PR1108/02	Sample ID:	RP1
End Date:	18/02/2014 12:10	Lab ID:	6462	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 126	Test Species:	MS-Melanotaenia splendida
Comments:					

		Auxiliary Data Summary					
Conc-%	**Parameter**	**Mean**	**Min**	**Max**	**SD**	**CV%**	**N**
DMW Control	% Un-affected	90.00	60.00	100.00	20.00	4.97	4
Diluent Control		95.00	80.00	100.00	10.00	3.33	4
0.016		86.67	80.00	100.00	11.55	3.92	3
0.031		100.00	100.00	100.00	0.00	0.00	4
0.063		80.00	60.00	100.00	16.33	5.05	4
0.125		53.33	20.00	100.00	35.28	11.14	4
0.25		45.00	20.00	80.00	30.00	12.17	4
0.5		20.00	0.00	40.00	16.33	20.21	4
DMW Control	pH	8.10	8.10	8.10	0.00	0.00	1
Diluent Control		6.70	6.70	6.70	0.00	0.00	1
0.016		6.60	6.60	6.60	0.00	0.00	1
0.031		6.50	6.50	6.50	0.00	0.00	1
0.063		6.40	6.40	6.40	0.00	0.00	1
0.125		6.40	6.40	6.40	0.00	0.00	1
0.25		6.40	6.40	6.40	0.00	0.00	1
0.5		6.30	6.30	6.30	0.00	0.00	1
DMW Control	DO %	100.30	100.30	100.30	0.00	0.00	1
Diluent Control		94.50	94.50	94.50	0.00	0.00	1
0.016		97.30	97.30	97.30	0.00	0.00	1
0.031		97.70	97.70	97.70	0.00	0.00	1
0.063		97.90	97.90	97.90	0.00	0.00	1
0.125		98.70	98.70	98.70	0.00	0.00	1
0.25		99.30	99.30	99.30	0.00	0.00	1
0.5		99.40	99.40	99.40	0.00	0.00	1
DMW Control	Conductivity uS/cm	170.80	170.80	170.80	0.00	0.00	1
Diluent Control		13.40	13.40	13.40	0.00	0.00	1
0.016		10.60	10.60	10.60	0.00	0.00	1
0.031		11.00	11.00	11.00	0.00	0.00	1
0.063		11.40	11.40	11.40	0.00	0.00	1
0.125		12.90	12.90	12.90	0.00	0.00	1
0.25		15.90	15.90	15.90	0.00	0.00	1
0.5		21.60	21.60	21.60	0.00	0.00	1

ecotox
SERVICES AUSTRALASIA

Statistical Printouts for the Freshwater Shrimp Tests

Freshwater Shrimp Acute Toxicity Test-96 hr Survival

Start Date:	7/02/2014 15:30	Test ID:	PR1108/02	Sample ID:	RP1
End Date:	11/02/2014 08:15	Lab ID:	6462	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 123	Test Species:	PSP-Paratya australiensis
Comments:					

Conc-%	1	2	3	4
DMW Control	1.0000	1.0000	1.0000	1.0000
Diluent Control	1.0000	0.8000	1.0000	0.8000
0.031	0.8000	0.6000	0.8000	1.0000
0.063	1.0000	0.8000	0.8000	0.8000
0.125	1.0000	1.0000	1.0000	1.0000
0.25	1.0000	1.0000	1.0000	0.8000
0.5	1.0000	0.8000	1.0000	0.8000
1	0.2000	0.6000	0.8000	0.6000

			Transform: Arcsin Square Root					Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Resp	Number
DMW Control	1.0000	1.1111	1.3453	1.3453	1.3453	0.000	4		
Diluent Control	0.9000	1.0000	1.2262	1.1071	1.3453	11.212	4	2	20
0.031	0.8000	0.8889	1.1114	0.8861	1.3453	16.874	4	4	20
0.063	0.8500	0.9444	1.1667	1.1071	1.3453	10.206	4	3	20
0.125	1.0000	1.1111	1.3453	1.3453	1.3453	0.000	4	0	20
0.25	0.9500	1.0556	1.2857	1.1071	1.3453	9.261	4	1	20
0.5	0.9000	1.0000	1.2262	1.1071	1.3453	11.212	4	2	20
1	0.5500	0.6111	0.8357	0.4636	1.1071	32.195	4	9	20

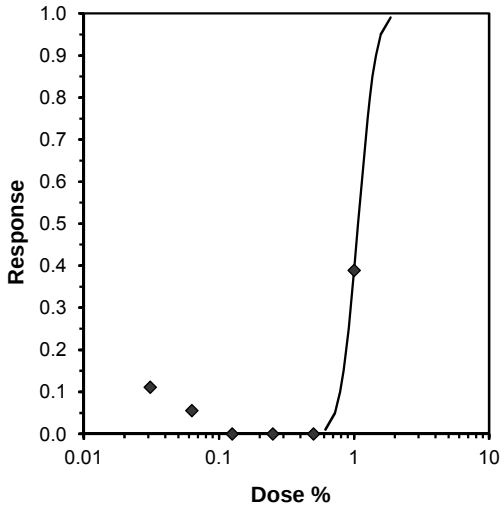
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.05)	0.972625	0.924	-0.44498	0.844567
Equality of variance cannot be confirmed				
The control means are not significantly different (p = 0.13)	1.732051	2.446912		

Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits	
Slope	9.6458	41.90229	-72.4827	91.77429
Intercept	4.717783	0.327432	4.076017	5.359549
TSCR	0.1	0.030001	0.041198	0.158802

Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
0.1	5.555299	9.487729	0.23	0.029258	0.103672	5

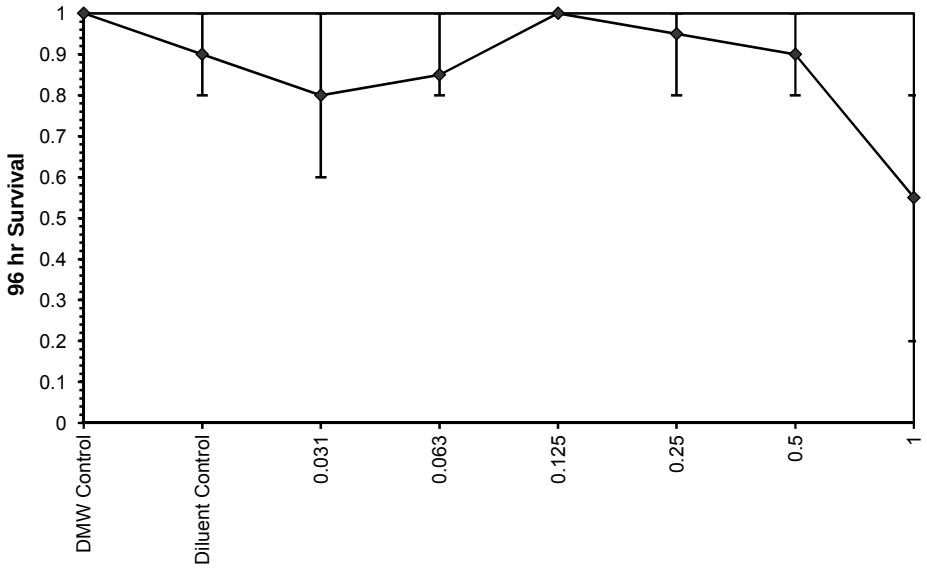
Point	Probits	%	95% Fiducial Limits	
EC01	2.674	0.613876		
EC05	3.355	0.722325		
EC10	3.718	0.787765		
EC15	3.964	0.835235		
EC20	4.158	0.874995		
EC25	4.326	0.91061		
EC40	4.747	1.006915		
EC50	5.000	1.06969		
EC60	5.253	1.136379		
EC75	5.674	1.256562		
EC80	5.842	1.307708		
EC85	6.036	1.369958		
EC90	6.282	1.45251		
EC95	6.645	1.584103		
EC99	7.326	1.863955		



Freshwater Shrimp Acute Toxicity Test-96 hr Survival

Start Date:	7/02/2014 15:30	Test ID:	PR1108/02	Sample ID:	RP1
End Date:	11/02/2014 08:15	Lab ID:	6462	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 123	Test Species:	PSP-Paratya australiensis
Comments:					

Dose-Response Plot



Freshwater Shrimp Acute Toxicity Test-96 hr Survival

Start Date:	7/02/2014 15:30	Test ID:	PR1108/02	Sample ID:	RP1
End Date:	11/02/2014 08:15	Lab ID:	6462	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 123	Test Species:	PSP-Paratya australiensis
Comments:					

		Auxiliary Data Summary					
Conc-%	**Parameter**	**Mean**	**Min**	**Max**	**SD**	**CV%**	**N**
DMW Control	% Survival	100.00	100.00	100.00	0.00	0.00	4
Diluent Control		90.00	80.00	100.00	11.55	3.78	4
0.031		80.00	60.00	100.00	16.33	5.05	4
0.063		85.00	80.00	100.00	10.00	3.72	4
0.125		100.00	100.00	100.00	0.00	0.00	4
0.25		95.00	80.00	100.00	10.00	3.33	4
0.5		90.00	80.00	100.00	11.55	3.78	4
1		55.00	20.00	80.00	25.17	9.12	4
DMW Control	pH	8.10	8.10	8.10	0.00	0.00	1
Diluent Control		7.10	7.10	7.10	0.00	0.00	1
0.031		6.80	6.80	6.80	0.00	0.00	1
0.063		6.70	6.70	6.70	0.00	0.00	1
0.125		6.60	6.60	6.60	0.00	0.00	1
0.25		6.50	6.50	6.50	0.00	0.00	1
0.5		6.40	6.40	6.40	0.00	0.00	1
1		6.20	6.20	6.20	0.00	0.00	1
DMW Control	Cond uS/cm	169.30	169.30	169.30	0.00	0.00	1
Diluent Control		10.90	10.90	10.90	0.00	0.00	1
0.031		11.60	11.60	11.60	0.00	0.00	1
0.063		11.20	11.20	11.20	0.00	0.00	1
0.125		12.80	12.80	12.80	0.00	0.00	1
0.25		15.70	15.70	15.70	0.00	0.00	1
0.5		21.60	21.60	21.60	0.00	0.00	1
1		33.30	33.30	33.30	0.00	0.00	1
DMW Control	DO %	100.20	100.20	100.20	0.00	0.00	1
Diluent Control		95.90	95.90	95.90	0.00	0.00	1
0.031		98.70	98.70	98.70	0.00	0.00	1
0.063		98.80	98.80	98.80	0.00	0.00	1
0.125		98.70	98.70	98.70	0.00	0.00	1
0.25		98.90	98.90	98.90	0.00	0.00	1
0.5		99.20	99.20	99.20	0.00	0.00	1
1		100.40	100.40	100.40	0.00	0.00	1

Appendix B – Chemistry Report

Envirolab Services Pty Ltd
ABN 37 112 535 645
12 Ashley St Chatswood NSW 2067
ph 02 9910 6200 fax 02 9910 6201
enquiries@envirolabservices.com.au
www.envirolabservices.com.au

CERTIFICATE OF ANALYSIS 104464

Client:
Vista Gold Australia Pty Ltd
Mt Todd Mine Site
Edith Falls Rd
NT 2067

Attention: Austin Brandis

Sample log in details:

Your Reference:	**Ecotox-Vista Gold**
No. of samples:	2 Waters
Date samples received / completed instructions received	04/02/2014 / 04/02/2014

Analysis Details:
Please refer to the following pages for results, methodology summary and quality control data.
Samples were analysed as received from the client. Results relate specifically to the samples as received.
Results are reported on a dry weight basis for solids and on an as received basis for other matrices.
Please refer to the last page of this report for any comments relating to the results.

Report Details:

Date results requested by: / Issue Date:	6/02/14 / 6/02/14
Date of Preliminary Report:	Not issued

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Accredited for compliance with ISO/IEC 17025. **Tests not covered by NATA are denoted with *.**

Results Approved By:

Jacinta Hurst
Laboratory Manager

HM in water - dissolved Our Reference: Your Reference Type of sample	UNITS ---------- ----------	104464-1 SW2 Water	104464-2 RP1 Water
Date prepared	-	05/02/2014	05/02/2014
Date analysed	-	05/02/2014	05/02/2014
Aluminium-Dissolved	µg/L	90	23,000
Cadmium-Dissolved	µg/L	<0.1	73
Chromium-Dissolved	µg/L	<1	<1
Copper-Dissolved	µg/L	<1	5,600
Cobalt-Dissolved	µg/L	<1	870
Iron-Dissolved	µg/L	130	210
Manganese-Dissolved	µg/L	<5	10,000
Nickel-Dissolved	µg/L	<1	900
Lead-Dissolved	µg/L	<1	47
Mercury-Dissolved	µg/L	<0.05	<0.05
Zinc-Dissolved	µg/L	2	20,000

Cations in water Dissolved Our Reference: Your Reference Type of sample	UNITS ---------- ----------	104464-1 SW2 Water	104464-2 RP1 Water
Date digested	-	05/02/2014	05/02/2014
Date analysed	-	05/02/2014	05/02/2014
Calcium - Dissolved	mg/L	<0.5	66
Magnesium - Dissolved	mg/L	<0.5	160

Miscellaneous Inorganics Our Reference: Your Reference Type of sample	UNITS ---------- ----------	104464-1 SW2 Water	104464-2 RP1 Water
Date prepared	-	04/02/2014	04/02/2014
Date analysed	-	04/02/2014	04/02/2014
pH	pH Units	6.2	3.8
Electrical Conductivity	µS/cm	10	1,600
Sulphate, SO4	mg/L	<1	830

Method ID	Methodology Summary
Metals-022 ICP-MS	Determination of various metals by ICP-MS.
Metals-021 CV-AAS	Determination of Mercury by Cold Vapour AAS.
Metals-020 ICP-AES	Determination of various metals by ICP-AES.
Inorg-001	pH - Measured using pH meter and electrode in accordance with APHA 22nd ED, 4500-H+.
Inorg-112	Dissolved Oxygen using membrane electrode. The method is based upon APHA 4500-O G. Note this analysis should ideally be carried out immediately after sampling.
Inorg-002	Conductivity and Salinity - measured using a conductivity cell and dedicated meter, in accordance with APHA 22nd ED 2510 and Rayment & Lyons.
Inorg-081	Anions - a range of Anions are determined by Ion Chromatography, in accordance with APHA 22nd ED, 4110 -B.

Client Reference: Ecotox-Vista Gold

QUALITY CONTROL HM in water - dissolved	UNITS	PQL	METHOD	Blank	Duplicate Sm#	Duplicate results Base II Duplicate II %RPD	Spike Sm#	Spike % Recovery
Date prepared	-			05/02/2014	104464-1	05/02/2014 || 05/02/2014	LCS-W1	05/02/2014
Date analysed	-			05/02/2014	104464-1	05/02/2014 || 05/02/2014	LCS-W1	05/02/2014
Aluminium-Dissolved	µg/L	10	Metals-022 ICP-MS	<10	104464-1	90 || 90 || RPD: 0	LCS-W1	92%
Cadmium-Dissolved	µg/L	0.1	Metals-022 ICP-MS	<0.1	104464-1	<0.1 || <0.1	LCS-W1	87%
Chromium-Dissolved	µg/L	1	Metals-022 ICP-MS	<1	104464-1	<1 || <1	LCS-W1	87%
Copper-Dissolved	µg/L	1	Metals-022 ICP-MS	<1	104464-1	<1 || <1	LCS-W1	88%
Cobalt-Dissolved	µg/L	1	Metals-022 ICP-MS	<1	104464-1	<1 || <1	LCS-W1	92%
Iron-Dissolved	µg/L	10	Metals-022 ICP-MS	<10	104464-1	130 || 130 || RPD: 0	LCS-W1	100%
Manganese-Dissolved	µg/L	5	Metals-022 ICP-MS	<5	104464-1	<5 || <5	LCS-W1	92%
Nickel-Dissolved	µg/L	1	Metals-022 ICP-MS	<1	104464-1	<1 || <1	LCS-W1	89%
Lead-Dissolved	µg/L	1	Metals-022 ICP-MS	<1	104464-1	<1 || <1	LCS-W1	86%
Mercury-Dissolved	µg/L	0.05	Metals-021 CV-AAS	<0.05	104464-1	<0.05 || [N/T]	LCS-W1	100%
Zinc-Dissolved	µg/L	1	Metals-022 ICP-MS	<1	104464-1	2 || 2 || RPD: 0	LCS-W1	90%

Client Reference: Ecotox-Vista Gold

QUALITY CONTROL Cations in water Dissolved	UNITS	PQL	METHOD	Blank	Duplicate Sm#	Duplicate results Base II Duplicate II %RPD	Spike Sm#	Spike % Recovery
Date digested	-			05/02/2014	[NT]	[NT]	LCS-W1	05/02/2014
Date analysed	-			05/02/2014	[NT]	[NT]	LCS-W1	05/02/2014
Calcium - Dissolved	mg/L	0.5	Metals-020 ICP-AES	<0.5	[NT]	[NT]	LCS-W1	108%
Magnesium - Dissolved	mg/L	0.5	Metals-020 ICP-AES	<0.5	[NT]	[NT]	LCS-W1	110%

QUALITY CONTROL Miscellaneous Inorganics	UNITS	PQL	METHOD	Blank	Duplicate Sm#	Duplicate results Base II Duplicate II %RPD	Spike Sm#	Spike % Recovery
Date prepared	-			04/02/2014	[NT]	[NT]	LCS-W1	04/02/2014
Date analysed	-			04/02/2014	[NT]	[NT]	LCS-W1	04/02/2014
pH	pH Units		Inorg-001	[NT]	[NT]	[NT]	LCS-W1	101%
Dissolved Oxygen*	mg/L	0.1	Inorg-112	<0.1	[NT]	[NT]	[NR]	[NR]
Electrical Conductivity	µS/cm	1	Inorg-002	<1	[NT]	[NT]	LCS-W1	101%
Sulphate, SO4	mg/L	1	Inorg-081	<1	[NT]	[NT]	LCS-W1	87%

QUALITY CONTROL HM in water - dissolved	UNITS	Dup. Sm#	Duplicate Base + Duplicate + %RPD	Spike Sm#	Spike % Recovery
Date prepared	-	[NT]	[NT]	104464-2	05/02/2014
Date analysed	-	[NT]	[NT]	104464-2	05/02/2014
Aluminium-Dissolved	µg/L	[NT]	[NT]	104464-2	#
Cadmium-Dissolved	µg/L	[NT]	[NT]	104464-2	99%
Chromium-Dissolved	µg/L	[NT]	[NT]	104464-2	91%
Copper-Dissolved	µg/L	[NT]	[NT]	104464-2	#
Cobalt-Dissolved	µg/L	[NT]	[NT]	104464-2	100%
Iron-Dissolved	µg/L	[NT]	[NT]	104464-2	83%
Manganese-Dissolved	µg/L	[NT]	[NT]	104464-2	#
Nickel-Dissolved	µg/L	[NT]	[NT]	104464-2	110%
Lead-Dissolved	µg/L	[NT]	[NT]	104464-2	95%
Mercury-Dissolved	µg/L	[NT]	[NT]	[NR]	[NR]
Zinc-Dissolved	µg/L	[NT]	[NT]	104464-2	#

Report Comments:

Trace metals: # Percent recovery is not possible to report due to the high concentration of the element/s in the sample/s. However an acceptable recovery was obtained for the LCS.

Asbestos ID was analysed by Approved Identifier:	Not applicable for this job
Asbestos ID was authorised by Approved Signatory:	Not applicable for this job

INS: Insufficient sample for this test	PQL: Practical Quantitation Limit	NT: Not tested
NA: Test not required	RPD: Relative Percent Difference	NA: Test not required
<: Less than	>: Greater than	LCS: Laboratory Control Sample

Quality Control Definitions

Blank: This is the component of the analytical signal which is not derived from the sample but from reagents, glassware etc, can be determined by processing solvents and reagents in exactly the same manner as for samples.

Duplicate: This is the complete duplicate analysis of a sample from the process batch. If possible, the sample selected should be one where the analyte concentration is easily measurable.

Matrix Spike : A portion of the sample is spiked with a known concentration of target analyte. The purpose of the matrix spike is to monitor the performance of the analytical method used and to determine whether matrix interferences exist.

LCS (Laboratory Control Sample) : This comprises either a standard reference material or a control matrix (such as a blank sand or water) fortified with analytes representative of the analyte class. It is simply a check sample.

Surrogate Spike: Surrogates are known additions to each sample, blank, matrix spike and LCS in a batch, of compounds which are similar to the analyte of interest, however are not expected to be found in real samples.

Laboratory Acceptance Criteria

Duplicate sample and matrix spike recoveries may not be reported on smaller jobs, however, were analysed at a frequency to meet or exceed NEPM requirements. All samples are tested in batches of 20. The duplicate sample RPD and matrix spike recoveries for the batch were within the laboratory acceptance criteria.
Filters, swabs, wipes, tubes and badges will not have duplicate data as the whole sample is generally extracted during sample extraction.
Spikes for Physical and Aggregate Tests are not applicable.
For VOCs in water samples, three vials are required for duplicate or spike analysis.

Duplicates: <5xPQL - any RPD is acceptable; >5xPQL - 0-50% RPD is acceptable.
Matrix Spikes, LCS and Surrogate recoveries: Generally 70-130% for inorganics/metals; 60-140% for organics and 10-140% for SVOC and speciated phenols is acceptable.

In circumstances where no duplicate and/or sample spike has been reported at 1 in 10 and/or 1 in 20 samples respectively, the sample volume submitted was insufficient in order to satisfy laboratory QA/QC protocols.

When samples are received where certain analytes are outside of recommended technical holding times (THTs), the analysis has proceeded. Where analytes are on the verge of breaching THTs, every effort will be made to analyse within the THT or as soon as practicable.

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Document Status

Rev No.	Author	Reviewer		Approved for Issue		
		Name	Signature	Name	Signature	Date
0	J.Woodworth	G.Metcalfe		N.Conroy		19/03/2014

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Vista Gold Australia Pty Ltd

Mt Todd Discharge

Treated Retention Pond 3 Ecotoxicological Report

February 2013

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1. Introduction

1.1 Project Background

Vista Gold Australia Pty Ltd (Vista Gold) received a Waste Discharge Licence (WDL 178-2) on 5 February 2013 from the Northern Territory Environment Protection Authority (NT EPA). The WDL outlines environmental requirements for protection of the Edith River from mine wastewater discharges. Previously, Vista Gold derived interim site specific trigger values (ISSTVs) (GHD 2012) to meet the 95% species protection trigger values downstream of the Retention Pond 1 (RP1) discharge site at SW10 (8.7 km from SW4) to meet the requirements of WDL 178-1. The discharge scenario from previous years where mine water was discharged from RP1 is no longer applicable to the site due to the need for dewatering and on-site water treatment.

The use of dilution factors derived from direct toxicity assessment (DTA) for three water bodies at the Mt Todd mine site has been selected as the most appropriate method for deriving safe concentrations of metals for environmental protection of the Edith River to meet the requirements of WDL 178-2. This method is recommended by ANZECC & ARMCANZ (2000) where chemicals are present in a complex mixture. DTA allows the determination of direct biological effects on appropriate species to be assessed and also enables metal concentrations to be established that would not cause adverse environmental effects.

DTA has been used throughout Australia to establish appropriate dilution factors for complex effluents. For example the Perth Desalination Plant (Geotechnical Services 2006, 2008) provided dilution factors to be met at the 80% species protection level within the Low Protection Area at the outfall, and the 90% species protection level at Moderate Protection Area in Cockburn Sound. All other desalination plants around Australia have used the DTA methodology to derive appropriate dilution factors and engineer the outfall configuration to meet the required dilutions (BHP Billiton 2009, Victorian Government 2008).

1.2 Objective

WDL 178-2 requires that ecotoxicological assessment be conducted for a wastewater source. This treated retention pond (RP)3 Ecotox DTA Report has been developed to address the relevant sections of the WDL 178-2 to derive a dilution factor for RP3 prior to discharging into the Edith River.

1.3 Scope of Work

This Report addresses the following aspects of the WDL 178-2:

- The methodology for calculating the 80% species protection ANZECC & ARMCANZ (2000) dilution factor for ecosystem protection applied at SW4 for mine discharges from RP3.
- Deriving Monitoring Values for the treated RP3 mine water to be met at SW4 to check that the dilution factor for the treated mine water is being met.

1.4 Limitations

This Vista Gold Australia Discharge Plan Revision 1 ("Report"):

1. *has been prepared by GHD Pty Ltd ("GHD") for Vista Gold Australia Pty Ltd (Vista Gold) and the NT EPA;*
2. *may only be used and relied on by Vista Gold and the NT EPA;*

3. *must not be copied to, used by, or relied on by any person other than Vista Gold without the prior written consent of GHD;*
4. *may only be used for the purpose of addressing WDL 178-2 requirements (and must not be used for any other purpose).*

GHD and its servants, employees and officers otherwise expressly disclaim responsibility to any person other than Vista Gold arising from or in connection with this Report.

To the maximum extent permitted by law, all implied warranties and conditions in relation to the services provided by GHD and the Report are excluded unless they are expressly stated to apply in this Report.

The services undertaken by GHD in connection with preparing this Report were limited to those specifically detailed in section 1.2 of this Report.

The opinions, conclusions and any recommendations in this Report are based on assumptions made by GHD when undertaking services and preparing the Report ("Assumptions"), including (but not limited to): Current accepted practices.

GHD expressly disclaims responsibility for any error in, or omission from, this Report arising from or in connection with any of the Assumptions being incorrect.

Subject to the paragraphs in this section of the Report, the opinions, conclusions and any recommendations in this Report are based on conditions encountered and information reviewed at the time of preparation and may be relied on until 6 months, after which time, GHD expressly disclaims responsibility for any error in, or omission from, this Report arising from or in connection with those opinions, conclusions and any recommendations.

1.5 Assumptions

The assumptions upon which this report is based are:

- The treated mine water tested was representative of the RP3 water at the time of testing; and
- SW2 water used in the DTA was representative of late dry/ early wet season Edith River water.

2. Direct Toxicity Methodology

2.1 Direct Toxicity Assessment (DTA)

DTA provides an integrated measure of the toxicity of chemicals within a complex mixture, and accounts for interactions between compounds which may be additive, synergistic or ameliorative. DTA provides a better representation of natural environmental conditions than single chemical testing, and is therefore recommended by ANZECC & ARMCANZ (2000) for assessing the environmental impact from complex effluents.

The Mt Todd DTA program was designed in line with the recommendations contained in ANZECC & ARMCANZ (2000). ANZECC & ARMCANZ (2000) states that a DTA program should address the following design considerations:

- Test species selection
- Receiving water selection
- Nature of contaminant(s)
- Test methods
- Statistical considerations.

2.1.1 Selected Species

ANZECC & ARMCANZ (2000) "Minimum requirements for DTA" (section 8.3.6.8) recommend that toxicity data from between three and five species representing at least four different taxonomic groups (where five species are used) is required for effluent DTA. Further information on species used in the DTAs conducted for Mt Todd discharge to the Edith River is shown in Table 1 below.

Often, due to laboratory issues, not all tests listed in Table 1 are available at the time of testing. DTAs conducted on RP1, RP7 and RP3 Pilot Trial used a maximum of seven species to provide confidence in the species sensitivity distribution calculation.

Tests were selected adhering to the following criteria:

- a minimum of five bioassays covering at least four taxa
- preferably chronic endpoints to avoid the used of application factors
- locally occurring species or species representative of the ecosystem
- dilution water sampled from SW2
- sufficient test dilutions to obtain a statistically valid EC10 (i.e. tight 95% confidence limits)
- bioassay suite sensitive to a broad range of contaminants.

The bioassays were conducted in two laboratories because not all bioassays are available from one laboratory. The cladoceran (*Moinodaphnia macleayi*) is the most sensitive bioassay species and is only able to be conducted by the ERISS Laboratory in Darwin. All other bioassays are conducted by Ecotox Services Australasia located in Sydney as shown in Table 1.

Table 1 Species used in the Ecotoxicological Assessment of Treated RP3 Discharge

Test Organism	Test Duration	Test Endpoint	Key Reference	Laboratory
Chlorella vulgaris (green alga)	72 hour (chronic)	Growth inhibition	USEPA Method 1003.0 (2002)	Ecotox Services
Lemna aequinoctialis (duckweed)	96 hour (chronic)	Growth (frond number)	OECD Method 221 (2006)	Ecotox Services
Moinodaphnia macleayi (water flea)	3 brood (chronic)	Reproduction	Riethmuller *et. al.* (2003)	ERISS
Hydra viridissima Pallas (green hydra)	96 hour (chronic)	Population growth	Riethmuller *et al.* (2003)	Ecotox Services
Chironomus tepperi (chironomid)	48 hour (acute)	Survival	USEPA (2002), OECD (2011)	Ecotox Services
Macrobrachium bullatum (shrimp)	96 hour (acute)	Survival	ESA SOP 123 (ESA 2012)	Ecotox Services
Melanotaenia splendida (rainbowfish)	10 day (chronic)	Embryonic development and post hatch survival	USEPA (2002)	Ecotox Services

2.1.2 Concentrations Tested

Edith River water sampled from the upstream site SW2 was used as the diluent to assess the toxicity of the treated RP3 mine water. A laboratory control was run concurrently with all tests to confirm that the natural diluent water was not confounding the bioassays (as can happen in many cases). All samples were serially diluted with the SW2 river water to achieve the required test concentrations. Concentrations differed between bioassays due to methodologies used and sensitivity of the species tested as determined by previous bioassays, as shown in Table 2.

Table 2 Concentrations of Treated RP3 used in Bioassays

Microalga % RP3	Duckweed % RP3	Cladoceran % RP3	Hydra % RP3	Chironomid % RP3	Shrimp %RP3	Fish % RP3
0	0	0	0	0	0	0
0.1	0.1	0.0078	0.06	6.3	0.3	0.03
0.2	0.2	0.0156	0.13	12.5	0.6	0.06
0.4	0.4	0.0312	0.25	25	1.3	0.13
0.8	0.8	0.0625	0.5	50	2.5	0.25
1.5	1.5	0.125	1.0	100	5.0	0.50
3	3.0	0.25	2.0	-	10.0	1.00
-	-	0.5	-	-	-	-
-	-	1.0	-	-	-	-

2.1.3 Dilution Factors

Dilution factors are derived from results of DTA using bioassays representative of the receiving ecosystem which incorporates all actions of the constituents of the effluent. These results are placed in the BurrliOZ (Campbell *et al.* 2000) statistics program for calculation of a species sensitivity distribution and the concentration of effluent is determined to protect 80% of the populations in the receiving water from a 10% decrease in growth and / or reproduction.

Concentrations of individual chemicals cannot be extrapolated from DTAs for use as trigger values. However, individual chemical concentrations can be used for monitoring purposes to ensure that the dilution factors are met at the appropriate monitoring site. If the dilution factors are not met, the investigation procedure shown in Figure 1 (GHD 2013) will be followed.

2.2 Surface Water Chemistry

Samples from SW2 (diluent) and treated RP3 water were analysed for the suite of analytes shown in Table 3.

Table 3 Analytes for SW2 and Treated RP3

	Analytes
In-Situ	DO, temperature, EC, pH
Metals (total and dissolved i.e. 0.45 µm)	Al, Cd, Co, Cu, Cr, Fe, Pb, Mg, Mn, Hg, Ni, Zn
Others	NO3, PO4, SO4, bicarbonate, alkalinity, hardness, TDS, TSS, TS, Na, Cl, Ca, WAD cyanide, TOC and DOC

2.3 Sample Sites

RP3 and SW2 sampling locations are shown in Table 4. The treated RP3 water was sampled at 6 metres below the RP surface. This depth is the proposed pumping depth for discharge and provides the treated water with the highest pH and lowest metal concentrations.

Table 4 Sampling Sites

Site Name	Site Description	Easting (UTM)	Northing	Latitude (degree, decimal)	Longitude
SW2	Edith River at bridge on Edith Falls Road	0189088	8431347	-14.1718	132.12
RP3	Batman Pit	0187055	8434993	-14.1388	132.1015

3. Results

3.1 Ecotoxicology Testing of RP3 Treated Water (in-situ)

The species used were appropriate for the site and the EC10 and LC50 results were of sufficient quality to be used in the BurrliOZ (Campbell *et al.,* 2000) program for species sensitivity distribution (SSD) calculations. Not all species that were used in the ERISS (2005) toxicity testing program were available at the time of testing.

A copy of the Ecotox Report by Ecotox Services, "*Toxicity Assessment of a Treated Water Sample, January 2013*" and the ERISS Test Report are located in Appendix A. A summary of the results is provided in Table 5. The results of the site specific DTA testing performed by Ecotox Services used in this assessment do meet the laboratory NATA requirements for quality assurance/quality control (QA/QC) parameters. It must be noted however, that the majority of the reference toxicant results are in the lower 10% of the Cusum chart limits, indicating that the organisms used were more sensitive to the toxicants than is usual (Appendix A). This sensitivity means that the results from this treated RP3 DTA are very conservative.

ERISS does not hold NATA accreditation, but does perform similar QA/QC on toxicity tests, as is required for NATA accreditation.

Table 5 Summary of Treated RP3 Ecotox Results

Test	EC/IC10 and LC10 (95% confidence limits) % Treated RP3	EC/IC50 and LC50 (95% confidence limits) % Treated RP3
Microalgal 72-hour growth inhibition	0.2 (0.1-0.3)	0.3 (0.3-0.4)
Duckweed 7-day growth inhibition	0.30 (0.1-0.4)	0.6 (0.5-0.6)
Cladoceran 6-day reproduction	0.019 (0.016-0.020)	0.14 (0.01-0.20)
Hydra 96-hour growth	0.27 (0.06-0.39)	0.42 (0.35-0.49)
Chironomid 96-hour survival	7.4 (6.4-10.6)	9.5 (8.7-10.5)
Shrimp 96-hour survival	0.5 **	1.0 (0.7-1.2)
Fish 10-day post hatch survival	0.28 (0.14-0.37)	0.56 (0.43-0.71)

**No observable effect concentration (NOEC) ** 95% confidence limits not reliable*

The results show that the cladoceran is the most sensitive species to the treated RP3 water. Algae and duckweed usually show similar sensitivities to most toxicants and Gausman (2006) suggests that the two tests can be used interchangeably. Gausman (2006) also cites literature that shows that unicellular alga is more sensitive than duckweed to some metals as is shown in this suite of bioassay results.

In this case, copper, manganese and zinc are present in the treated RP3 mine water and may be causing the toxicity observed in the cladoceran, duckweed and algal bioassays. Cobalt, nickel, lead and cadmium are also present in concentrations that have potential to cause toxicity to unicellular alga, as the alga is more sensitive than duckweed to these metals.

The cladoceran is sensitive to the treated RP3 water, which may be a reflection of the toxicity of the treated RP3 water to their food source (i.e. the unicellular alga).

3.2 Acute to Chronic Ratios (ACR)

Two of the bioassays used in this study were acute bioassays where survival was the end point. The chironomid and shrimp are acute bioassays and the LC50 results need to be adjusted so that they can be incorporated into the statistics to calculate the SSD. ANZECC & ARMCANZ (2000) recommend an acute to chronic factor of 10 to go from an acute LC50 to a chronic NOEC or EC10. This acute to chronic ratio (ACR) was applied to the chironomid and shrimp LC50 data to obtain a chronic EC10.

3.3 Concentrations used to calculate the SSD

The acute bioassays required an ACR adjustment, applied to the LC50 values for the chironomid (LC50 = 9.5%) and shrimp (LC50 = 1.0%). The values used to calculate the SSD and dilution factors are shown in Table 6.

Table 6 Values used to Calculate SSD

Bioassay	EC10 % RP3
Microalgal 72-hour growth inhibition	0.2
Duckweed 96-hour growth inhibition	0.30
Cladoceran 6-day reproduction	0.02
Hydra 96-hour growth	0.27
Chironomid 48-hour survival	0.95
Shrimp 96-hour survival	0.1
Fish 96-hour imbalance	0.28

3.4 Species Protection Values

The EC10 and LC50/10 values from Table 6 were input into the BurrliOZ (Campbell *et al*., 2000) statistics program for calculation of the 80% species protection value using the SSD. The calculated value and the dilution factor required to reach the concentration are provided in Table 7.

Table 7 Species Protection Values

Species Protection Level	Concentration of Treated RP3 (%)	Dilution Factor
80%	0.089	1,123

3.5 Dilution Factor

The 80% species protection was selected as appropriate to provide a level of protection for the Edith River from the discharge of treated RP3 water over the dewatering period. A dilution factor of 1:1,123 for the RP3 treated water (as at time of testing, 23/01/13) discharged into the Edith River will provide 80% of the species with protection from a 10% decrease in reproduction or growth (Table 7). The concentrations of chemicals at this dilution are shown in Table 8. The levels of copper and zinc have been selected as monitoring values to be met to ensure that the dilution of the treated RP3 water is at 1:1,123 to meet the requirements of WDL 178-2.

3.6 80 Percent Species Protection Monitoring Values

The chemistry of the RP3 treated water at the 1:1,123 dilution is shown in Table 8. The complete chemistry results are located in Appendix B. The concentrations of copper at 3.0 µg/L and zinc at 33.8 µg/L should be met at SW4 to ensure the correct dilution is being achieved. If this dilution is not met then action is triggered as shown in Figure 1 of the Discharge Plan (GHD 2013).

Table 8 Dilution Rates of RP3 Treated Water (in-situ) to Meet 80 percent SSD Dilution (1:1,370)

Analytes (metals 0.45 µm)	SW2 23/01/13	RP3 Treated Water 23/01/13	Conc. in RP3 treated water at 1:1,123 to be met at SW4	ANZECC 80% species protection TVs/ISSTV	ANZECC 95% species protection TVs/ISSTV
pH		-	-	6-8	6-8
DO (%)		-	-	85-120	85-120
Conductivity (µS/cm)				20-250	20-250
Mg (mg/L)	0.5	200	0.17	2.5	2.5
SO_4 (mg/L)	<1	1,800	1.6	129	129
Al (µg/L)	110	<10	<10	150	149
Cd (µg/L)	<0.1	130	0.12	0.8	0.2
Co (µg/L)	<1	1,300	1.16	90	90
Cr (µg/L)	<1	<1	<1	40	1.0
Cu (µg/L)	<1	3,400	3.0	2.5	1.4
Fe (µg/L)	290	<10	<10	300	300
Mn (µg/L)	<5	19,000	16.9	3,600	1,700
Ni (µg/L)	<1	1,300	1.16	17	11
Pb (µg/L)	<1	65	0.06	9.4	3.4
Hg (µg/L)	<0.05	<0.05	<0.05	5.4	0.6
Zn (µg/L)	2	38,000	33.8	31	8.0

Note. Cells in green are below the ISSTVs and ANZECC & ARMCANZ (2000) default 95% trigger values

Table 9 shows the Monitoring Values for each discharge point as of 28 February 2013.

RP3 treated water will be assessed for toxicity during the 2012/2013 wet season and a revised dilution factor and Monitoring Values calculated. RP1 and RP7 mine waters will be assessed for toxicity prior to the 2013/2014 wet season.

Table 9 Monitoring Values for SW4 (February 2013)

Discharge	Chemical	Concentration (µg/L) 0.45 µm filtered
RP3 Treated Water	Cu	3.0
	Zn	33.8
RP1 Untreated Water	Cu	4.3
RP7 Untreated Water	Cu	4.2

4. Conclusions and Recommendations

4.1 Conclusions

Copper and zinc are the only metals present in the treated RP3 mine water above the 95% species protection ISSTVs at the 1:1,123 dilution factor. However the dilution factor calculated using the SSD seems to be conservative for ecosystem protection of the Edith River at SW4 because the chemistry of RP3 has improved significantly since the DTA was conducted (Table 10).

Both the zinc and copper concentrations are above the ANZECC & ARMCANZ 80% species protection trigger values. Again, the dilution factor of 1:1,123 and monitoring values for copper of 3µg/L and zinc of 33.8 µg/L are very conservative, because the mean concentrations of copper and zinc at SW4 during the 2011/2012 wet season were much higher at 20 µg/L and 102 µg/L respectively, with maximum concentrations of 77 µg/L copper and 300 µg/L zinc recorded. Monitoring conducted during the 2012 dry season showed that there were no adverse environmental impacts at SW4 or downstream of SW4 from the 2011/2012 wet season discharge from RP1.

Table 10 RP3 Chemistry (Top 15 metres)

Analyte Dissolved µg/L	20 Oct 12	23 Nov 12	20 Dec 12	17 Jan 13	14 Feb 13
Aluminium	57,000	31,000	1,700	270	<10
Chromium	2	2	1	<1	<1
Copper	10,000	11,000	9,900	5,400	140
Lead	210	190	160	86	<1
Cadmium	140	140	140	130	45
Zinc	36,000	34,000	38,000	39,000	8,400

Treatment of RP3 is on-going and the water quality is constantly improving with elevated pH and reduced metal concentrations (Table 10). The dilution factor of 1: 1,123 calculated in this report will be applied until the results of the next DTA become available. Due to the improvement of the RP3 water quality since the DTA, the dilution factor calculated in this report is very conservative.

It must be noted that the SW2 Edith River water sampled at the end of the dry season used as the dilution water for the DTA was not representative of wet season river water, when TOC and DOC are elevated. Therefore, the DTA conducted on this sample would have been a worst case scenario as increased DOC has the ability to reduce the toxicity of metals in the mine water.

4.2 Recommendations

To meet the 80% species protection level required by WDL 178-2, based on ecotoxicological testing, it is recommended that a dilution factor of 1:1,123 be applied to the discharge of RP3 treated mine water, with the following Monitoring Values applied at SW4:

- Cu 3.0 µg/L
- Zn 33.8 µg/L

These monitoring values will apply until a further round of ecotoxicological testing is conducted to derive a new dilution factor and Monitoring Values.

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Appendices

Appendix A – Ecotox Reports

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Cladoceran Reproduction Test Report (1317D)

Client: Vista Gold Pty Ltd.

Project: Ecotoxicological assessment of Mt Todd Gold Mine discharge waters

Test Performed: BTT-D, 3 brood reproduction test using *Moinodaphnia macleayi*

Samples Collected:	15th January 2013		
Samples Received:	16th January 2012	**Test Initiated:**	17th January 2013
ERISS Sample ID.	**Sample Name**	**Sample Description**	
Reference water	Edith River (SW2)	Edith River upstream reference site	
Toxicant	Treated RP3 water (RP3)	Lime treated water from Retention Pond 3	

Comments: Waters received 16th January 2012 and refrigerated at 4 ºC. Waters were filtered (3 µm pore size) on the 17th January

Sample Physico-Chemistry and Preparation:

Sample	**Physico-chemistry**		
	pH	**EC** [a]	**DO** [b]
QA Magela Creek water control	5.7	17	84.2
Edith River – reference water	6.4	17	80.8
Mt Todd treated RP3 water	6.5	2770	79.1

[a] EC = Electrical Conductivity (µS cm^{-1}) unless other units provided; [b] DO = Dissolved Oxygen (% saturation)

Test Method: Cladoceran neonates (<6 h old) were exposed to a Magela Creek water QA control, a Reference/control water (Edith River, SW2) and 8 concentrations of Mt Todd Mine treated RP3 water , diluted with the reference water, for a period of 144 h. All waters were filtered (3 µm pore size) prior to testing.

The test was completed when >80% of control cladocera had released their third brood offspring. Observations of the appearance and number of neonates produced by each cladoceran were recorded at 24 h intervals, after which the cladocera were transferred to fresh test solution containing food. The detailed test protocol is presented in Riethmuller et al. (2003).

Linear interpolation analysis was used to determine point estimates of Inhibitory Concentrations (ICs) that reduced neonate production by 10% and 50% (i.e. IC_{10} and IC_{50}) relative to the reference site control responses (CETIS v1.8.1.2).

Comments: A larger concentration range was achieved by reducing treatment replication from 10 to 5 per treatment. Control replication was not modified (i.e. 10 replicates). This allows greater characterisation of the concentration-response of the cladoceran.

Results*

Sample	Mean neonate production ± SEM	% of Reference
QA Magela Creek water control	29.8 ± 0.6	98
SW2 - Reference water	30.3 ± 1.4	100
0.0078% Treated RP3 water	30.0 ± 0.9	99
0.0156% Treated RP3 water	32.6 ± 1.3	107
0.0313% Treated RP3 water	17.6 ± 0.4	58
0.0625% Treated RP3 water	16.2 ± 2.2	53
0.125% Treated RP3 water	16.8 ± 1.5	55
0.25% Treated RP3 water	10.4 ± 1.4	34
0.5% Treated RP3 water	0.4 ± 0.4	1.3
1.0% Treated RP3 water	0 ± 0	0

Toxicity Estimates

IC10 (95% Confidence limits)	IC50 (95% Confidence limits)
0.019 (0.016 – 0.020) % RP3 water	0.14 (0.01 – 0.20) % RP3 water

Comments: A full concentration-response relationship was established for the treated RP3 water (Figure 1). Individuals exposed to 0.078 and 0.0156% treated RP3 water had neonate counts of > 30 neonates adult^{-1}. Individuals exposed to 0.0313, 0.0625, 0.125 and 0.25% treated RP3 water produced 42, 47, 45 and 66% less neonates compared to the SW2 reference control, respectively. Exposure to 0.5% treated RP3 water resulted in 80% mortality with 2 neonates produced. 100% mortality of test organisms following exposure to 1% treated RP3 water, resulting in no reproduction.

Toxicity of treated RP3 water lies between the toxicity responses of RP1 and RP7, where IC10's were 0.1 and 0.008%, respectively. This was consistent with the different metal concentrations found in the three water types. The toxicity of this water was higher than treated RP3 water that was tested in August 2011 (IC10=1.3%), which may be due to the extended storage time (6 weeks) of that sample.

* See Attachment A for test raw data and statistical analysis report.

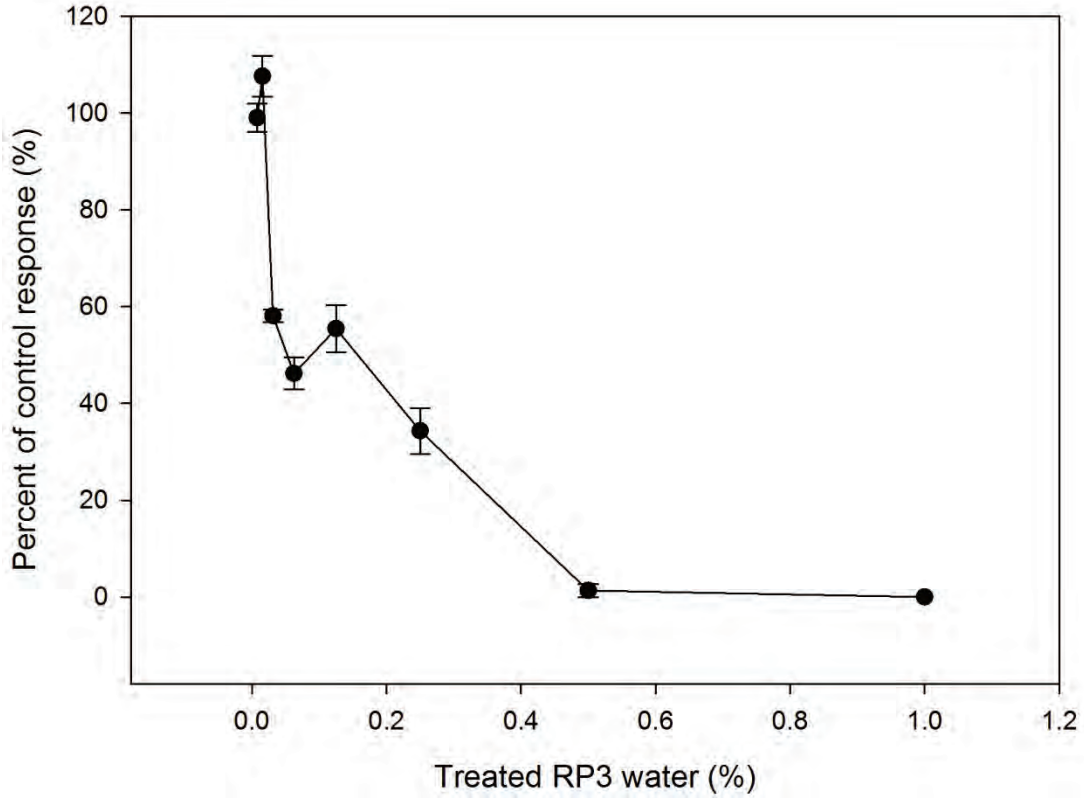


Figure 1 Effect of Mt Todd Retention Pond 3 water to *Moinodaphnia macleayi.* Results are represented as mean±standard error of neonate production (5 replicates) compared to control response (10 replicates). Mean control response = 30.3 neonates

Quality Control *	**Criterion**	**NMCW**	**SW2**	**Criterion Met?**
Mean # neonates in control	>30	29.8	30.3	Yes
% co-efficient of variation of control	<20	7	15	Yes
pH (Magela Creek water control)	<1 unit variation	<1	<1	Yes
DO concentration (throughout test)	>70% saturation	86.4%	86.4%	Yes
EC	<10% or < 3 µS cm^{-1} variation	1 µS cm^{-1}	2 µS cm^{-1}	Yes
Reference toxicant EC50 (µg U L^{-1})	±2 SD of running mean ie. 76±132	171		No

Comments: NMCW control produced less than the acceptability criterion of >30 neonates whereas SW2 reference water reached the criterion. Hence, this test was valid. The most recent reference toxicity test indicated reduced sensitivity of *M. macleayi* to uranium. This is currently under investigation.

* See Attachment B for detailed test QC physico-chemical data.

References:

Riethmuller N, Camilleri C, Franklin N, Hogan AC, King A, Koch A, Markich SJ, Turley C & van Dam R 2003. *Ecotoxicological testing protocols for Australian tropical freshwater ecosystems*. Supervising Scientist Report 173, Supervising Scientist, Darwin NT. http://www.environment.gov.au/ssd/publications/ssr/pubs/ssr173-print-quality.pdf

Test carried out by:	Kim Cheng and Claire Costello
Test supervised by:	Rick van Dam and Andrew Harford
Test report prepared by:	Kim Cheng
	Professional Officer (ph: 08 8920 1361)
Test report authorised by:	Andrew Harford
	A/g Program Leader (ph: 08 8920 1175)
Date:	12/02/13

Attachment A – Test raw data and statistical analysis report

CETIS Analytical Report

Report Date:	24 Jan-13 09:30 (p 1 of 2)
Test Code:	1317D | 09-3585-5883

Cladoceran Reproduction Test — **eriss ecotoxicology lab**

Analysis ID:	18-4815-7504	**Endpoint:**	Total neonates	**CETIS Version:**	CETISv1.8.7
Analyzed:	24 Jan-13 9:30	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Batch ID:	19-4868-4197	**Test Type:**	Cladoceran reproduction	**Analyst:**	Kim Cheng
Start Date:	17 Jan-13	**Protocol:**	Clad (chronic) eriss tropical freshwater	**Diluent:**	Upstream of Discharge
Ending Date:	23 Jan-13	**Species:**	Moinodaphnia macleayi	**Brine:**	Not Applicable
Duration:	6d 0h	**Source:**	In-House Culture	**Age:**	

Sample ID:	06-4638-2752	**Code:**	1317D	**Client:**	Vista Gold
Sample Date:	15 Jan-13	**Material:**	RP7	**Project:**	Mt Todd
Receive Date:	16 Jan-13	**Source:**	RP3		
Sample Age:	48h	**Station:**			

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	1477154	200	Yes	Two-Point Interpolation

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Extreme Value	Grubbs Extreme Value	3.499	3.128	0.0092	Outlier Detected

Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
IC5	0.01742	0.001629	0.01779	5740	5621	61380
IC10	0.01922	0.01634	0.01996	5202	5010	6121
IC15	0.02103	0.01853	0.02213	4756	4518	5397
IC20	0.02284	0.0207	0.02431	4379	4113	4830
IC25	0.02465	0.0227	0.02649	4058	3774	4406
IC40	0.0301	0.02836	0.123	3323	812.8	3526
IC50	0.1449	0.006486	0.1959	690	510.4	15420

Total neonates Summary

			Calculated Variate						
C-%	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Upstream Contro	10	30.3	20	37	1.453	4.596	15.2%	0.0%
0.0078		5	30	27	32	0.8944	2	6.67%	0.99%
0.015625		5	32.6	28	35	1.288	2.881	8.84%	-7.59%
0.03125		5	17.6	17	19	0.4	0.8944	5.08%	41.9%
0.0625		5	16.2	11	24	2.177	4.868	30.1%	46.5%
0.125		5	16.8	13	22	1.463	3.271	19.5%	44.6%
0.25		5	10.4	7	15	1.435	3.209	30.9%	65.7%
0.5		5	0.4	0	2	0.4	0.8944	224.0%	98.7%
1		5	0	0	0	0	0		100.0%

Total neonates Detail

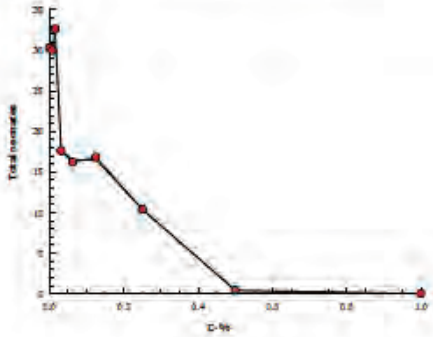
C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Upstream Control	33	26	33	30	30	32	30	32	37	20
0.0078		29	27	32	31	31					
0.015625		28	35	32	33	35					
0.03125		19	17	18	17	17					
0.0625		17	15	24	11	14					
0.125		16	17	16	13	22					
0.25		7	8	10	12	15					
0.5		0	2	0	0	0					
1		0	0	0	0	0					

000-428-181-4 CETIS™ v1.8.7.4 Analyst:_____ QA:_____

Cladoceran Reproduction Test — eriss ecotoxicology lab

Analysis ID:	18-4815-7504	**Endpoint:**	Total neonates	**CETIS Version:**	CETISv1.8.7
Analyzed:	24 Jan-13 9:30	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Graphics



Attachment B - Physico-chemical measurements of the test solutions for test 1317D

Treatment (%)		MCW		SW2		0.01		0.02		0.03		0.06		0.13		0.25		0.5		1.0	
Parameter		**0 h**	**24 h**	**0 h**	**24 h**	**0 h**	**24 h**	**0 h**	**24 h**	**0 h**	**24 h**	**0 h**	**24 h**	**0 h**	**24 h**	**0 h**	**24 h**	**0 h**	**24 h**	**0 h**	**24 h**
Day 0	pH	5.8	6.3	6.5	6.6	6.5	6.6	6.5	6.8	6.6	6.7	6.5	6.8	6.5	6.7	6.5	6.7	6.5	6.7	6.5	6.7
	EC (µS cm^{-1})	21	21	21	20	20	22	21	22	21	22	24	24	24	27	32	33	44	44	66	67
	DO (%)	102	90	98	89	103	88	101	90	104	89	100	91	99	91	101	90	101	91	98	88
	Temp (°C)	23.1	23.5	23.3	23.7	22.6	23.6	22.6	22.7	22.5	22.8	22.6	22.7	22.6	22.4	22.5	22.4	22.2	22.2	22.2	22.3
Day 1	pH	6.0	6.4	6.5	6.8	6.6	6.7	6.6	6.8	6.6	6.8	6.6	6.8	6.6	6.8	6.5	6.8	6.6	6.8	6.6	N.M
	EC (µS cm^{-1})	21	21	20	20	20	21	21	23	22	22	23	24	26	26	32	35	44	44	66	N.M
	DO (%)	97	91	105	89	106	90	103	87	102	91	103	92	105	90	103	90	102	90	99	N.M
	Temp (°C)	23.0	22.5	22.6	22.3	22.1	22.1	22.8	22.2	21.8	22.0	21.4	22.1	21.2	22.0	21.3	22.0	21.1	21.8	20.9	N.M
Day 2	pH	5.9	6.5	6.5	6.8	6.5	6.8	6.5	6.8	6.5	6.8	6.5	6.8	6.5	6.8	6.5	6.8	6.5	6.7	N.M	N.M
	EC (µS cm^{-1})	20	21	19	20	19	20	20	22	21	22	22	23	24	26	31	33	44	44	N.M	N.M
	DO (%)	106	89	111	89	116	93	116	91	111	90	114	94	115	90	113	92	114	91	N.M	N.M
	Temp (°C)	23.3	21.1	23.3	21.1	23.8	21.4	23.7	21.6	23.3	21.4	23.3	22.1	23.1	20.9	23.0	22.1	22.8	21.3	N.M	N.M
Day 3	pH	6.1	6.8	6.8	6.7	6.7	6.8	6.7	6.9	6.7	6.8	6.7	6.8	6.7	6.8	6.7	6.8	6.7	6.7	N.M	N.M
	EC (µS cm^{-1})	21	21	20	19	20	20	20	20	20	21.0		23	25	26	31	33	44	42	N.M	N.M
	DO (%)	101	86	102	86	107	86	109	85	107	90	107	88	110	86	102	85	106	90	N.M	N.M
	Temp (°C)	23.1	21.9	22.3	22.2	22.9	22.0	23.2	22.0	23.4	21.9	23.0	21.8	22.1	21.6	21.7	21.6	21.4	21.1	N.M	N.M
Day 4	pH	5.9	6.5	6.7	6.7	6.7	6.9	6.6	6.8	6.6	6.8	6.6	6.8	6.6	6.8	6.8	6.8	6.7	6.8	N.M	N.M
	EC (µS cm^{-1})	20	21	19	20	18	21	18	22	21	23	22	24	26	26	32	32	44	44	N.M	N.M
	DO (%)	103	90	113	89	111	90	112	90	112	92	107	93	101	91	104	92	104	93	N.M	N.M
	Temp (°C)	23.3	24.5	22.4	23.2	23.7	22.8	23.4	23.6	23.9	24.3	23.7	24.7	22.9	24.5	22.0	24.2	21.7	24.2	N.M	N.M
Day 5	pH	6.0	6.4	6.6	6.7	6.8	6.9	6.8	6.9	6.8	6.9	6.8	6.9	6.7	6.9	6.7	6.8	6.7	6.8	N.M	N.M
	EC (µS cm^{-1})	24	21	21	19	21	21	20	21	22	22	23	24	25	26	31	32	44	44	N.M	N.M
	DO (%)	103	88	99	90	102	88	106	89	101	91	106	90	108	92	106	92	104	90	N.M	N.M
	Temp (°C)	23.5	22.7	23.4	23.0	22.0	23.7	21.5	22.6	21.6	22.4	21.5	24.5	21.4	24.1	21.4	23.9	21.0	22.7	N.M	N.M

N.M- not measured due to 100% mortality in treatment

Attachment C - Metal and major ion analyses of QA/QC waters

		Sample ID	
Elements	Units	**1317D Pr Blk**	**1317D Blk**
Aluminium-Total	µg L^{-1}	0.26	0.32
Cadmium-Total	µg L^{-1}	<0.02	<0.02
Cobalt-Total	µg L^{-1}	<0.01	<0.01
Chromium-Total	µg L^{-1}	<0.1	<0.1
Copper-Total	µg L^{-1}	<0.01	0.025
Iron-Total	µg L^{-1}	<1	<1
Manganese-Total	µg L^{-1}	<0.01	<0.01
Nickel-Total	µg L^{-1}	0.047	0.047
Lead-Total	µg L^{-1}	0.082	<0.01
Selenium-Total	µg L^{-1}	<0.2	<0.2
Uranium-Total	µg L^{-1}	0.45	0.11
Zinc-Total	µg L^{-1}	<0.1	<0.1
Calcium - Total	mg L^{-1}	<0.1	<0.1
Magnesium - Total	mg L^{-1}	<0.1	<0.1
Sodium - Total	mg L^{-1}	<0.1	<0.1
Sulphate, SO4	mg L^{-1}	<0.5	<0.5

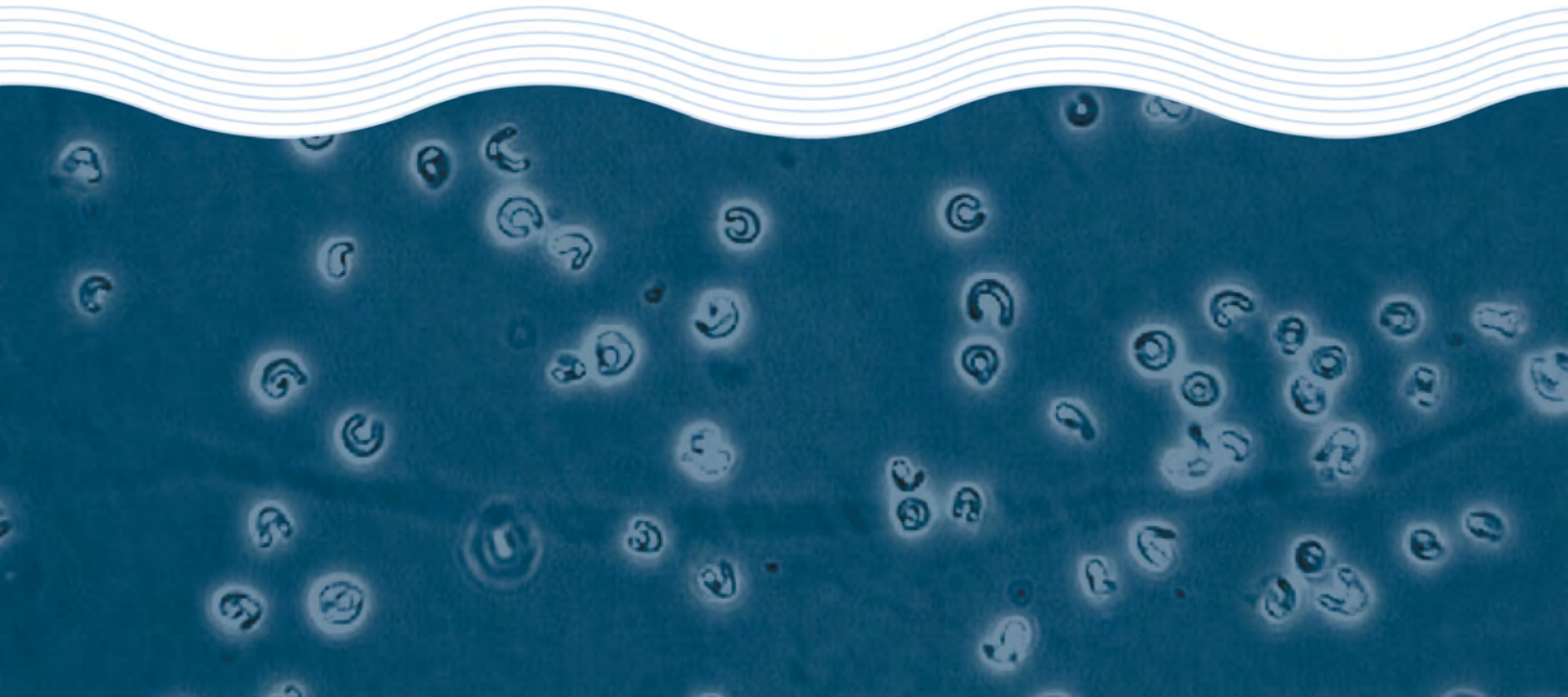
ecotox
SERVICES AUSTRALASIA

Toxicity Assessment of a Treated Water Sample

Vista Gold Australia Pty Ltd

Test Report

January 2013



Toxicity Assessment of a Treated Water Sample

Vista Gold Australia Pty Ltd

Test Report

January 2013

ECOTOX Services Australasia Pty Ltd ABN>45 094 714 904
unit 27/2 chaplin drive lane cove nsw 2066 T>61 2 9420 9481 F>61 2 9420 9484 W>www.ecotox.com.au

Toxicity Test Report: TR0989/1

Client:	Vista Gold Pty Ltd PO Box 1616 Katherine NT 0851	**ESA Job #:** **Date Sampled:** **Date Received:**	PR0989 Not supplied 16 January 2013
Attention:	Andrew Sawicki	**Sampled By:**	Client
Client Ref:	Not supplied	**ESA Quote #:**	PL0989_q01

Lab ID No.:	Sample Name:	Sample Description:
5822	SW2	Aqueous sample, pH 7.0, conductivity 23.2µS/cm, total ammonia <2.0mg/L*. Sample received at room temperature in apparent good condition.
5823	RP3	Aqueous sample, pH 6.4, conductivity 2590µS/cm, total ammonia <2.0mg/L*. Sample received at room temperature in apparent good condition.

*Ammonia analysis is not covered by Ecotox Services Australasia's scope of accreditation

Test Performed:	48-hr acute (survival) toxicity test using the freshwater chironomid *Chironomus tepperi*
Test Protocol:	ESA SOP 121 (ESA 2012), based on OECD (2011) USEPA (2002) and Bailey *et al*. (2000)
Test Temperature:	The test was performed at 25±1°C.
Deviations from Protocol:	Nil
Comments on Solution Preparation:	The sample was serially diluted with Sample 5822 'SW2' to achieve the test concentrations. A DMW and diluent control (SW2) were tested concurrently with the sample.
Source of Test Organisms:	ESA Laboratory culture
Age of Test Organisms:	8-14 days
Test Initiated:	29 January 2013 at 1430h

Sample 5823: *RP3*		*Vacant*	*Vacant*
Concentration (%)	**% Survival (Mean ± SD)**		
DMW Control	95.0 ± 10.0		
Diluent Control	90.0 ± 11.6		
6.3	100 ± 0.0		
12.5	10.0 ± 11.6 *		
25	0.0 ± 0.0		
50	0.0 ± 0.0		
100	0.0 ± 0.0		
48-hr IC10 = 7.4 (6.4-10.6)% **48-hr EC50 = 9.5 (8.7-10.5)%** **NOEC = 6.3%** **LOEC = 12.5%**			

*Significantly lower percent survival compared with the Diluent Control (Steel's Many-One Rank Test, 1-tailed, P=0.05)

Toxicity Test Report: TR0989/1

QA/QC Parameter	Criterion	This Test	Criterion met?
Control mean % survival	≥85.0%	95.0%	Yes
Reference Toxicant within cusum chart limits	16.5-3474.6 µg Cu/L	268.9µg Cu/L	Yes

* Cusum chart limits are not available due to limited testing with *C. tepperi*

Test Report Authorised by: Dr Rick Krassoi, Director on 22 February 2013

NATA Accredited Laboratory Number: 14709

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Citations:

Bailey, H.C., Krassoi, R., Elphick, J.R., Mulhall, A., Hunt, 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* 19:88-93.

ESA (2012) *SOP 121 – Acute toxicity test using Chironomus tepperi*. Issue No. 1. Ecotox Services Australasia, Sydney, New South Wales.

OECD (2011) OECD Guideline for the Testing of Chemicals. Test Guideline 235: *Chironomus sp*, Acute Immobilisation Test.

USEPA (2002) *Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms. 4th Ed.* United States Environmental Protection Agency, Office of Water, Washington DC.

Toxicity Test Report: TR0989/2

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Client:	Vista Gold Pty Ltd PO Box 1616 Katherine NT 0851	**ESA Job #:** **Date Sampled:** **Date Received:**	PR0989 Not supplied 16 January 2013
Attention:	Andrew Sawicki	**Sampled By:**	Client
Client Ref:	Not supplied	**ESA Quote #:**	PL0989_q01

Lab ID No.:	Sample Name:	Sample Description:
5822	SW2	Aqueous sample, pH 7.0, conductivity 23.2µS/cm, total ammonia <2.0mg/L*. Sample received at room temperature in apparent good condition.
5823	RP3	Aqueous sample, pH 6.4, conductivity 2590µS/cm, total ammonia <2.0mg/L*. Sample received at room temperature in apparent good condition.

*Ammonia analysis is not covered by Ecotox Services Australasia's scope of accreditation

Test Performed:	96-hr Growth inhibition of the freshwater aquatic duckweed *Lemna aequinoctialis*
Test Protocol:	ESA SOP 112 (ESA 2011), based on OECD method 221 (2006)
Test Temperature:	The test was performed at 29±2°C.
Deviations from Protocol:	Nil
Comments on Solution Preparation:	The highest concentration was prepared by diluting Sample 5823 'RP3' with Sample 5822 'SW2', then serially diluting to achieve the test concentrations. A DMW and diluent control (SW2) were tested concurrently with the sample.
Source of Test Organisms:	ESA Laboratory culture
Test Initiated:	17 January 2013 at 1230h

Sample 5823: *RP3*		*Vacant*	*Vacant*
Concentration (%)	**Specific Growth Rate (Mean ± SD)**		
CAAC Control	0.24 ± 0.02		
Diluent Control	0.26 ± 0.03		
0.1	0.26 ± 0.03		
0.2	0.25 ± 0.02		
0.4	0.22 ± 0.01*		
0.8	0.00 ± 0.00		
1.5	0.00 ± 0.00		
3.0	0.00 ± 0.00		
96-hr IC10 = 0.3 (0.1-0.4)% **96-hr IC50 = 0.6 (0.5-0.6)%** **NOEC = 0.2%** **LOEC = 0.4%**			

*Significantly lower specific growth rate compared with the Diluent Control (Dunnett's Test, 1-tailed, P=0.05)

Toxicity Test Report: TR0989/2

QA/QC Parameter	Criterion	This Test	Criterion met?
Control frond doubling time	<3.0 days	2.9 days	Yes
Reference Toxicant within cusum chart limits	7.8-62.4mg $MgSO_4$/L	13.7mg $MgSO_4$/L	Yes

Test Report Authorised by: Dr Rick Krassoi, Director on 22 February 2013

Results are based on the samples in the condition as received by ESA.

NATA Accredited Laboratory Number: 14709

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Citations:

ESA (2011) *SOP 112 – Duckweed Growth Inhibition Test.* Issue No. 3. Ecotox Services Australasia, Sydney NSW

OECD (2006) *Lemna sp*. Growth Inhibition Test. Method 221. OECD Guideline for the Testing of Chemicals. Organisation for Economic Cooperation and Development, Paris

Toxicity Test Report: TR0989/3

Client:	Vista Gold Pty Ltd PO Box 1616 Katherine NT 0851 Andrew Sawicki	**ESA Job #:** **Date Sampled:** **Date Received:** **Sampled By:**	PR0989 Not supplied 16 January 2013 Client
Attention:	Not supplied	**ESA Quote #:**	PL0989_q01

Lab ID No.:	Sample Name:	Sample Description:
5822	SW2	Aqueous sample, pH 7.0, conductivity 23.2µS/cm, total ammonia <2.0mg/L*. Sample received at room temperature in apparent good condition.
5823	RP3	Aqueous sample, pH 6.4, conductivity 2590µS/cm, total ammonia <2.0mg/L*. Sample received at room temperature in apparent good condition.

*Ammonia analysis is not covered by Ecotox Services Australasia's scope of accreditation

Test Performed:	96-hr acute survival test using the freshwater shrimp *Macrobrachium bullatum*
Test Protocol:	ESA SOP 123 (ESA 2012), based on USEPA (1996)
Test Temperature:	The test was performed at 25±1°C.
Deviations from Protocol:	Nil
Comments on Solution Preparation:	The highest concentration was prepared by diluting Sample 5823 'RP3' with Sample 5822 'SW2', then serially diluting to achieve the test concentrations. A DMW and diluent control were tested concurrently with the sample.
Source of Test Organisms:	Hatchery reared, NT
Test Initiated:	25 January 2013 at 1600h

Sample 5823: *RP3*		*Vacant*	*Vacant*
Concentration (%)	**% Un-affected (Mean ± SD)**		
DMW Control	90.0 ± 11.6		
Diluent Control	95.0 ± 10.0		
0.3	95.0 ± 10.0		
0.6	70.0 ± 25.8		
1.3	40.0 ± 36.5		
2.5	0.0 ± 0.0		
5.0	0.0 ± 0.0		
10.0	0.0 ± 0.0		
96-hr EC10 = 0.5%**			
96-hr EC50 = 1.0 (0.7-1.2)%			
NOEC = 0.6%			
LOEC = 1.3%			

*Significantly lower percentage of un-affected shrimp compared with the Diluent Control (Dunnett's Test, 1-tailed, P=0.05)
**95% confidence limits not reliable

QA/QC Parameter	Criterion	This Test	Criterion met?
Control mean % un-affected	≥90.0%	90.0%	Yes
Reference Toxicant within cusum chart limits	30.1-287.8µg Cu/L	54.8 µg Cu/L	Yes

Toxicity Test Report: TR0989/3

Test Report Authorised by: Dr Rick Krassoi, Director on 22 February 2013

Results are based on the samples in the condition as received by ESA

NATA Accredited Laboratory Number: 14709

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Citations:

ESA (2012) SOP 123 –*Acute Toxicity Test Using Freshwater Shrimp.* Issue No 1. Ecotox Services Australasia, Sydney, NSW

USEPA (1996) Ecological Effects Test Guidelines: OPPTS 850.1035 Mysid Acute Toxicity Test. Public Draft. United States Environmental Protection Agency, Washington DC, USA.

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Toxicity Test Report: TR0989/4

This document is issued in accordance with NATA's accreditation requirements

Client:	Vista Gold Pty Ltd PO Box 1616 Katherine NT 0851	**ESA Job #:** **Date Sampled:** **Date Received:**	PR0989 Not supplied 16 January 2013
Attention:	Andrew Sawicki	**Sampled By:**	Client
Client Ref:	Not supplied	**ESA Quote #:**	PL0989_q01

Lab ID No.:	Sample Name:	Sample Description:
5822	SW2	Aqueous sample, pH 7.0, conductivity 23.2µS/cm, total ammonia <2.0mg/L*. Sample received at room temperature in apparent good condition.
5823	RP3	Aqueous sample, pH 6.4, conductivity 2590µS/cm, total ammonia <2.0mg/L*. Sample received at room temperature in apparent good condition.

*Ammonia analysis is not covered by Ecotox Services Australasia's scope of accreditation

Test Performed:	72-hr microalgal growth inhibition test using the green alga *Chlorella vulgaris*
Test Protocol:	ESA SOP 103 (ESA 2011), based on USEPA (2002)
Test Temperature:	The test was performed at 25±1°C.
Deviations from Protocol:	Nil
Comments on Solution Preparation:	The highest concentration was prepared by diluting Sample 5823 'RP3' with Sample 5822 'SW2', then serially diluting to achieve the test concentrations. Sample 5822 'SW2' was filtered to 0.45µm prior to use. A DMW and diluent control (SW2) were tested concurrently with the sample.
Source of Test Organisms:	ESA Laboratory culture, originally sourced from CSIRO Microalgal Supply Service, TAS
Test Initiated:	18 January 2013 at 1640h

Sample 5823: *RP3*		*Vacant*	*Vacant*
Concentration (%)	**Cell Yield x10^4 cells/mL (Mean ± SD)**		
USEPA Control	305.0 ± 58.0		
Diluent Control	325.3 ± 20.3		
0.1	325.5 ± 31.1		
0.2	272.5 ± 47.0		
0.4	92.0 ± 19.3 *		
0.8	0.4 ± 0.8 *		
1.5	0.0 ± 0.0		
3.0	0.0 ± 0.0		
72-hr IC10 = 0.2 (0.1-0.3)% **72-hr IC50 =0.3 (0.3-0.4)%** **NOEC = 0.2%** **LOEC = 0.4%**			

*Significantly lower cell yield compared with the Diluent Control (Steel's Many-One Rank Test, 1-tailed, P=0.05)

Toxicity Test Report: TR0989/4

QA/QC Parameter	Criterion	This Test	Criterion met?
Control mean cell density	≥16.0x10^4 cells/mL	305.0x10^4 cells/mL	Yes
Control coefficient of variation	<20%	19.0%	Yes
Reference Toxicant within cusum chart limits	N/A*	2.0g KCl/L	N/A

*Cusum chart not available due to limited testing with this species

Test Report Authorised by: Dr Rick Krassoi, Director on 22 February 2013

Results are based on the samples in the condition as received by ESA.

Citations:

ESA (2011) *ESA SOP 103 – Green Alga, Selenastrum capricornutum, Growth Test*. Issue No 9. Ecotox Services Australasia, Sydney, NSW.

USEPA (2002) *Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms*. Fourth Edition. EPA-821-R-02-013. United States Environmental Protection Agency, Office of Research and Development, Washington DC, USA,

Toxicity Test Report: TR0989/5

This document is issued in accordance with NATA's accreditation requirements

Client:	Vista Gold Pty Ltd PO Box 1616 Katherine NT 0851	**ESA Job #:** **Date Sampled:** **Date Received:**	PR0989 Not supplied 16 January 2013
Attention:	Andrew Sawicki	**Sampled By:**	Client
Client Ref:	Not supplied	**ESA Quote #:**	PL0989_q01

Lab ID No.:	Sample Name:	Sample Description:
5822	SW2	Aqueous sample, pH 7.0, conductivity 23.2µS/cm, total ammonia <2.0mg/L*. Sample received at room temperature in apparent good condition.
5823	RP3	Aqueous sample, pH 6.4, conductivity 2590µS/cm, total ammonia <2.0mg/L*. Sample received at room temperature in apparent good condition.

*Ammonia analysis is not covered by Ecotox Services Australasia's scope of accreditation

Test Performed:	96-hr fish imbalance toxicity test using the eastern rainbowfish *Melanotaenia splendida splendida*
Test Protocol:	ESA SOP 117 (ESA 2011), based on USEPA (2002)
Test Temperature:	The test was performed at 25±1°C.
Deviations from Protocol:	Three replicates were tested for the control treatment rather than four
Comments on Solution Preparation:	The highest concentration was prepared by diluting sample 5823 'RP3' with sample 5822 'SW2'. This concentration was then serially diluted with Sample 5822 'SW2' to achieve the test concentrations. A DMW control and a diluent control (SW2) were tested concurrently with the sample.
Source of Test Organisms:	In-house cultures
Test Initiated:	31 January 2013 at 1530h

Sample 5823: *RP3*

Concentration (%)	% Un-affected (Mean ± SD)
DMW Control	100 ± 0.0
Dilunt Control	80.0 ± 20.0
0.06	85.0 ± 10.0
0.13	50.0 ± 11.6
0.25	25.0 ± 25.2 *
0.50	0.0 ± 0.0
1.00	0.0 ± 0.0

96-hr IC10 = 0.08%**
96-hr EC50 = 0.17 (0.13-0.20)%
NOEC = 0.13%
LOEC = 0.25%

*Significantly lower percentage of un-affected larval fish compared with the DMW Control (Bonferroni t Test, 1-tailed, P=0.05)
**95% confidence limits not reliable

QA/QC Parameter	Criterion	This Test	Criterion met?
Control mean % un-affected	≥80.0%	100%	Yes
Reference Toxicant within cusum chart limits	4.8-74.1µg Cu/L	12.7µg Cu/L	Yes

Toxicity Test Report: TR0989/5

Test Report Authorised by: Dr Rick Krassoi, Director on 22 February 2013

Results are based on the samples in the condition as received by ESA.

NATA Accredited Laboratory Number: 14709

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Citations:

ESA (2011) SOP 117 –*Freshwater and Marine Fish Imbalance Test*. Issue No 7. Ecotox Services Australasia, Sydney, NSW

USEPA (2002) Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms. Fifth edition EPA-821-R-02-012. United States Environmental Protection Agency, Office of Research and Development, Washington FC, USA

Toxicity Test Report: TR0989/6

This document is issued in accordance with NATA's accreditation requirements

Client:	Vista Gold Pty Ltd PO Box 1616 Katherine NT 0851	**ESA Job #:** **Date Sampled:** **Date Received:**	PR0989 Not supplied 16 January 2013
Attention:	Andrew Sawicki	**Sampled By:**	Client
Client Ref:	Not supplied	**ESA Quote #:**	PL0989_q01

Lab ID No.:	Sample Name:	Sample Description:
5822	SW2	Aqueous sample, pH 7.0, conductivity 23.2µS/cm, total ammonia <2.0mg/L*. Sample received at room temperature in apparent good condition.
5823	RP3	Aqueous sample, pH 6.4, conductivity 2590µS/cm, total ammonia <2.0mg/L*. Sample received at room temperature in apparent good condition.

*Ammonia analysis is not covered by Ecotox Services Australasia's scope of accreditation

Test Performed:	96-hr acute toxicity test using the freshwater hydra *hydra viridissima*
Test Protocol:	ESA SOP 125 (2012), based on Riethmuller et al. (2003)
Test Temperature:	The test was performed at 27±1°C.
Deviations from Protocol:	Nil
Comments on Solution Preparation:	The highest concentration was prepared by diluting sample 5823 'RP3' with sample 5822 'SW2'. This concentration was then serially diluted with Sample 5822 'SW2' to achieve the test concentrations. A laboratory water control and a diluent control (SW2) were tested concurrently with the sample.
Source of Test Organisms:	ESA Laboratory culture
Test Initiated:	4 February 2013 at 1330h

Sample 5823: *RP3*					*Vacant*
Concentration (%)	**Population Growth Rate (Mean ± SD)**				
Laboratory Water Control	0.30	±	0.03		
Diluent Control	0.30	±	0.03		
0.06	0.30	±	0.03		
0.13	0.30	±	0.03		
0.25	0.29	±	0.03		
0.50	0.07	±	0.07	*	
1.00	0.0	±	0.0		
2.00	0.0	±	0.0		
96-hr IC10 = 0.27 (0.06-0.30)% **96-hr IC50 = 0.42 (0.35-0.49)%** **NOEC = 0.25%** **LOEC = 0.5%**					

*Significantly lower population growth rate compared with the Diluent Control (Dunnett's Test, 1-tailed, P=0.05)

QA/QC Parameter	Criterion	This Test	Criterion met?
Control mean population growth rate	≥0.25	0.30	Yes
Reference Toxicant within cusum chart limits	1.3-15.9 µg Cu/L	2.8µg Cu/L	Yes

Toxicity Test Report: TR0989/6

Test Report Authorised by: Dr Rick Krassoi, Director on 22 February 2013

Results are based on the samples in the condition as received by ESA.

NATA Accredited Laboratory Number: 14709

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Citations:

ESA (2012) SOP 125 –*Hydra Population Growt Test.* Issue No 1. Ecotox Services Australasia, Sydney, NSW

Riethmuller N, Camilleri C, Franklin N, Hogan A, King A, Koch A, Markich SJ, Turley C and van Dam R (2003). Green Hydra Population Growth Test. In: *Ecotoxicological testing protocols for Australian tropical freshwater ecosystems*. Supervising Scientist Report 173, Supervising Scientist, Darwin NT.

Toxicity Test Report: TR0989/7

Client:	Vista Gold Pty Ltd PO Box 1616 Katherine NT 0851	**ESA Job #:** **Date Sampled:** **Date Received:**	PR0989 Not supplied 16 January 2013
Attention:	Andrew Sawicki	**Sampled By:**	Client
Client Ref:	Not supplied	**ESA Quote #:**	PL0989_q01

Lab ID No.:	Sample Name:	Sample Description:
5822	SW2	Aqueous sample, pH 7.0, conductivity 23.2µS/cm, total ammonia <2.0mg/L*. Sample received at room temperature in apparent good condition.
5823	RP3	Aqueous sample, pH 6.4, conductivity 2590µS/cm, total ammonia <2.0mg/L*. Sample received at room temperature in apparent good condition.

*Ammonia analysis is not covered by Ecotox Services Australasia's scope of accreditation

Test Performed:	Rainbowfish embryo hatching test using *Melanotaenia splendida splendida*
Test Protocol:	ESA SOP 126 (2012), based on USEPA (2002), but adapted for use with native rainbowfish
Test Temperature:	The test was performed at 25±1°C.
Deviations from Protocol:	Test solutions were renewed every 48 hours instead of 24 hours
Comments on Solution Preparation:	The highest concentration was prepared by diluting sample 5823 'RP3' with sample 5822 'SW2'. This concentration was then serially diluted with Sample 5822 'SW2' to achieve the test concentrations. A DMW control and a diluent control (SW2) were tested concurrently with the sample.
Source of Test Organisms:	ESA Laboratory culture
Test Initiated:	6 February 2013 at 1515 h

Sample 5823: *RP3*

Concentration (%)	% Un-Affected (Mean ± SD)	
DMW Control	100 ± 0.0	
Diluent Control	90.0 ± 11.6	
0.03	100 ± 0.0	
0.06	100 ± 0.0	
0.13	100 ± 0.0	
0.25	90.0 ± 20.0	
0.50	55.0 ± 19.2	
1.00	15.0 ± 10.0	*

10 day EC10 = 0.28 (0.14-0.37)%
10 day EC50 = 0.56 (0.43-0.71)%
NOEC = 0.5%
LOEC = 1.0%

Vacant

*Significantly lower percentage of un-affected fish compared with the diluent control (Steel's Many-One Rank Test, 1-tailed, P=0.05)

Toxicity Test Report: TR0989/7

QA/QC Parameter	Criterion	This Test	Criterion met?
Control mean % survival	≥80.0%	100%	Yes
Reference Toxicant within cusum chart limit	20.7-401.3µg Cu/L	78.9µg Cu/L	Yes

Test Report Authorised by: Dr Rick Krassoi, Director on 22 February 2013

Results are based on the samples in the condition as received by ESA.

NATA Accredited Laboratory Number: 14709

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Citations:

ESA (2012) *SOP 126- Rainbowfish Embryo Hatching Test*. Issue N°2. Ecotox Services Australasia, Sydney NSW

USEPA (2002) *Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms.4th Ed.* United States Environmental Protection Agency, Office of Water, Washington DC.

Chain-of-Custody Documentation

Datasheet ID: 601.2
Last Revised: 30 April 2009

Sample Receipt Notification

Attention : Andrew Sawicki

Client : Vista Gold Pty Ltd
PO Box 1616
Katherine NT 0851

Email : asawicki@vistagoldaustralia.com.au
Telephone :
Facsimile :

Date : 16/01/2013

Re : Receipt of Sample **Pages :** **2**

ESA Project : PR0989 ☑ For Review ☐ Additional Documentation Required - Please Respond

Sample Delivery Details

Completed Chain of Custody accompanied samples: **NO - Documentation Required**
Samples received in apparent good condition and correctly bottled: **YES**
Security seals on sample bottles and esky intact: **YES**

Date samples received : 16/01/2013
Time samples received : 10:00
No. of samples received : 1
Sample matrix : aqueous
Sample temperature : room temperature

Comments : Includes 2 x 30L SW2 (ESA ID# 5822) and 1 x 30L RP3 (ESA ID# 5823)

Contact Details

Customer Services Officer : Tina Micevska
Telephone : 61 2 9420 9481
Facsimile : 61 2 9420 9484
Email : tmicevska@ecotox.com.au

Please contact customer services officer for all queries or issues regarding samples

Note that the chain-of-custody provides definitive information on the tests to be performed

Ecotox Services Australia
ABN 45 094 714 904
Unit 27, 2 Chaplin Drive
Lane Cove NSW 2066 Australia

Phone : 61 2 9420 9481
Fax : 61 2 9420 9484
Email : info@ecotox.com.au

ecotox
SERVICES AUSTRALASIA

Statistical Printouts for the Acute Test with *Chironomus tepperi*

Chironomid Acute Toxicity Test-48hr Survival

Start Date:	29/01/2013 14:30	Test ID:	PR0989/02	Sample ID:	SW2
End Date:	31/01/2013 13:00	Lab ID:	5823	Sample Type:	Diluent Control
Sample Date:		Protocol:	ESA 121	Test Species:	CT-Chironomus tepperi
Comments:					

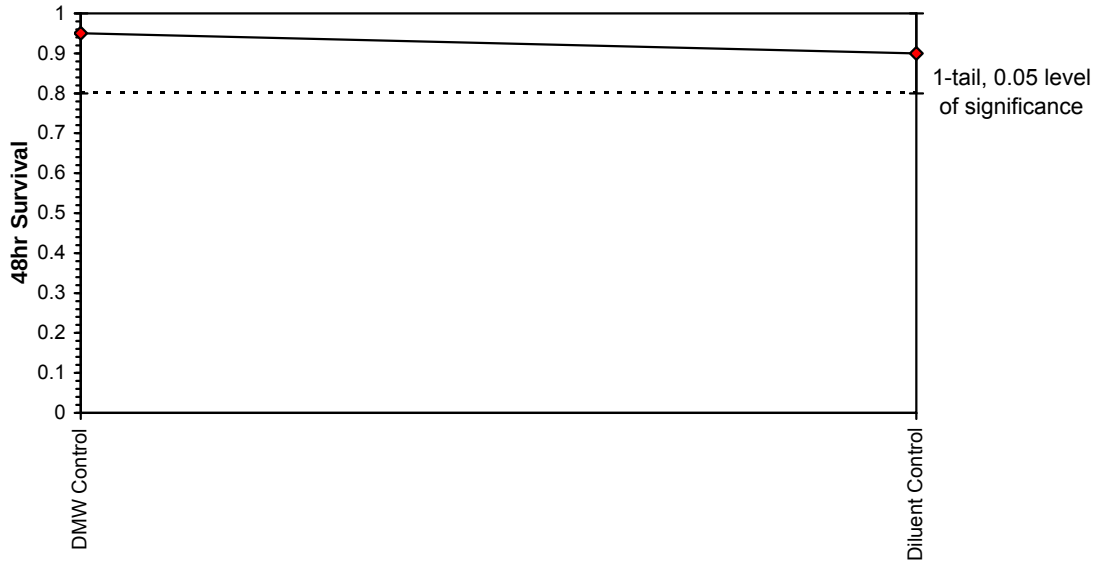
Conc-	1	2	3	4
DMW Control	0.8000	1.0000	1.0000	1.0000
Diluent Control	0.8000	0.8000	1.0000	1.0000

			Transform: Arcsin Square Root						1-Tailed	
Conc-	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD
DMW Control	0.9500	1.0000	1.2857	1.1071	1.3453	9.261	4			
Diluent Control	0.9000	0.9474	1.2262	1.1071	1.3453	11.212	4	0.655	1.943	0.1767

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.05)	0.827836	0.818	-0.57143	-1.72857
F-Test indicates equal variances (p = 0.82)	1.333333	47.46723		

Hypothesis Test (1-tail, 0.05)	MSDu	MSDp	MSB	MSE	F-Prob	df
Homoscedastic t Test indicates no significant differences Treatments vs DMW Control	0.119415	0.129669	0.007088	0.01654	0.536963	1, 6

Dose-Response Plot



 ToxCalc v5.0.23 Reviewed by:_____

Chironomid Acute Toxicity Test-48hr Survival

Start Date:	29/01/2013 14:30	Test ID:	PR0989/02	Sample ID:	SW2
End Date:	31/01/2013 13:00	Lab ID:	5823	Sample Type:	Diluent Control
Sample Date:		Protocol:	ESA 121	Test Species:	CT-Chironomus tepperi
Comments:					

Auxiliary Data Summary

Conc-	Parameter	Mean	Min	Max	SD	CV%	N
DMW Control	% Survival	95.00	80.00	100.00	10.00	3.33	4
Diluent Control		90.00	80.00	100.00	11.55	3.78	4
DMW Control	pH	8.10	8.10	8.10	0.00	0.00	1
Diluent Control		7.50	7.50	7.50	0.00	0.00	1
DMW Control	Cond uS/cm	297.00	297.00	297.00	0.00	0.00	1
Diluent Control		69.40	69.40	69.40	0.00	0.00	1
DMW Control	DO %	97.70	97.70	97.70	0.00	0.00	1
Diluent Control		93.10	93.10	93.10	0.00	0.00	1

Chironomid Acute Toxicity Test-48hr Survival

Start Date:	29/01/2013 14:30	Test ID:	PR0989/03	Sample ID:	RP3
End Date:	31/01/2013 13:00	Lab ID:	5823	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 121	Test Species:	CT-Chironomus tepperi
Comments:					

Conc-%	1	2	3	4
DMW Control	0.8000	1.0000	1.0000	1.0000
Diluent Control	0.8000	0.8000	1.0000	1.0000
6.3	1.0000	1.0000	1.0000	1.0000
12.5	0.2000	0.0000	0.2000	0.0000
25	0.0000	0.0000	0.0000	0.0000
50	0.0000	0.0000	0.0000	0.0000
100	0.0000	0.0000	0.0000	0.0000

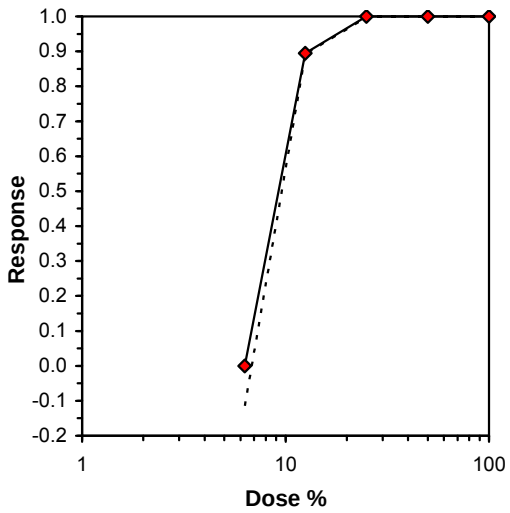
			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
DMW Control	0.9500	1.0556	1.2857	1.1071	1.3453	9.261	4				
Diluent Control	0.9000	1.0000	1.2262	1.1071	1.3453	11.212	4	*		2	20
6.3	1.0000	1.1111	1.3453	1.3453	1.3453	0.000	4	22.00	11.00	0	20
*12.5	0.1000	0.1111	0.3446	0.2255	0.4636	39.900	4	10.00	11.00	18	20
25	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	4			20	20
50	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	4			20	20
100	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	4			20	20

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.05)	0.810646	0.859	3.39E-16	-1.65
Equality of variance cannot be confirmed				
The control means are not significantly different (p = 0.54)	0.654654	2.446912		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	6.3	12.5	8.87412	15.87302
Treatments vs Diluent Control				

Trimmed Spearman-Karber

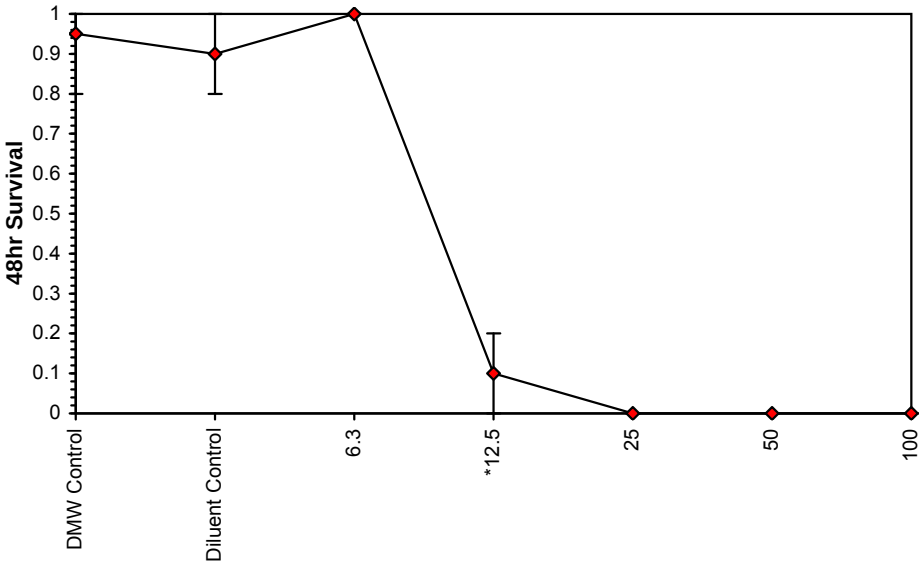
Trim Level	EC50	95% CL	
0.0%	9.5418	8.6807	10.4884
5.0%	9.3308	8.4993	10.2436
10.0%	9.2400	8.6559	9.8636
20.0%	9.2391	8.7121	9.7980
Auto-0.0%	9.5418	8.6807	10.4884



Chironomid Acute Toxicity Test-48hr Survival

Start Date:	29/01/2013 14:30	Test ID:	PR0989/03	Sample ID:	RP3
End Date:	31/01/2013 13:00	Lab ID:	5823	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 121	Test Species:	CT-Chironomus tepperi
Comments:					

Dose-Response Plot



ToxCalc v5.0.23

Reviewed by:_____

Chironomid Acute Toxicity Test-48hr Survival

Start Date:	29/01/2013 14:30	Test ID:	PR0989/03	Sample ID:	RP3
End Date:	31/01/2013 13:00	Lab ID:	5823	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 121	Test Species:	CT-Chironomus tepperi
Comments:					

		Auxiliary Data Summary					
Conc-%	**Parameter**	**Mean**	**Min**	**Max**	**SD**	**CV%**	**N**
DMW Control	% Survival	95.00	80.00	100.00	10.00	3.33	4
Diluent Control		90.00	80.00	100.00	11.55	3.78	4
6.3		100.00	100.00	100.00	0.00	0.00	4
12.5		10.00	0.00	20.00	11.55	33.98	4
25		0.00	0.00	0.00	0.00		4
50		0.00	0.00	0.00	0.00		4
100		0.00	0.00	0.00	0.00		4
DMW Control	pH	8.10	8.10	8.10	0.00	0.00	1
Diluent Control		7.50	7.50	7.50	0.00	0.00	1
6.3		6.70	6.70	6.70	0.00	0.00	1
12.5		6.50	6.50	6.50	0.00	0.00	1
25		6.40	6.40	6.40	0.00	0.00	1
50		6.40	6.40	6.40	0.00	0.00	1
100		6.40	6.40	6.40	0.00	0.00	1
DMW Control	Cond uS/cm	297.00	297.00	297.00	0.00	0.00	1
Diluent Control		69.40	69.40	69.40	0.00	0.00	1
6.3		314.00	314.00	314.00	0.00	0.00	1
12.5		527.00	527.00	527.00	0.00	0.00	1
25		873.00	873.00	873.00	0.00	0.00	1
50		1471.00	1471.00	1471.00	0.00	0.00	1
100		2380.00	2380.00	2380.00	0.00	0.00	1
DMW Control	DO %	97.70	97.70	97.70	0.00	0.00	1
Diluent Control		93.10	93.10	93.10	0.00	0.00	1
6.3		95.60	95.60	95.60	0.00	0.00	1
12.5		95.00	95.00	95.00	0.00	0.00	1
25		94.60	94.60	94.60	0.00	0.00	1
50		94.30	94.30	94.30	0.00	0.00	1
100		92.10	92.10	92.10	0.00	0.00	1

Chironomid Acute Toxicity Test-48hr Survival

Start Date:	29/01/2013 14:30	Test ID:	PR0989/03	Sample ID:	RP3
End Date:	31/01/2013 13:00	Lab ID:	5823	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 121	Test Species:	CT-Chironomus tepperi
Comments:					

Conc-%	1	2	3	4
DMW Control	0.8000	1.0000	1.0000	1.0000
Diluent Control	0.8000	0.8000	1.0000	1.0000
6.3	1.0000	1.0000	1.0000	1.0000
12.5	0.2000	0.0000	0.2000	0.0000
25	0.0000	0.0000	0.0000	0.0000
50	0.0000	0.0000	0.0000	0.0000
100	0.0000	0.0000	0.0000	0.0000

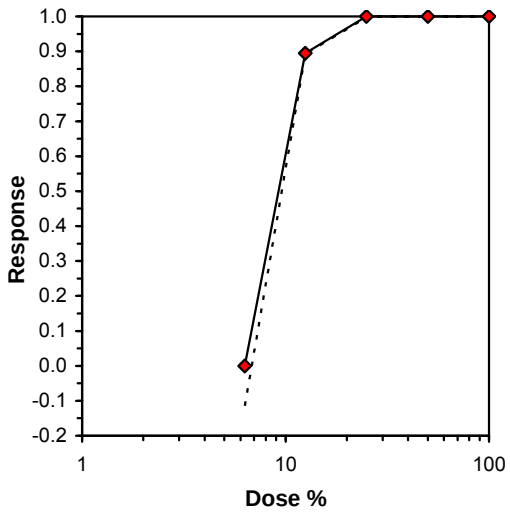
			Transform: Arcsin Square Root					Rank	1-Tailed	Isotonic	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Mean	N-Mean
DMW Control	0.9500	1.0556	1.2857	1.1071	1.3453	9.261	4				
Diluent Control	0.9000	1.0000	1.2262	1.1071	1.3453	11.212	4	*		0.9500	1.0000
6.3	1.0000	1.1111	1.3453	1.3453	1.3453	0.000	4	22.00	11.00	0.9500	1.0000
*12.5	0.1000	0.1111	0.3446	0.2255	0.4636	39.900	4	10.00	11.00	0.1000	0.1053
25	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	4			0.0000	0.0000
50	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	4			0.0000	0.0000
100	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	4			0.0000	0.0000

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.05)	0.810646	0.859	3.39E-16	-1.65
Equality of variance cannot be confirmed				
The control means are not significantly different (p = 0.54)	0.654654	2.446912		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	6.3	12.5	8.87412	15.87302
Treatments vs Diluent Control				

Log-Logit Interpolation (200 Resamples)

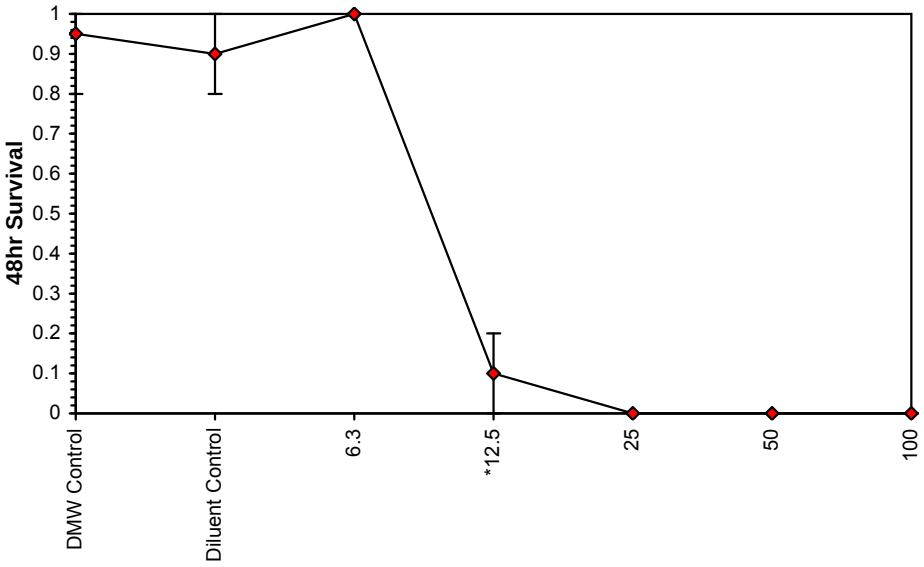
Point	%	SD	95% CL(Exp)		Skew
IC05	6.9555	0.4893	6.3368	10.2624	3.4147
IC10	7.3963	0.5225	6.3508	10.6156	2.6264
IC15	7.7452	0.5407	6.3563	10.8006	1.9757
IC20	8.0442	0.5558	6.3572	10.9242	1.3985
IC25	8.3132	0.5710	6.3551	11.0171	0.8767
IC40	9.0368	0.6271	6.3371	11.2112	-0.3875
IC50	9.5058	0.6786	6.3163	11.3076	-0.9815



Chironomid Acute Toxicity Test-48hr Survival

Start Date:	29/01/2013 14:30	Test ID:	PR0989/03	Sample ID:	RP3
End Date:	31/01/2013 13:00	Lab ID:	5823	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 121	Test Species:	CT-Chironomus tepperi
Comments:					

Dose-Response Plot



ToxCalc v5.0.23

Reviewed by:_____

Chironomid Acute Toxicity Test-48hr Survival

Start Date:	29/01/2013 14:30	Test ID:	PR0989/03	Sample ID:	RP3
End Date:	31/01/2013 13:00	Lab ID:	5823	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 121	Test Species:	CT-Chironomus tepperi
Comments:					

		Auxiliary Data Summary					
Conc-%	**Parameter**	**Mean**	**Min**	**Max**	**SD**	**CV%**	**N**
DMW Control	% Survival	95.00	80.00	100.00	10.00	3.33	4
Diluent Control		90.00	80.00	100.00	11.55	3.78	4
6.3		100.00	100.00	100.00	0.00	0.00	4
12.5		10.00	0.00	20.00	11.55	33.98	4
25		0.00	0.00	0.00	0.00		4
50		0.00	0.00	0.00	0.00		4
100		0.00	0.00	0.00	0.00		4
DMW Control	pH	8.10	8.10	8.10	0.00	0.00	1
Diluent Control		7.50	7.50	7.50	0.00	0.00	1
6.3		6.70	6.70	6.70	0.00	0.00	1
12.5		6.50	6.50	6.50	0.00	0.00	1
25		6.40	6.40	6.40	0.00	0.00	1
50		6.40	6.40	6.40	0.00	0.00	1
100		6.40	6.40	6.40	0.00	0.00	1
DMW Control	Cond uS/cm	297.00	297.00	297.00	0.00	0.00	1
Diluent Control		69.40	69.40	69.40	0.00	0.00	1
6.3		314.00	314.00	314.00	0.00	0.00	1
12.5		527.00	527.00	527.00	0.00	0.00	1
25		873.00	873.00	873.00	0.00	0.00	1
50		1471.00	1471.00	1471.00	0.00	0.00	1
100		2380.00	2380.00	2380.00	0.00	0.00	1
DMW Control	DO %	97.70	97.70	97.70	0.00	0.00	1
Diluent Control		93.10	93.10	93.10	0.00	0.00	1
6.3		95.60	95.60	95.60	0.00	0.00	1
12.5		95.00	95.00	95.00	0.00	0.00	1
25		94.60	94.60	94.60	0.00	0.00	1
50		94.30	94.30	94.30	0.00	0.00	1
100		92.10	92.10	92.10	0.00	0.00	1

Statistical Printouts for the Duckweed Growth Inhibition Tests

Duckweed Growth Inhibtion Test-Specific Growth Rate

Start Date:	17/01/2013 12:30	Test ID:	PR0989/05	Sample ID:	SW2
End Date:	21/01/2013 14:00	Lab ID:	5822	Sample Type:	Diluent Control
Sample Date:		Protocol:	ESA 112	Test Species:	LA-Lemna aequinoctialis
Comments:					

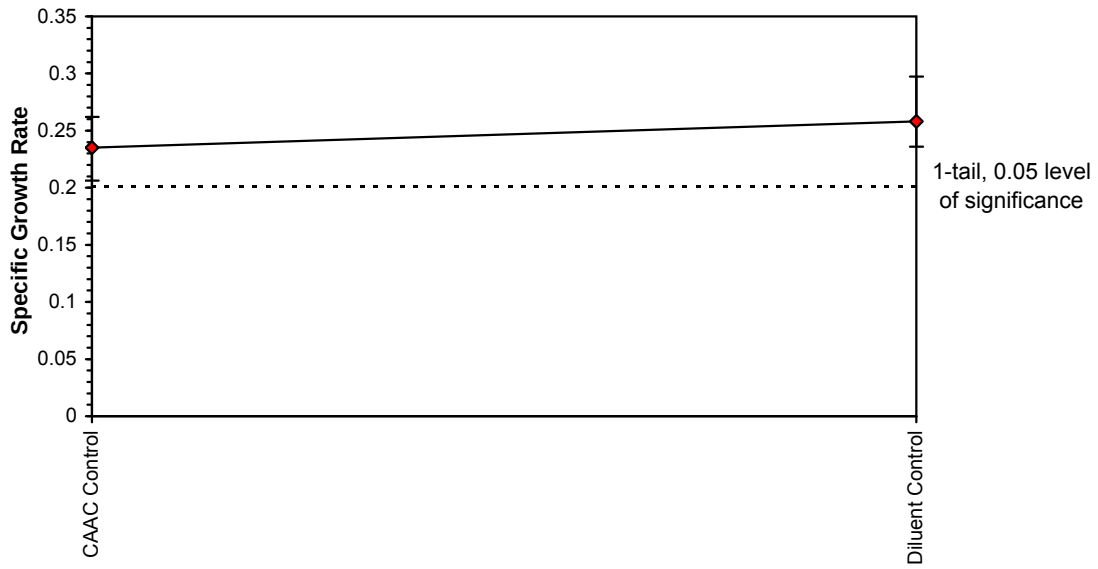
Conc-	1	2	3	4
CAAC Control	0.2361	0.2361	0.2067	0.2625
Diluent Control	0.2361	0.2974	0.2496	0.2496

			Transform: Untransformed						1-Tailed	
Conc-	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD
CAAC Control	0.2353	1.0000	0.2353	0.2067	0.2625	9.685	4			
Diluent Control	0.2582	1.0971	0.2582	0.2361	0.2974	10.419	4	-1.296	1.943	0.0343

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.05)	0.926865	0.818	0.6909	-0.27791
F-Test indicates equal variances (p = 0.79)	1.392974	47.46723		

Hypothesis Test (1-tail, 0.05)	MSDu	MSDp	MSB	MSE	F-Prob	df
Homoscedastic t Test indicates no significant differences Treatments vs CAAC Control	0.034256	0.14556	0.001045	0.000622	0.242447	1, 6

Dose-Response Plot



Duckweed Growth Inhibtion Test-Specific Growth Rate

Start Date:	17/01/2013 12:30	Test ID:	PR0989/05	Sample ID:	SW2
End Date:	21/01/2013 14:00	Lab ID:	5822	Sample Type:	Diluent Control
Sample Date:		Protocol:	ESA 112	Test Species:	LA-Lemna aequinoctialis
Comments:					

Auxiliary Data Summary

Conc-	Parameter	Mean	Min	Max	SD	CV%	N
CAAC Control	Specific Growth Rate	0.24	0.21	0.26	0.02	64.15	4
Diluent Control		0.26	0.24	0.30	0.03	63.52	4
CAAC Control	pH	6.00	6.00	6.00	0.00	0.00	1
Diluent Control		6.60	6.60	6.60	0.00	0.00	1
CAAC Control	Cond uS/cm	169.70	169.70	169.70	0.00	0.00	1
Diluent Control		51.90	78.10	78.10	0.00	0.00	1

Duckweed Growth Inhibtion Test-Specific Growth Rate

Start Date:	17/01/2013 12:30	Test ID:	PR0989/06	Sample ID:	RP3
End Date:	21/01/2013 14:00	Lab ID:	5823	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 112	Test Species:	LA-Lemna aequinoctialis
Comments:					

Conc-	1	2	3	4
CAAC Control	0.2361	0.2361	0.2067	0.2625
Diluent Control	0.2361	0.2974	0.2496	0.2496
0.1	0.2361	0.2496	0.2863	0.2863
0.2	0.2496	0.2218	0.2625	0.2747
0.4	0.2218	0.2218	0.2218	0.2067
0.8	0.0000	0.0000	0.0000	0.0000
1.5	0.0000	0.0000	0.0000	0.0000
3	0.0000	0.0000	0.0000	0.0000

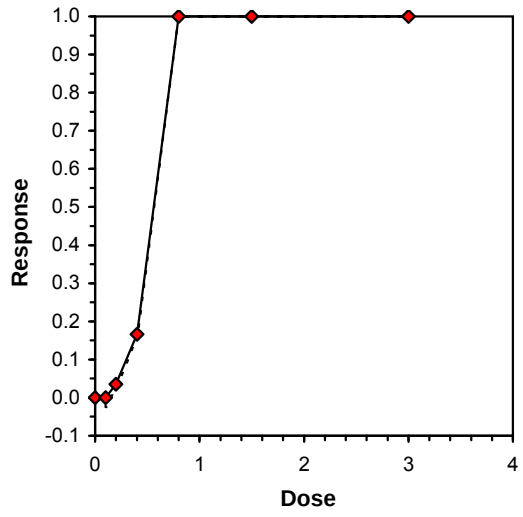
			Transform: Untransformed						1-Tailed		Isotonic	
Conc-	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
CAAC Control	0.2353	0.9115	0.2353	0.2067	0.2625	9.685	4					
Diluent Control	0.2582	1.0000	0.2582	0.2361	0.2974	10.419	4	*			0.2614	1.0000
0.1	0.2646	1.0247	0.2646	0.2361	0.2863	9.699	4	-0.409	2.290	0.0358	0.2614	1.0000
0.2	0.2521	0.9766	0.2521	0.2218	0.2747	8.981	4	0.387	2.290	0.0358	0.2521	0.9646
*0.4	0.2180	0.8445	0.2180	0.2067	0.2218	3.476	4	2.571	2.290	0.0358	0.2180	0.8342
0.8	0.0000	0.0000	0.0000	0.0000	0.0000	0.000	4				0.0000	0.0000
1.5	0.0000	0.0000	0.0000	0.0000	0.0000	0.000	4				0.0000	0.0000
3	0.0000	0.0000	0.0000	0.0000	0.0000	0.000	4				0.0000	0.0000

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.05)	0.967965	0.887	0.252787	-0.51339
Bartlett's Test indicates equal variances (p = 0.30)	3.680906	11.34487		
The control means are not significantly different (p = 0.24)	1.296453	2.446912		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	0.2	0.4	0.282843		0.035775	0.138559	0.001725	0.000488	0.04843	3, 12
Treatments vs Diluent Control										

Linear Interpolation (200 Resamples)

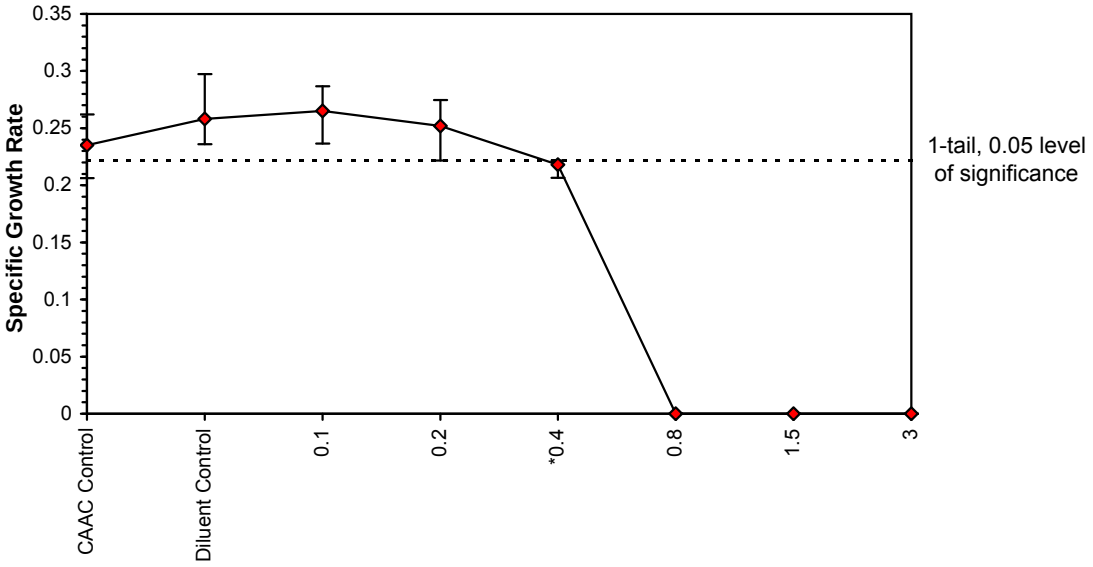
Point		SD	95% CL(Exp)		Skew
IC05	0.2224	0.0652	0.0000	0.3156	-0.5460
IC10	0.2991	0.0636	0.0663	0.4185	-0.6772
IC15	0.3757	0.0563	0.0915	0.4406	-1.4159
IC20	0.4164	0.0308	0.3078	0.4524	-4.1359
IC25	0.4404	0.0150	0.3978	0.4741	-1.7631
IC40	0.5123	0.0109	0.4783	0.5393	-0.5818
IC50	0.5602	0.0091	0.5319	0.5827	-0.5818



Duckweed Growth Inhibtion Test-Specific Growth Rate

Start Date:	17/01/2013 12:30	Test ID:	PR0989/06	Sample ID:	RP3
End Date:	21/01/2013 14:00	Lab ID:	5823	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 112	Test Species:	LA-Lemna aequinoctialis
Comments:					

Dose-Response Plot



ToxCalc v5.0.23

Reviewed by:_____

Duckweed Growth Inhibtion Test-Specific Growth Rate

Start Date:	17/01/2013 12:30	Test ID:	PR0989/06	Sample ID:	RP3
End Date:	21/01/2013 14:00	Lab ID:	5823	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 112	Test Species:	LA-Lemna aequinoctialis
Comments:					

		Auxiliary Data Summary					
Conc-	**Parameter**	**Mean**	**Min**	**Max**	**SD**	**CV%**	**N**
CAAC Control	Specific Growth Rate	0.24	0.21	0.26	0.02	64.15	4
Diluent Control		0.26	0.24	0.30	0.03	63.52	4
0.1		0.26	0.24	0.29	0.03	60.55	4
0.2		0.25	0.22	0.27	0.02	59.68	4
0.4		0.22	0.21	0.22	0.01	39.93	4
0.8		0.00	0.00	0.00	0.00		4
1.5		0.00	0.00	0.00	0.00		4
3		0.00	0.00	0.00	0.00		4
CAAC Control	pH	6.00	6.00	6.00	0.00	0.00	1
Diluent Control		6.60	6.60	6.60	0.00	0.00	1
0.1		6.70	6.70	6.70	0.00	0.00	1
0.2		6.70	6.70	6.70	0.00	0.00	1
0.4		6.70	6.70	6.70	0.00	0.00	1
0.8		6.60	6.60	6.60	0.00	0.00	1
1.5		6.50	6.50	6.50	0.00	0.00	1
3		6.30	6.30	6.30	0.00	0.00	1
CAAC Control	Cond uS/cm	169.70	169.70	169.70	0.00	0.00	1
Diluent Control		51.90	78.10	78.10	0.00	0.00	1
0.1		59.90	59.90	59.90	0.00	0.00	1
0.2		63.00	63.00	63.00	0.00	0.00	1
0.4		68.30	68.30	68.30	0.00	0.00	1
0.8		87.00	87.00	87.00	0.00	0.00	1
1.5		115.70	115.70	115.70	0.00	0.00	1
3		173.60	173.60	173.60	0.00	0.00	1

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Statistical Printouts for the Freshwater Shrimp Tests

Freshwater Shrimp Acute Toxicity Test-96 hr Survival

Start Date:	25/01/2013 16:00	Test ID:	PR0989/02	Sample ID:	SW2
End Date:	29/01/2013 16:00	Lab ID:	5822	Sample Type:	Diluent Control
Sample Date:		Protocol:	ESA 123	Test Species:	MB-Macrobrachium bullatum
Comments:					

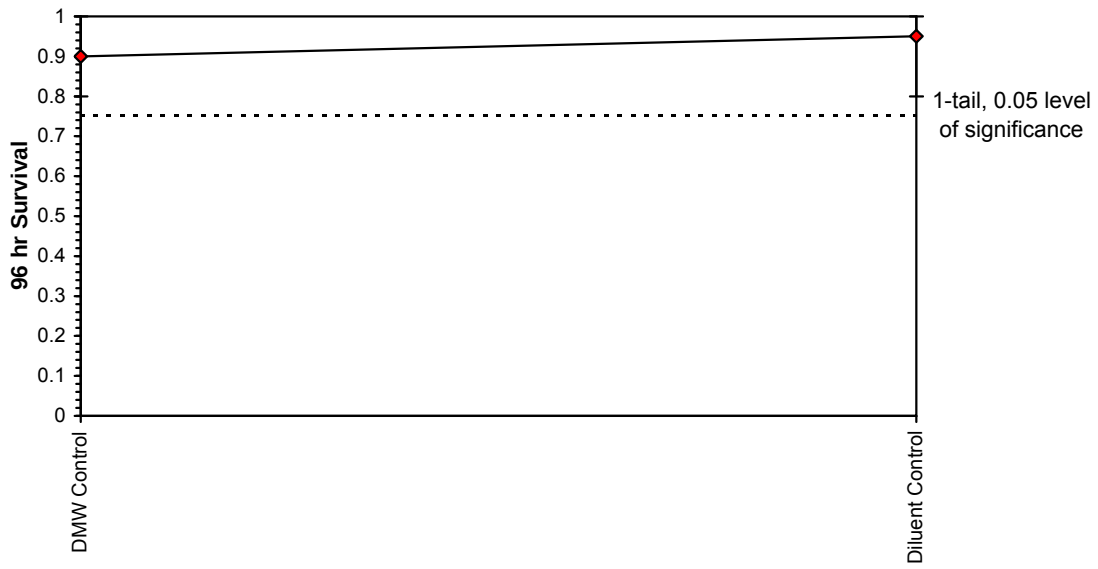
Conc-	1	2	3	4
DMW Control	0.8000	0.8000	1.0000	1.0000
Diluent Control	1.0000	1.0000	1.0000	0.8000

			Transform: Arcsin Square Root						1-Tailed	
Conc-	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD
DMW Control	0.9000	1.0000	1.2262	1.1071	1.3453	11.212	4			
Diluent Control	0.9500	1.0556	1.2857	1.1071	1.3453	9.261	4	-0.655	1.943	0.1767

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.05)	0.827836	0.818	-0.57143	-1.72857
F-Test indicates equal variances (p = 0.82)	1.333333	47.46723		

Hypothesis Test (1-tail, 0.05)	MSDu	MSDp	MSB	MSE	F-Prob	df
Homoscedastic t Test indicates no significant differences Treatments vs DMW Control	0.133894	0.151141	0.007088	0.01654	0.536963	1, 6

Dose-Response Plot



 ToxCalc v5.0.23 Reviewed by:_____

Freshwater Shrimp Acute Toxicity Test-96 hr Survival

Start Date:	25/01/2013 16:00	Test ID:	PR0989/02	Sample ID:	SW2
End Date:	29/01/2013 16:00	Lab ID:	5822	Sample Type:	Diluent Control
Sample Date:		Protocol:	ESA 123	Test Species:	MB-Macrobrachium bullatum
Comments:					

Auxiliary Data Summary

Conc-	Parameter	Mean	Min	Max	SD	CV%	N
DMW Control	% Survival	90.00	80.00	100.00	11.55	3.78	4
Diluent Control		95.00	80.00	100.00	10.00	3.33	4
DMW Control	pH	8.20	8.20	8.20	0.00	0.00	1
Diluent Control		6.90	6.90	6.90	0.00	0.00	1
DMW Control	Cond uS/cm	174.20	174.20	174.20	0.00	0.00	1
Diluent Control		22.40	22.40	22.40	0.00	0.00	1
DMW Control	DO %	102.40	102.40	102.40	0.00	0.00	1
Diluent Control		104.40	104.40	104.40	0.00	0.00	1

Freshwater Shrimp Acute Toxicity Test-96 hr Survival

Start Date:	25/01/2013 16:00	Test ID:	PR0989/02	Sample ID:	RP3
End Date:	29/01/2013 16:00	Lab ID:	5823	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 123	Test Species:	MB-Macrobrachium bullatum
Comments:					

Conc-%	1	2	3	4
DMW Control	0.8000	0.8000	1.0000	1.0000
Diluent Control	1.0000	1.0000	1.0000	0.8000
0.3	0.8000	1.0000	1.0000	1.0000
0.6	0.6000	0.4000	0.8000	1.0000
1.3	0.0000	0.6000	0.2000	0.8000
2.5	0.0000	0.0000	0.0000	0.0000
5	0.0000	0.0000	0.0000	0.0000
10	0.0000	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
DMW Control	0.9000	0.9474	1.2262	1.1071	1.3453	11.212	4					
Diluent Control	0.9500	1.0000	1.2857	1.1071	1.3453	9.261	4	*			1	20
0.3	0.9500	1.0000	1.2857	1.1071	1.3453	9.261	4	0.000	2.290	0.4196	1	20
0.6	0.7000	0.7368	1.0058	0.6847	1.3453	28.293	4	1.528	2.290	0.4196	6	20
*1.3	0.4000	0.4211	0.6706	0.2255	1.1071	59.520	4	3.357	2.290	0.4196	12	20
2.5	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	4				20	20
5	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	4				20	20
10	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	4				20	20

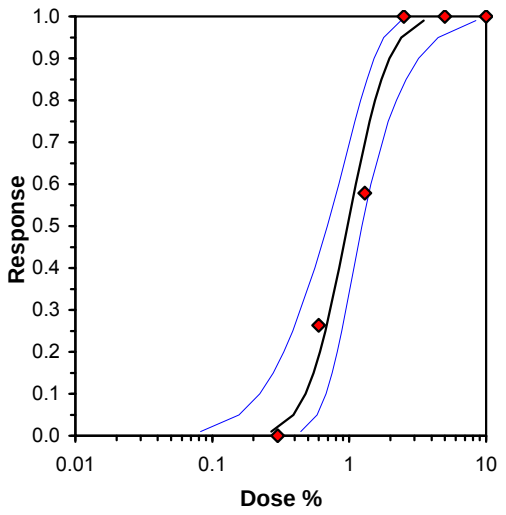
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.05)	0.952861	0.887	-0.05271	-0.05443
Bartlett's Test indicates equal variances (p = 0.14)	5.42661	11.34487		
The control means are not significantly different (p = 0.54)	0.654654	2.446912		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	0.6	1.3	0.883176	166.6667	0.340569	0.369812	0.341976	0.067162	0.01677	3, 12
Treatments vs Diluent Control										

Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	4.179646	0.925532	2.365603	5.993689	0.05	2.82232	9.487729	0.59	-0.01265	0.239255	5
Intercept	5.052875	0.228508	4.604999	5.500752							
TSCR	0.048377	0.039808	-0.02965	0.1264							

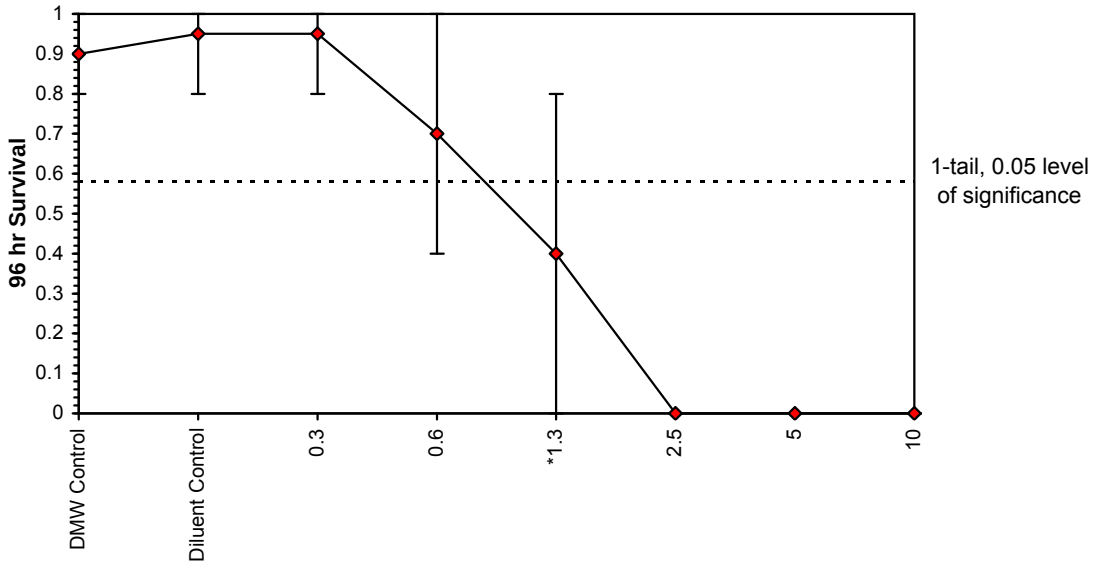
Point	Probits	%	95% Fiducial Limits	
EC01	2.674	0.269625	0.082172	0.442411
EC05	3.355	0.392473	0.157388	0.58259
EC10	3.718	0.479438	0.221704	0.677254
EC15	3.964	0.548754	0.278653	0.75158
EC20	4.158	0.610924	0.333464	0.818172
EC25	4.326	0.669845	0.388207	0.881792
EC40	4.747	0.844763	0.562027	1.078858
EC50	5.000	0.971291	0.692263	1.235421
EC60	5.253	1.11677	0.838948	1.43786
EC75	5.674	1.408394	1.104703	1.934184
EC80	5.842	1.544227	1.214411	2.207564
EC85	6.036	1.719178	1.345233	2.596188
EC90	6.282	1.967735	1.516054	3.213163
EC95	6.645	2.403744	1.787779	4.461947
EC99	7.326	3.498961	2.3857	8.433532



Freshwater Shrimp Acute Toxicity Test-96 hr Survival

Start Date:	25/01/2013 16:00	Test ID:	PR0989/02	Sample ID:	RP3
End Date:	29/01/2013 16:00	Lab ID:	5823	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 123	Test Species:	MB-Macrobrachium bullatum
Comments:					

Dose-Response Plot



 ToxCalc v5.0.23 Reviewed by:_____

Freshwater Shrimp Acute Toxicity Test-96 hr Survival

Start Date:	25/01/2013 16:00	Test ID:	PR0989/02	Sample ID:	RP3
End Date:	29/01/2013 16:00	Lab ID:	5823	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 123	Test Species:	MB-Macrobrachium bullatum
Comments:					

		Auxiliary Data Summary					
Conc-%	**Parameter**	**Mean**	**Min**	**Max**	**SD**	**CV%**	**N**
DMW Control	% Survival	90.00	80.00	100.00	11.55	3.78	4
Diluent Control		95.00	80.00	100.00	10.00	3.33	4
0.3		95.00	80.00	100.00	10.00	3.33	4
0.6		70.00	40.00	100.00	25.82	7.26	4
1.3		40.00	0.00	80.00	36.51	15.11	4
2.5		0.00	0.00	0.00	0.00		4
5		0.00	0.00	0.00	0.00		4
10		0.00	0.00	0.00	0.00		4
DMW Control	pH	8.20	8.20	8.20	0.00	0.00	1
Diluent Control		6.90	6.90	6.90	0.00	0.00	1
0.3		6.90	6.90	6.90	0.00	0.00	1
0.6		6.80	6.80	6.80	0.00	0.00	1
1.3		6.70	6.70	6.70	0.00	0.00	1
2.5		6.60	6.60	6.60	0.00	0.00	1
5		6.60	6.60	6.60	0.00	0.00	1
10		6.60	6.60	6.60	0.00	0.00	1
DMW Control	Cond uS/cm	174.20	174.20	174.20	0.00	0.00	1
Diluent Control		22.40	22.40	22.40	0.00	0.00	1
0.3		35.60	35.60	35.60	0.00	0.00	1
0.6		47.80	47.80	47.80	0.00	0.00	1
1.3		74.00	74.00	74.00	0.00	0.00	1
2.5		129.00	129.00	129.00	0.00	0.00	1
5		227.00	227.00	227.00	0.00	0.00	1
10		400.00	400.00	400.00	0.00	0.00	1
DMW Control	DO %	102.40	102.40	102.40	0.00	0.00	1
Diluent Control		104.40	104.40	104.40	0.00	0.00	1
0.3		109.40	109.40	109.40	0.00	0.00	1
0.6		106.10	106.10	106.10	0.00	0.00	1
1.3		102.40	102.40	102.40	0.00	0.00	1
2.5		104.20	104.20	104.20	0.00	0.00	1
5		104.20	104.20	104.20	0.00	0.00	1
10		103.50	103.50	103.50	0.00	0.00	1

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Statistical Printouts for the *Chlorella* Growth Inhibition Tests

Microalgal Cell Yield-Cell Yield

Start Date:	18/01/2013 16:15	Test ID:	PR0989/02	Sample ID:	SW2
End Date:	21/01/2013 16:40	Lab ID:	5822	Sample Type:	Diluent Control
Sample Date:		Protocol:	ESA 103	Test Species:	CV-Chlorella vulgaris
Comments:					

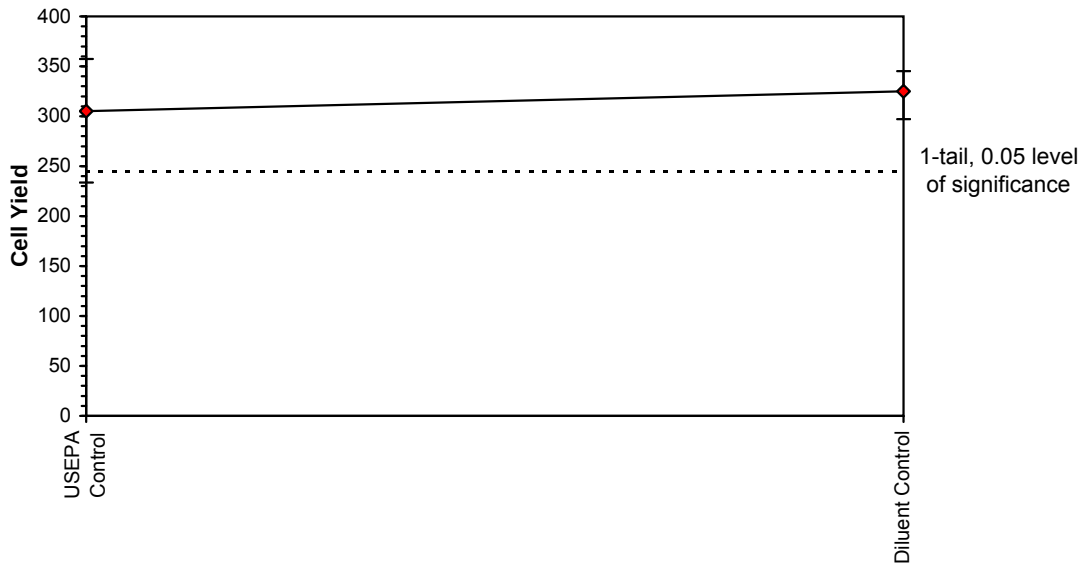
Conc-	1	2	3	4
USEPA Control	233.52	282.52	346.52	357.52
Diluent Control	325.52	332.52	345.52	297.52

			Transform: Untransformed						1-Tailed	
Conc-	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD
USEPA Control	305.02	1.0000	305.02	233.52	357.52	19.020	4			
Diluent Control	325.27	1.0664	325.27	297.52	345.52	6.232	4	-0.659	1.943	59.71

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.05)	0.969389	0.818	-0.5073	0.036526
F-Test indicates equal variances (p = 0.12)	8.190631	47.46723		

Hypothesis Test (1-tail, 0.05)	MSDu	MSDp	MSB	MSE	F-Prob	df
Homoscedastic t Test indicates no significant differences Treatments vs USEPA Control	59.70802	0.195751	820.125	1888.292	0.534336	1, 6

Dose-Response Plot



 ToxCalc v5.0.23 Reviewed by:_____

Microalgal Cell Yield-Cell Yield

Start Date:	18/01/2013 16:15	Test ID:	PR0989/02	Sample ID:	SW2
End Date:	21/01/2013 16:40	Lab ID:	5822	Sample Type:	Diluent Control
Sample Date:		Protocol:	ESA 103	Test Species:	CV-Chlorella vulgaris
Comments:					

Auxiliary Data Summary

Conc-	Parameter	Mean	Min	Max	SD	CV%	N
USEPA Control	Cell Yield	305.02	233.52	357.52	58.01	2.50	4
Diluent Control		325.27	297.52	345.52	20.27	1.38	4
USEPA Control	pH	7.60	7.60	7.60	0.00	0.00	1
Diluent Control		7.40	7.40	7.40	0.00	0.00	1
USEPA Control	Conductivity uS/cm	462.00	462.00	462.00	0.00	0.00	1
Diluent Control		111.40	111.40	111.40	0.00	0.00	1

Microalgal Cell Yield-Cell Yield

Start Date:	18/01/2013 16:15	Test ID:	PR0989/03	Sample ID:	RP3
End Date:	21/01/2013 16:40	Lab ID:	5823	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 103	Test Species:	CV-Chlorella vulgaris
Comments:					

Conc-%	1	2	3	4
USEPA Control	233.52	282.52	346.52	357.52
Diluent Control	325.52	332.52	345.52	297.52
0.1	340.52	359.52	313.52	288.52
0.2	232.52	340.52	256.52	260.52
0.4	77.52	101.52	114.52	74.52
0.8	0.00	0.00	1.52	0.00
1.5	0.00	0.00	0.00	0.00
3	0.00	0.00	0.00	0.00

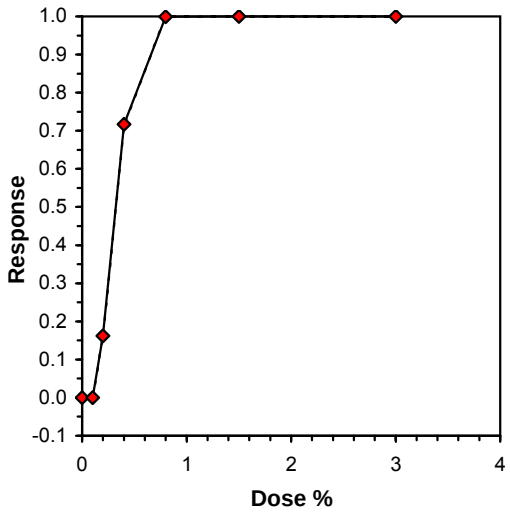
			Transform: Untransformed					Rank	1-Tailed	Isotonic	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Mean	N-Mean
USEPA Control	305.02	0.9377	305.02	233.52	357.52	19.020	4				
Diluent Control	325.27	1.0000	325.27	297.52	345.52	6.232	4	*		325.39	1.0000
0.1	325.52	1.0008	325.52	288.52	359.52	9.541	4	18.00	10.00	325.39	1.0000
0.2	272.52	0.8378	272.52	232.52	340.52	17.243	4	13.00	10.00	272.52	0.8375
*0.4	92.02	0.2829	92.02	74.52	114.52	20.932	4	10.00	10.00	92.02	0.2828
*0.8	0.38	0.0012	0.38	0.00	1.52	200.000	4	10.00	10.00	0.38	0.0012
1.5	0.00	0.0000	0.00	0.00	0.00	0.000	4			0.00	0.0000
3	0.00	0.0000	0.00	0.00	0.00	0.000	4			0.00	0.0000

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.05)	0.947384	0.905	0.847102	1.735358
Bartlett's Test indicates unequal variances (p = 5.95E-04)	19.61403	13.2767		
The control means are not significantly different (p = 0.53)	0.65903	2.446912		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	0.2	0.4	0.282843	500
Treatments vs Diluent Control				

Linear Interpolation (200 Resamples)

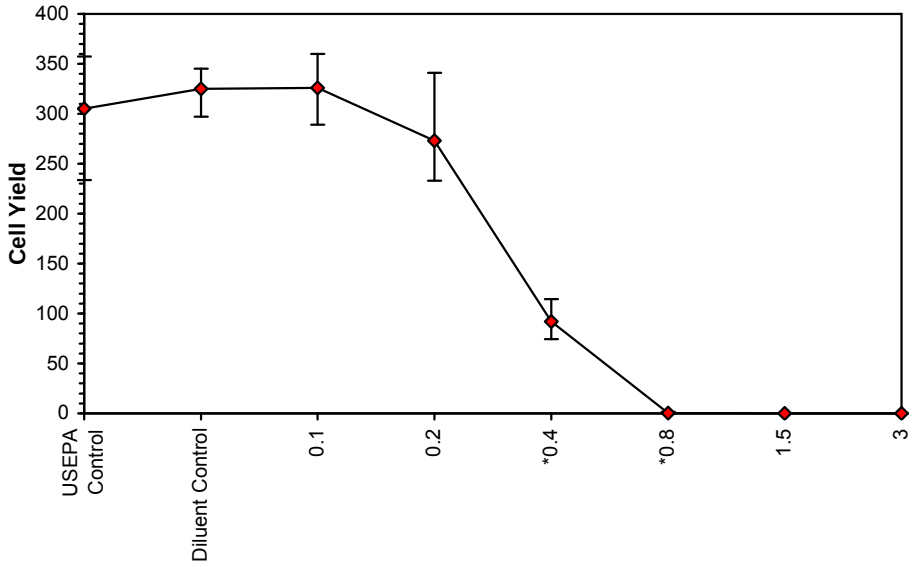
Point	%	SD	95% CL(Exp)		Skew
IC05	0.1308	0.0317	0.0085	0.2479	0.2079
IC10	0.1615	0.0310	0.0729	0.2533	0.1965
IC15	0.1923	0.0268	0.1139	0.2592	0.0498
IC20	0.2135	0.0228	0.1427	0.2691	-0.1078
IC25	0.2315	0.0206	0.1661	0.2824	-0.1349
IC40	0.2856	0.0149	0.2356	0.3220	-0.1175
IC50	0.3217	0.0117	0.2848	0.3510	-0.0865



Microalgal Cell Yield-Cell Yield

Start Date:	18/01/2013 16:15	Test ID:	PR0989/03	Sample ID:	RP3
End Date:	21/01/2013 16:40	Lab ID:	5823	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 103	Test Species:	CV-Chlorella vulgaris
Comments:					

Dose-Response Plot



Microalgal Cell Yield-Cell Yield

Start Date:	18/01/2013 16:15	Test ID:	PR0989/03	Sample ID:	RP3
End Date:	21/01/2013 16:40	Lab ID:	5823	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 103	Test Species:	CV-Chlorella vulgaris
Comments:					

Auxiliary Data Summary

Conc-%	Parameter	Mean	Min	Max	SD	CV%	N
USEPA Control	Cell Yield	305.02	233.52	357.52	58.01	2.50	4
Diluent Control		325.27	297.52	345.52	20.27	1.38	4
0.1		325.52	288.52	359.52	31.06	1.71	4
0.2		272.52	232.52	340.52	46.99	2.52	4
0.4		92.02	74.52	114.52	19.26	4.77	4
0.8		0.38	0.00	1.52	0.76	229.44	4
1.5		0.00	0.00	0.00	0.00		4
3		0.00	0.00	0.00	0.00		4
USEPA Control	pH	7.60	7.60	7.60	0.00	0.00	1
Diluent Control		7.40	7.40	7.40	0.00	0.00	1
0.1		7.30	7.30	7.30	0.00	0.00	1
0.2		7.30	7.30	7.30	0.00	0.00	1
0.4		7.30	7.30	7.30	0.00	0.00	1
0.8		7.20	7.20	7.20	0.00	0.00	1
1.5		7.20	7.20	7.20	0.00	0.00	1
3		7.10	7.10	7.10	0.00	0.00	1
USEPA Control	Conductivity uS/cm	462.00	462.00	462.00	0.00	0.00	1
Diluent Control		111.40	111.40	111.40	0.00	0.00	1
0.1		122.00	122.00	122.00	0.00	0.00	1
0.2		102.50	102.50	102.50	0.00	0.00	1
0.4		160.80	160.80	160.80	0.00	0.00	1
0.8		170.00	170.00	170.00	0.00	0.00	1
1.5		191.90	191.90	191.90	0.00	0.00	1
3		259.00	259.00	259.00	0.00	0.00	1

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Statistical Printouts for the Larval Fish Imbalance Tests

Fish Imbalance Test-96 hr Imbalance

Start Date:	31/01/2013 15:30	Test ID:	PR0989	Sample ID:	RP3
End Date:	4/02/2013 15:30	Lab ID:	5823	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 117	Test Species:	MS-Melanotaenia splendida
Comments:					

Conc-%	1	2	3	4
DMW Control	1.0000	1.0000	1.0000	
Diluent control	1.0000	0.6000	0.8000	
0.0625	0.8000	0.8000	1.0000	0.8000
0.125	0.6000	0.6000	0.4000	0.4000
0.25	0.2000	0.2000	0.0000	0.6000
0.5	0.0000	0.0000	0.0000	0.0000
1	0.0000	0.0000	0.0000	0.0000

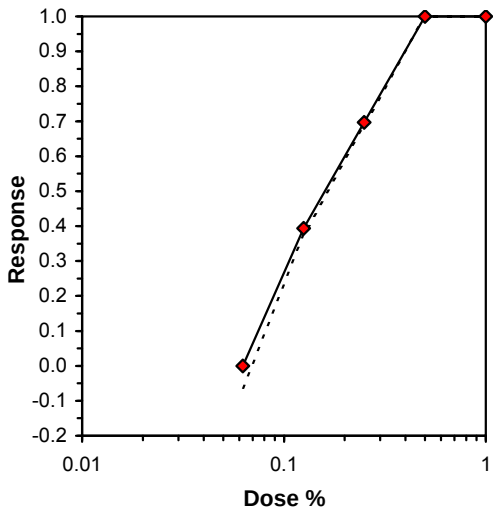
			Transform: Arcsin Square Root						1-Tailed		Isotonic	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
DMW Control	1.0000	1.2500	1.3453	1.3453	1.3453	0.000	3					
Diluent control	0.8000	1.0000	1.1128	0.8861	1.3453	20.637	3	*			0.8250	1.0000
0.0625	0.8500	1.0625	1.1667	1.1071	1.3453	10.206	4	-0.363	2.431	0.3608	0.8250	1.0000
0.125	0.5000	0.6250	0.7854	0.6847	0.8861	14.802	4	2.207	2.431	0.3608	0.5000	0.6061
*0.25	0.2500	0.3125	0.5097	0.2255	0.8861	53.926	4	4.064	2.431	0.3608	0.2500	0.3030
0.5	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	4				0.0000	0.0000
1	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	4				0.0000	0.0000

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.05)	0.945272	0.881	0.582816	0.431517
Bartlett's Test indicates equal variances (p = 0.42)	2.826027	11.34487		
The control means are not significantly different (p = 0.15)	1.7531	2.776445		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test	0.125	0.25	0.176777	800	0.337845	0.419929	0.358531	0.037748	0.002182	3, 11
Treatments vs Diluent control										

Log-Logit Interpolation (200 Resamples)

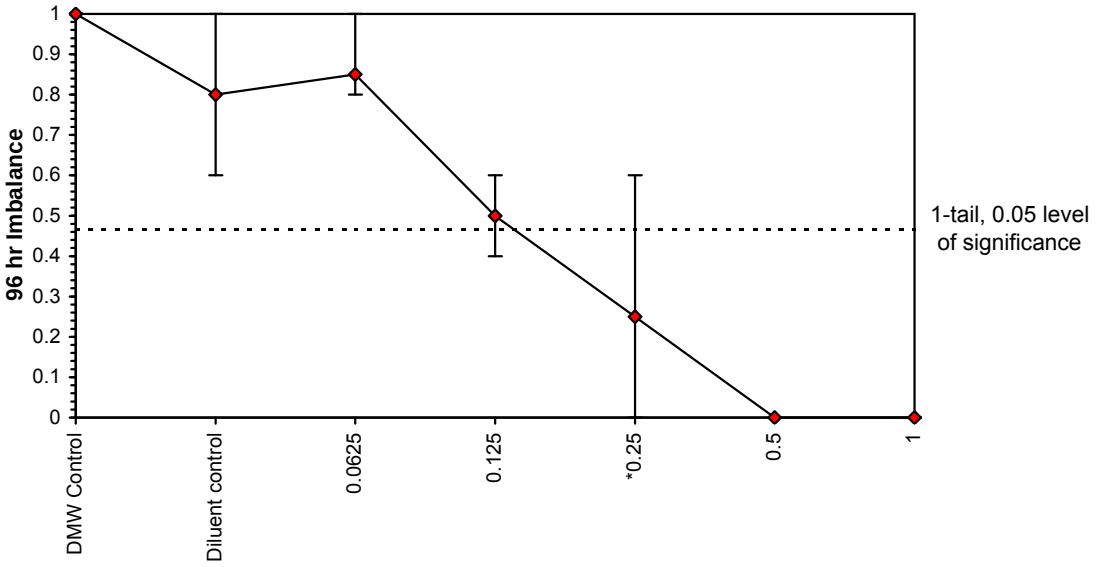
Point	%	SD	95% CL(Exp)		Skew
IC05	0.0728	0.0124	0.0195	0.0798	-1.8162
IC10	0.0819	0.0087	0.0399	0.0937	-1.6352
IC15	0.0902	0.0086	0.0484	0.1079	-1.0796
IC20	0.0978	0.0089	0.0628	0.1225	-0.4082
IC25	0.1051	0.0094	0.0751	0.1371	0.2729
IC40	0.1272	0.0241	0.0983	0.2270	2.3662
IC50	0.1638	0.0348	0.0938	0.3076	0.9942



Fish Imbalance Test-96 hr Imbalance

Start Date:	31/01/2013 15:30	Test ID:	PR0989	Sample ID:	RP3
End Date:	4/02/2013 15:30	Lab ID:	5823	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 117	Test Species:	MS-Melanotaenia splendida
Comments:					

Dose-Response Plot



Fish Imbalance Test-96 hr Imbalance

Start Date:	31/01/2013 15:30	Test ID:	PR0989	Sample ID:	RP3
End Date:	4/02/2013 15:30	Lab ID:	5823	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 117	Test Species:	MS-Melanotaenia splendida
Comments:					

Auxiliary Data Summary

Conc-%	Parameter	Mean	Min	Max	SD	CV%	N
DMW Control	% Un-affected	100.00	100.00	100.00	0.00	0.00	3
Diluent control		80.00	60.00	100.00	20.00	5.59	3
0.0625		85.00	80.00	100.00	10.00	3.72	4
0.125		50.00	40.00	60.00	11.55	6.80	4
0.25		25.00	0.00	60.00	25.17	20.07	4
0.5		0.00	0.00	0.00	0.00		4
1		0.00	0.00	0.00	0.00		4
DMW Control	pH	8.00	8.00	8.00	0.00	0.00	1
Diluent control		6.50	6.50	6.50	0.00	0.00	1
0.0625		6.50	6.50	6.50	0.00	0.00	1
0.125		6.50	6.50	6.50	0.00	0.00	1
0.25		6.40	6.40	6.40	0.00	0.00	1
0.5		6.40	6.40	6.40	0.00	0.00	1
1		6.30	6.30	6.30	0.00	0.00	1
DMW Control	DO %	97.30	97.30	97.30	0.00	0.00	1
Diluent control		86.30	86.30	86.30	0.00	0.00	1
0.0625		92.50	92.50	92.50	0.00	0.00	1
0.125		93.70	93.70	93.70	0.00	0.00	1
0.25		94.30	94.30	94.30	0.00	0.00	1
0.5		93.60	93.60	93.60	0.00	0.00	1
1		89.30	89.30	89.30	0.00	0.00	1
DMW Control	Conductivity uS/cm	168.40	168.40	168.40	0.00	0.00	1
Diluent control		18.40	18.40	18.40	0.00	0.00	1
0.0625		21.00	21.00	21.00	0.00	0.00	1
0.125		24.10	24.10	24.10	0.00	0.00	1
0.25		30.40	30.40	30.40	0.00	0.00	1
0.5		42.40	42.40	42.40	0.00	0.00	1
1		64.80	64.80	64.80	0.00	0.00	1

Fish Imbalance Test-96 hr Imbalance

Start Date:	31/01/2013 15:30	Test ID:	PR0989	Sample ID:	RP3
End Date:	4/02/2013 15:30	Lab ID:	5823	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 117	Test Species:	MS-Melanotaenia splendida
Comments:					

Conc-%	1	2	3	4
DMW Control	1.0000	1.0000	1.0000	
Diluent control	1.0000	0.6000	0.8000	
0.0625	0.8000	0.8000	1.0000	0.8000
0.125	0.6000	0.6000	0.4000	0.4000
0.25	0.2000	0.2000	0.0000	0.6000
0.5	0.0000	0.0000	0.0000	0.0000
1	0.0000	0.0000	0.0000	0.0000

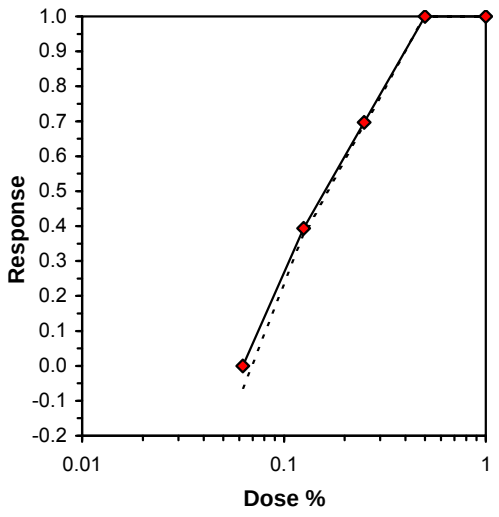
			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
DMW Control	1.0000	1.2500	1.3453	1.3453	1.3453	0.000	3					
Diluent control	0.8000	1.0000	1.1128	0.8861	1.3453	20.637	3	*			3	15
0.0625	0.8500	1.0625	1.1667	1.1071	1.3453	10.206	4	-0.363	2.431	0.3608	3	20
0.125	0.5000	0.6250	0.7854	0.6847	0.8861	14.802	4	2.207	2.431	0.3608	10	20
*0.25	0.2500	0.3125	0.5097	0.2255	0.8861	53.926	4	4.064	2.431	0.3608	15	20
0.5	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	4				20	20
1	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	4				20	20

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.05)	0.945272	0.881	0.582816	0.431517
Bartlett's Test indicates equal variances (p = 0.42)	2.826027	11.34487		
The control means are not significantly different (p = 0.15)	1.7531	2.776445		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test	0.125	0.25	0.176777	800	0.337845	0.419929	0.358531	0.037748	0.002182	3, 11
Treatments vs Diluent control										

Trimmed Spearman-Karber

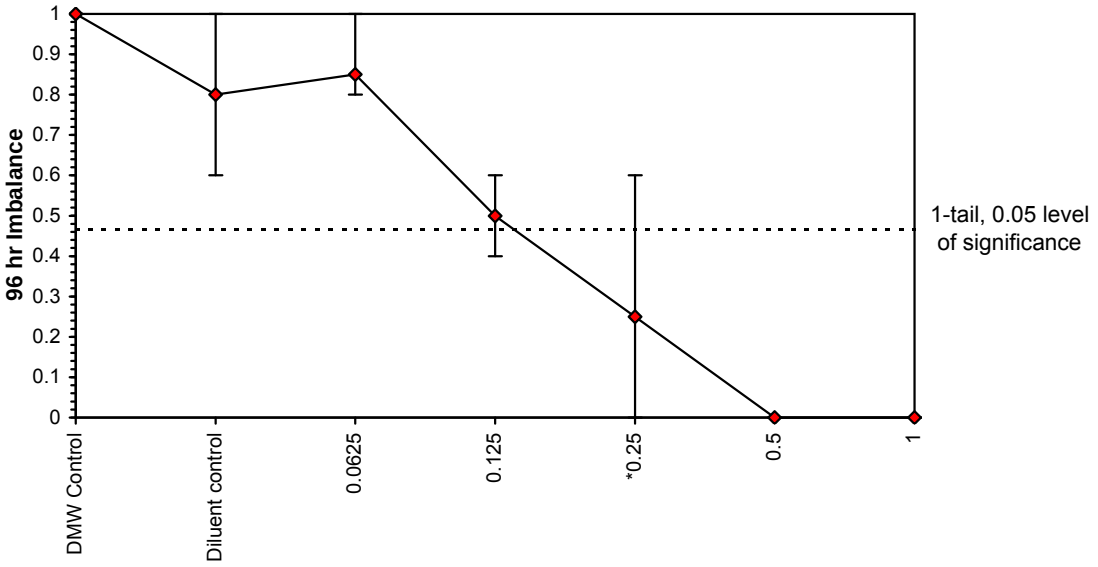
Trim Level	EC50	95% CL	
0.0%	0.1660	0.1348	0.2043
5.0%	0.1649	0.1312	0.2073
10.0%	0.1639	0.1278	0.2102
20.0%	0.1620	0.1215	0.2160
Auto-0.0%	0.1660	0.1348	0.2043



Fish Imbalance Test-96 hr Imbalance

Start Date:	31/01/2013 15:30	Test ID:	PR0989	Sample ID:	RP3
End Date:	4/02/2013 15:30	Lab ID:	5823	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 117	Test Species:	MS-Melanotaenia splendida
Comments:					

Dose-Response Plot



Fish Imbalance Test-96 hr Imbalance

Start Date:	31/01/2013 15:30	Test ID:	PR0989	Sample ID:	RP3
End Date:	4/02/2013 15:30	Lab ID:	5823	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 117	Test Species:	MS-Melanotaenia splendida
Comments:					

Auxiliary Data Summary

Conc-%	Parameter	Mean	Min	Max	SD	CV%	N
DMW Control	% Un-affected	100.00	100.00	100.00	0.00	0.00	3
Diluent control		80.00	60.00	100.00	20.00	5.59	3
0.0625		85.00	80.00	100.00	10.00	3.72	4
0.125		50.00	40.00	60.00	11.55	6.80	4
0.25		25.00	0.00	60.00	25.17	20.07	4
0.5		0.00	0.00	0.00	0.00		4
1		0.00	0.00	0.00	0.00		4
DMW Control	pH	8.00	8.00	8.00	0.00	0.00	1
Diluent control		6.50	6.50	6.50	0.00	0.00	1
0.0625		6.50	6.50	6.50	0.00	0.00	1
0.125		6.50	6.50	6.50	0.00	0.00	1
0.25		6.40	6.40	6.40	0.00	0.00	1
0.5		6.40	6.40	6.40	0.00	0.00	1
1		6.30	6.30	6.30	0.00	0.00	1
DMW Control	DO %	97.30	97.30	97.30	0.00	0.00	1
Diluent control		86.30	86.30	86.30	0.00	0.00	1
0.0625		92.50	92.50	92.50	0.00	0.00	1
0.125		93.70	93.70	93.70	0.00	0.00	1
0.25		94.30	94.30	94.30	0.00	0.00	1
0.5		93.60	93.60	93.60	0.00	0.00	1
1		89.30	89.30	89.30	0.00	0.00	1
DMW Control	Conductivity uS/cm	168.40	168.40	168.40	0.00	0.00	1
Diluent control		18.40	18.40	18.40	0.00	0.00	1
0.0625		21.00	21.00	21.00	0.00	0.00	1
0.125		24.10	24.10	24.10	0.00	0.00	1
0.25		30.40	30.40	30.40	0.00	0.00	1
0.5		42.40	42.40	42.40	0.00	0.00	1
1		64.80	64.80	64.80	0.00	0.00	1

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Statistical Printouts for *Hydra* Population Growth Tests

Hydra Population Growth Test-Growth Rate

Start Date:	4/02/2013 13:30	Test ID:	PR0989/10	Sample ID:	RP3
End Date:	8/02/2013 14:00	Lab ID:	5823	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 125	Test Species:	HV-Hydra viridissima
Comments:					

Conc-%	1	2	3	4
Lab Control	0.3033	0.2894	0.3289	0.2590
Diluent Control	0.3289	0.2670	0.3227	0.2670
0.063	0.3227	0.2964	0.3164	0.2670
0.125	0.3099	0.2508	0.3289	0.3033
0.25	0.2964	0.3099	0.2508	0.2894
0.5	0.0775	0.1088	0.1366	0.0000
1	0.0000	0.0000	0.0000	0.0000
2	0.0000	0.0000	0.0000	0.0000

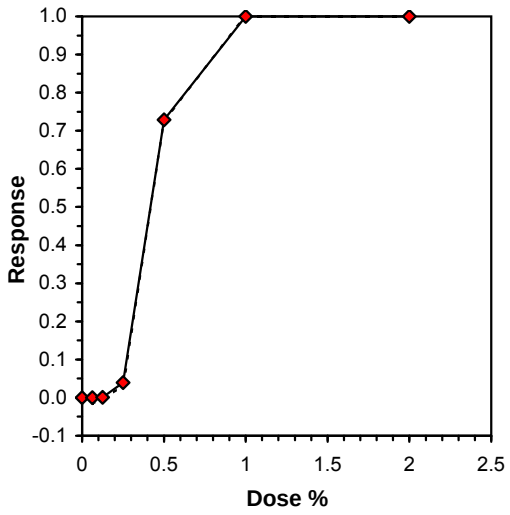
			Transform: Untransformed						1-Tailed		Isotonic	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
Lab Control	0.2951	0.9958	0.2951	0.2590	0.3289	9.868	4					
Diluent Control	0.2964	1.0000	0.2964	0.2670	0.3289	11.499	4	*			0.2985	1.0000
0.063	0.3006	1.0143	0.3006	0.2670	0.3227	8.349	4	-0.160	2.360	0.0626	0.2985	1.0000
0.125	0.2982	1.0062	0.2982	0.2508	0.3289	11.207	4	-0.069	2.360	0.0626	0.2982	0.9991
0.25	0.2866	0.9670	0.2866	0.2508	0.3099	8.843	4	0.368	2.360	0.0626	0.2866	0.9602
*0.5	0.0808	0.2724	0.0808	0.0000	0.1366	73.063	4	8.132	2.360	0.0626	0.0808	0.2705
1	0.0000	0.0000	0.0000	0.0000	0.0000	0.000	4				0.0000	0.0000
2	0.0000	0.0000	0.0000	0.0000	0.0000	0.000	4				0.0000	0.0000

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.05)	0.944838	0.905	-0.72702	0.298269
Bartlett's Test indicates equal variances (p = 0.57)	2.916687	13.2767		
The control means are not significantly different (p = 0.96)	0.056037	2.446912		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	0.25	0.5	0.353553	400	0.062582	0.211144	0.036997	0.001406	1.3E-06	4, 15
Treatments vs Diluent Control										

Linear Interpolation (200 Resamples)

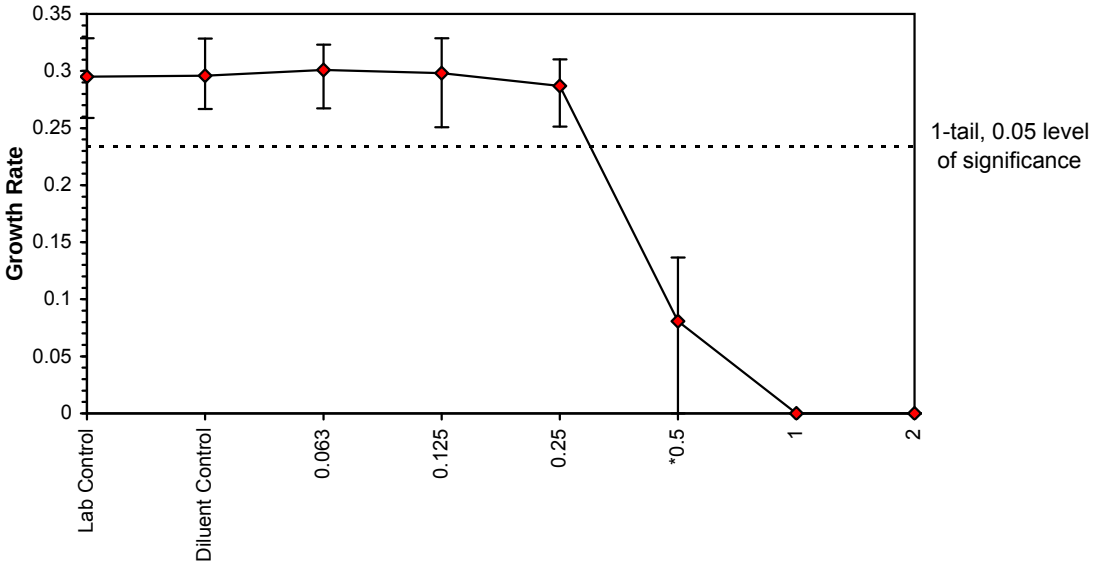
Point	%	SD	95% CL(Exp)		Skew
IC05	0.2537	0.0751	0.0000	0.2774	-0.7586
IC10	0.2718	0.0392	0.0627	0.2961	-2.7805
IC15	0.2899	0.0200	0.2235	0.3162	-3.5854
IC20	0.3081	0.0151	0.2452	0.3354	-0.6572
IC25	0.3262	0.0148	0.2678	0.3629	-0.2104
IC40	0.3806	0.0176	0.3255	0.4353	0.3395
IC50	0.4168	0.0214	0.3514	0.4887	0.4167



Hydra Population Growth Test-Growth Rate

Start Date:	4/02/2013 13:30	Test ID:	PR0989/10	Sample ID:	RP3
End Date:	8/02/2013 14:00	Lab ID:	5823	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 125	Test Species:	HV-Hydra viridissima
Comments:					

Dose-Response Plot



ToxCalc v5.0.23

Reviewed by:_____

Hydra Population Growth Test-Growth Rate

Start Date:	4/02/2013 13:30	Test ID:	PR0989/10	Sample ID:	RP3
End Date:	8/02/2013 14:00	Lab ID:	5823	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 125	Test Species:	HV-Hydra viridissima
Comments:					

		Auxiliary Data Summary					
Conc-%	**Parameter**	**Mean**	**Min**	**Max**	**SD**	**CV%**	**N**
Lab Control	Growth Rate	0.30	0.26	0.33	0.03	57.82	4
Diluent Control		0.30	0.27	0.33	0.03	62.29	4
0.063		0.30	0.27	0.32	0.03	52.70	4
0.125		0.30	0.25	0.33	0.03	61.30	4
0.25		0.29	0.25	0.31	0.03	55.55	4
0.5		0.07	-0.02	0.14	0.07	353.84	4
1		-0.60	-0.60	-0.60	0.00		4
2		-0.60	-0.60	-0.60	0.00		4
Lab Control	Conductivity	22.50	22.50	22.50	0.00	0.00	1
Diluent Control		16.40	16.40	16.40	0.00	0.00	1
0.063		18.80	18.80	18.80	0.00	0.00	1
0.125		21.30	21.30	21.30	0.00	0.00	1
0.25		26.40	26.40	26.40	0.00	0.00	1
0.5		37.10	37.10	37.10	0.00	0.00	1
1		58.20	58.20	58.20	0.00	0.00	1
2		99.50	99.50	99.50	0.00	0.00	1
Lab Control	pH	8.00	8.00	8.00	0.00	0.00	1
Diluent Control		6.90	6.90	6.90	0.00	0.00	1
0.063		6.80	6.80	6.80	0.00	0.00	1
0.125		6.70	6.70	6.70	0.00	0.00	1
0.25		6.70	6.70	6.70	0.00	0.00	1
0.5		6.60	6.60	6.60	0.00	0.00	1
1		6.50	6.50	6.50	0.00	0.00	1
2		6.50	6.50	6.50	0.00	0.00	1
Lab Control	DO, % sat	99.90	99.90	99.90	0.00	0.00	1
Diluent Control		95.20	95.20	95.20	0.00	0.00	1
0.063		98.60	98.60	98.60	0.00	0.00	1
0.125		99.50	99.50	99.50	0.00	0.00	1
0.25		100.20	100.20	100.20	0.00	0.00	1
0.5		100.10	100.10	100.10	0.00	0.00	1
1		99.80	99.80	99.80	0.00	0.00	1
2		99.60	99.60	99.60	0.00	0.00	1

Hydra Population Growth Test-Growth Rate

Start Date:	22/01/2013 15:30	Test ID:	PR0989/02	Sample ID:	RP3
End Date:	26/01/2013 14:30	Lab ID:	5823	Sample Type:	Aqueous
Sample Date:		Protocol:	ESA 125	Test Species:	HV-Hydra viridissima
Comments:					

Conc-%	1	2	3	4
Diluent Control	0.4479	0.3931	0.4765	0.4113
0.03	0.4199	0.4282	0.4113	
0.06	0.4591	0.4241	0.4363	
0.13	0.4441	0.4517	0.4282	
0.25	0.3735	0.3978	0.3577	
0.5	0.1733	0.2821	0.2670	

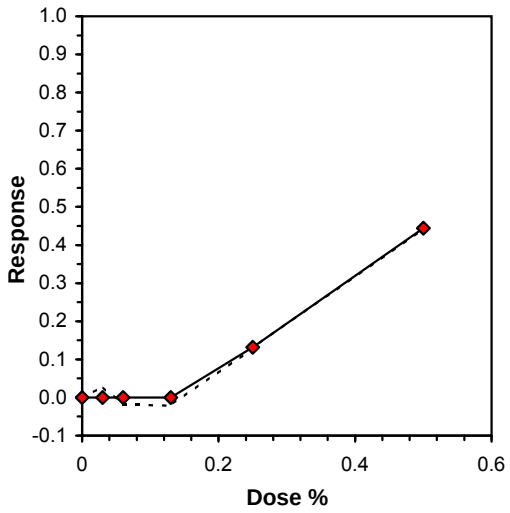
			Transform: Untransformed						1-Tailed		Isotonic	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
Diluent Control	0.4322	1.0000	0.4322	0.3931	0.4765	8.636	4				0.4333	1.0000
0.03	0.4198	0.9713	0.4198	0.4113	0.4282	2.020	3	0.513	2.650	0.0640	0.4333	1.0000
0.06	0.4398	1.0176	0.4398	0.4241	0.4591	4.032	3	-0.315	2.650	0.0640	0.4333	1.0000
0.13	0.4414	1.0211	0.4414	0.4282	0.4517	2.712	3	-0.378	2.650	0.0640	0.4333	1.0000
0.25	0.3763	0.8707	0.3763	0.3577	0.3978	5.366	3	2.314	2.650	0.0640	0.3763	0.8685
*0.5	0.2408	0.5571	0.2408	0.1733	0.2821	24.481	3	7.925	2.650	0.0640	0.2408	0.5557

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.05)	0.967023	0.901	-0.61284	1.063498
Bartlett's Test indicates equal variances (p = 0.14)	8.259734	15.08627		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test	0.25	0.5	0.353553	400	0.064022	0.148124	0.018446	0.001	1.7E-05	5, 13
Treatments vs Diluent Control										

Linear Interpolation (200 Resamples)

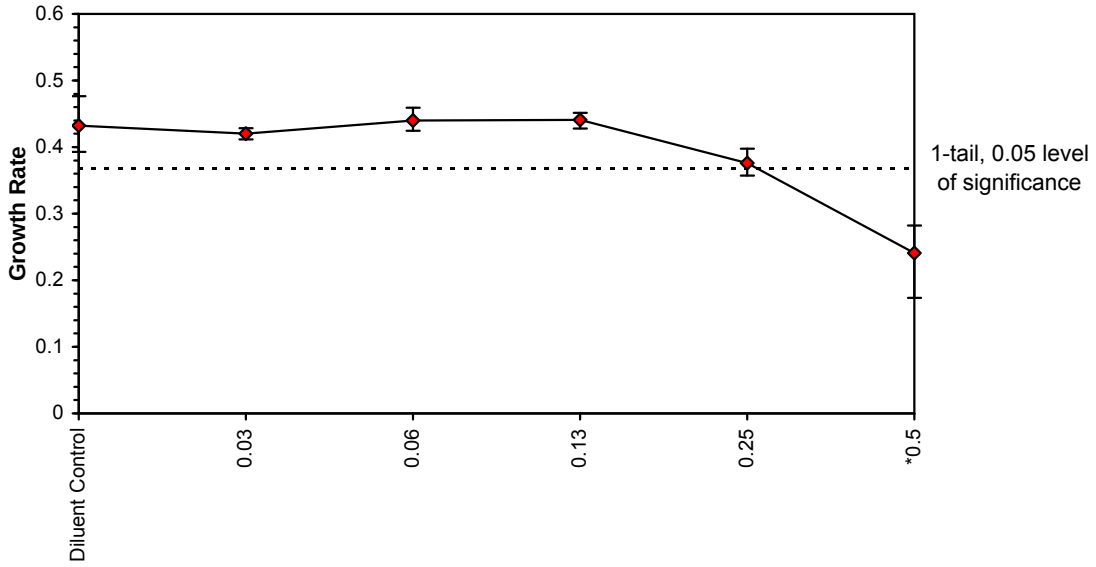
Point	%	SD	95% CL(Exp)		Skew
IC05	0.1756	0.0434	0.0000	0.2203	-2.2798
IC10	0.2212	0.0238	0.0858	0.2970	-0.1919
IC15	0.2648	0.0229	0.1455	0.3247	-0.3601
IC20	0.3047	0.0233	0.1875	0.3712	-0.2706
IC25	0.3447	0.0260	0.2250	0.4363	0.0698
IC40	0.4646				
IC50	>0.5				



Hydra Population Growth Test-Growth Rate

Start Date:	22/01/2013 15:30	Test ID:	PR0989/02	Sample ID:	RP3
End Date:	26/01/2013 14:30	Lab ID:	5823	Sample Type:	Aqueous
Sample Date:		Protocol:	ESA 125	Test Species:	HV-Hydra viridissima
Comments:					

Dose-Response Plot



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Reviewed by:_____

Hydra Population Growth Test-Growth Rate

Start Date:	22/01/2013 15:30	Test ID:	PR0989/02	Sample ID:	RP3
End Date:	26/01/2013 14:30	Lab ID:	5823	Sample Type:	Aqueous
Sample Date:		Protocol:	ESA 125	Test Species:	HV-Hydra viridissima
Comments:					

		Auxiliary Data Summary					
Conc-%	**Parameter**	**Mean**	**Min**	**Max**	**SD**	**CV%**	**N**
Diluent Control	Growth Rate	0.43	0.39	0.48	0.04	44.70	4
0.03		0.42	0.41	0.43	0.01	21.93	3
0.06		0.44	0.42	0.46	0.02	30.28	3
0.13		0.44	0.43	0.45	0.01	24.79	3
0.25		0.38	0.36	0.40	0.02	37.76	3
0.5		0.24	0.17	0.28	0.06	100.83	3
Diluent Control	Conductivity	17.70	17.70	17.70	0.00	0.00	1
0.03		20.50	20.50	20.50	0.00	0.00	1
0.06		21.50	21.50	21.50	0.00	0.00	1
0.13		26.20	26.20	26.20	0.00	0.00	1
0.25		30.40	30.40	30.40	0.00	0.00	1
0.5		43.10	43.10	43.10	0.00	0.00	1
Diluent Control	pH	7.30	7.30	7.30	0.00	0.00	1
0.03		7.30	7.30	7.30	0.00	0.00	1
0.06		7.20	7.20	7.20	0.00	0.00	1
0.13		7.20	7.20	7.20	0.00	0.00	1
0.25		7.10	7.10	7.10	0.00	0.00	1
0.5		7.00	7.00	7.00	0.00	0.00	1
Diluent Control	DO, % sat	100.10	100.10	100.10	0.00	0.00	1
0.03		100.10	100.10	100.10	0.00	0.00	1
0.06		100.10	100.10	100.10	0.00	0.00	1
0.13		99.40	99.40	99.40	0.00	0.00	1
0.25		99.90	99.90	99.90	0.00	0.00	1
0.5		99.50	99.50	99.50	0.00	0.00	1

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Statistical Printouts for the Rainbowfish Embryonic Hatching Survival Tests

Fish Embryonic Development and Post-Hatch Survival Test-Survival

Start Date:	6/02/2013 15:15	Test ID:	PR0989/01	Sample ID:	RP3
End Date:	16/02/2013 15:15	Lab ID:	5823	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 126	Test Species:	MS-Melanotaenia splendida
Comments:					

Conc-%	1	2	3	4
DMW Control	1.0000	1.0000	1.0000	1.0000
Diluent Control	0.8000	0.8000	1.0000	1.0000
0.03	1.0000	1.0000	1.0000	1.0000
0.06	1.0000	1.0000	1.0000	1.0000
0.13	1.0000	1.0000	1.0000	1.0000
0.25	1.0000	1.0000	1.0000	0.6000
0.5	0.4000	0.4000	0.6000	0.8000
1	0.2000	0.0000	0.2000	0.2000

			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
DMW Control	1.0000	1.1111	1.3453	1.3453	1.3453	0.000	4				
Diluent Control	0.9000	1.0000	1.2262	1.1071	1.3453	11.212	4	*		2	20
0.03	1.0000	1.1111	1.3453	1.3453	1.3453	0.000	4	22.00	10.00	0	20
0.06	1.0000	1.1111	1.3453	1.3453	1.3453	0.000	4	22.00	10.00	0	20
0.13	1.0000	1.1111	1.3453	1.3453	1.3453	0.000	4	22.00	10.00	0	20
0.25	0.9000	1.0000	1.2305	0.8861	1.3453	18.660	4	19.00	10.00	2	20
0.5	0.5500	0.6111	0.8407	0.6847	1.1071	23.960	4	11.00	10.00	9	20
*1	0.1500	0.1667	0.4041	0.2255	0.4636	29.464	4	10.00	10.00	17	20

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.05)	0.905671	0.924	-0.71785	1.957951
Equality of variance cannot be confirmed				
The control means are not significantly different (p = 0.13)	1.732051	2.446912		

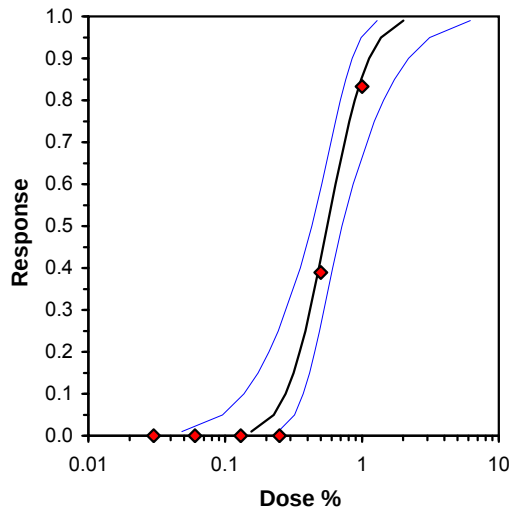
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	0.5	1	0.707107	200
Treatments vs Diluent Control				

Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits	
Slope	4.185474	0.990903	2.243305	6.127644
Intercept	6.060398	0.326545	5.42037	6.700427
TSCR	0.025408	0.01828	-0.01042	0.061236

Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
0.1	1.684102	9.487729	0.79	-0.25335	0.238922	5

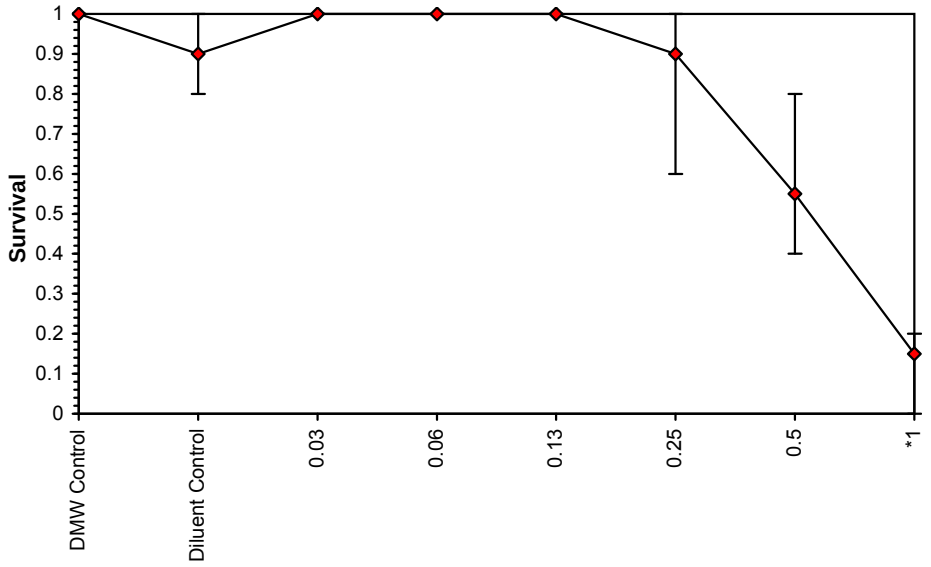
Point	Probits	%	95% Fiducial Limits	
EC01	2.674	0.155179	0.048118	0.244436
EC05	3.355	0.225765	0.095406	0.320557
EC10	3.718	0.275713	0.136695	0.372361
EC15	3.964	0.315516	0.173558	0.413563
EC20	4.158	0.35121	0.20909	0.451116
EC25	4.326	0.385033	0.244436	0.487789
EC40	4.747	0.48542	0.353546	0.608714
EC50	5.000	0.558018	0.429813	0.714266
EC60	5.253	0.641473	0.509122	0.860194
EC75	5.674	0.80872	0.642075	1.231103
EC80	5.842	0.886604	0.695959	1.435733
EC85	6.036	0.986903	0.760678	1.726198
EC90	6.282	1.129375	0.846273	2.188009
EC95	6.645	1.379237	0.984518	3.13022
EC99	7.326	2.006609	1.29287	6.197986



Fish Embryonic Development and Post-Hatch Survival Test-Survival

Start Date:	6/02/2013 15:15	Test ID:	PR0989/01	Sample ID:	RP3
End Date:	16/02/2013 15:15	Lab ID:	5823	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 126	Test Species:	MS-Melanotaenia splendida
Comments:					

Dose-Response Plot



ToxCalc v5.0.23

Reviewed by:_____

Fish Embryonic Development and Post-Hatch Survival Test-Survival

Start Date:	6/02/2013 15:15	Test ID:	PR0989/01	Sample ID:	RP3
End Date:	16/02/2013 15:15	Lab ID:	5823	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 126	Test Species:	MS-Melanotaenia splendida
Comments:					

		Auxiliary Data Summary					
Conc-%	**Parameter**	**Mean**	**Min**	**Max**	**SD**	**CV%**	**N**
DMW Control	% Survival	100.00	100.00	100.00	0.00	0.00	4
Diluent Control		90.00	80.00	100.00	11.55	3.78	4
0.03		100.00	100.00	100.00	0.00	0.00	4
0.06		100.00	100.00	100.00	0.00	0.00	4
0.13		100.00	100.00	100.00	0.00	0.00	4
0.25		90.00	60.00	100.00	20.00	4.97	4
0.5		55.00	40.00	80.00	19.15	7.96	4
1		15.00	0.00	20.00	10.00	21.08	4
DMW Control	pH	8.10	8.10	8.10	0.00	0.00	1
Diluent Control		6.90	6.90	6.90	0.00	0.00	1
0.03		6.80	6.80	6.80	0.00	0.00	1
0.06		6.80	6.80	6.80	0.00	0.00	1
0.13		6.70	6.70	6.70	0.00	0.00	1
0.25		6.70	6.70	6.70	0.00	0.00	1
0.5		6.60	6.60	6.60	0.00	0.00	1
1		6.50	6.50	6.50	0.00	0.00	1
DMW Control	Conductivity (uS/cm)	151.80	151.80	151.80	0.00	0.00	1
Diluent Control		17.20	17.20	17.20	0.00	0.00	1
0.03		19.50	19.50	19.50	0.00	0.00	1
0.06		21.60	21.60	21.60	0.00	0.00	1
0.13		25.00	25.00	25.00	0.00	0.00	1
0.25		31.40	31.40	31.40	0.00	0.00	1
0.5		43.20	43.20	43.20	0.00	0.00	1
1		66.10	66.10	66.10	0.00	0.00	1
DMW Control	DO (% sat)	105.80	105.80	105.80	0.00	0.00	1
Diluent Control		100.50	100.50	100.50	0.00	0.00	1
0.03		103.10	103.10	103.10	0.00	0.00	1
0.06		103.70	103.70	103.70	0.00	0.00	1
0.13		104.20	104.20	104.20	0.00	0.00	1
0.25		104.70	104.70	104.70	0.00	0.00	1
0.5		103.60	103.60	103.60	0.00	0.00	1
1		103.30	103.30	103.30	0.00	0.00	1

Appendix B – Chemistry Reports

Reference	Sample Description	Sample No.	Replicate	Date prepared	Aluminium Total	Cadmium Total	Cobalt Total	Chromium Total	Copper Total	Iron Total	Lead Total	Manganese Total	Mercury Total	Nickel Total	Zinc Total	Magnesium Total	Calcium Total	Sodium Total	Aluminium).45µm filtere	Cadmium).45µm filtere	Cobalt).45µm filtere
Units				-	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	mg/L	mg/L	mg/L	µg/L	µg/L	µg/L
PQL					10	0.1	1	1	1	10	1	5	0.05	1	1	0.5	0.5	0.5	10	0.1	1
Method					Metals-022 ICP-	tals-022 ICP-	tals-022 ICP-	tals-022 ICP-	tals-022 ICP-	tals-022 ICP-	tals-022 ICP-	tals-022 ICP-	tals-021 CV-A	tals-022 ICP-	tals-022 ICP-	:als-020 ICP-	;als-020 ICP-	;als-020 ICP-	tals-022 ICP-	tals-022 ICP-	tals-022 ICP-
84493	RP3 (sample)	1	0	23/01/2013	<10	180	1800	<1	4500	<10	100	23000	<0.05	2000	45000	240	350	56	<10	130	1300
84493	RP3 (sample)	1	1	23/01/2013	<10	190	1900	<1	4800	<10	100	25000	[NT]	2100	42000	240	340	56	<10	130	1300
84493	SW2 (diluent	2	0	23/01/2013	210	<0.1	<1	<1	<1	830	<1	<5	<0.05	<1	4	0.7	0.7	1.6	110	<0.1	<1

Chromium	Copper	Iron	Lead	Manganese	Mercury	Nickel	Zinc	Calcium	Magnesium	Hardness	Chloride, Cl	TSS	TDS	TS	›onate Alkalinonate Alkalinixide Alkalinity	˜otal Alkalinity	Sulphate, SO4	WAD CN	DOC
).45µm filtere	).45µm filtere	).45µm filtere	).45µm filtere	).45µm filtere	).45µm filtere	).45µm filtere	).45µm filtere	- Dissolved	0.45µm filtered)			@ 103-105°C	(grav)		$CaCO_3$	$CaCO_3$	as $CaCO_3$	as $CaCO_3$			
µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	mg/L	mg/L	$mgCaCO_{3/L}$	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
1	1	10	1	5	0.05	1	1	0.5	0.5	3	1	5	5	5	5	5	5	5	1	0.004	1
tals-022 ICP-	tals-022 ICP-	tals-022 ICP-	tals-022 ICP-	tals-022 ICP-	tals-021 CV-A	tals-022 ICP-	tals-022 ICP-	:als-020 ICP-	./:als-020 ICP-AES		Inorg-081	Inorg-019	Inorg-018	Inorg-041	Inorg-006	Inorg-006	Inorg-006	Inorg-006	Inorg-081	Inorg-083	Inorg-079
<1	3400	<10	65	19000	<0.05	1300	38000	300	200	1600	27	11	2000	2000	22	<5	<5	22	1800	<0.004	<1
<1	3400	<10	66	19000	[NT]	1300	38000	300	200	1600											
<1	<1	290	<1	<5	<0.05	<1	2	0.5	0.5	3	2	5	35	40	8	<5	<5	8	<1	<0.004	<1

TOC	Nitrate as N	hosphate as P
mg/L	mg/L	mg/L
1	0.005	0.005
Inorg-079	Inorg-055	Inorg-060
3	1.6	<0.005
3	0.008	<0.005

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Document Status

Rev No.	Author	Reviewer		Approved for Issue		
		Name	Signature	Name	Signature	Date
A	J. Woodworth	G. Metcalfe		K. Fitzpatrick		19/02/13
0	J. Woodworth	K. Fitzpatrick		K. Fitzpatrick		28/02/13

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Vista Gold Australia Pty Ltd

Mt Todd Discharge

Treated Retention Pond 3 Ecotoxicological Report

July 2013

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Appendices

1. Introduction

1.1 Project Background

Vista Gold Australia Pty Ltd (Vista Gold) received a Waste Discharge Licence (WDL 178-2) on 5 February 2013 from the Northern Territory Environment Protection Authority (NT EPA). The WDL outlines environmental requirements for protection of the Edith River from mine wastewater discharges. Previously, Vista Gold derived interim site specific trigger values (ISSTVs) (GHD 2012) to meet the 95% species protection trigger values downstream of the site at SW10 (8.7 km from SW4) to meet the requirements of WDL 178-1. However, the use of dilution factors derived from direct toxicity assessment (DTA) for three water bodies at the Mt Todd mine site have been selected as the most appropriate method for deriving safe concentrations of metals for environmental protection of the Edith River to meet the requirements of WDL 178-2.

The DTA method is recommended by ANZECC & ARMCANZ (2000) where chemicals are present in a complex mixture. DTA allows the determination of direct biological effects on appropriate species to be assessed and also enables metal concentrations to be established that would not cause adverse environmental effects.

DTA has been used throughout Australia to establish appropriate dilution factors for complex effluents. For example the Perth Desalination Plant (Geotechnical Services 2006, 2008) provided dilution factors to be met at the 80% species protection level within the Low Protection Area at the outfall, and the 90% species protection level at Moderate Protection Area in Cockburn Sound. All other desalination plants around Australia have used the DTA methodology to derive appropriate dilution factors and engineer the outfall configuration to meet the required dilutions (BHP Billiton 2009, Victorian Government 2008).

This report should be read in conjunction with the Waste Discharge Plan (GHD 2013) (http://mttodd.com.au/content/waste-discharge-licence).

1.2 Objective

WDL 178-2 requires that ecotoxicological assessment be conducted for a wastewater source. This treated retention pond (RP) 3 Ecotox DTA Report has been developed to address the relevant sections of the WDL 178-2 to derive a dilution factor for RP3 prior to discharging treated mine water into the Edith River.

1.3 Scope of Work

This Report addresses the following aspects of the WDL 178-2:

- The methodology for calculating the 80% species protection ANZECC & ARMCANZ (2000) dilution factor for ecosystem protection applied at SW4 for mine discharges from RP3.
- Deriving Monitoring Values for the treated RP3 mine water to be met at SW4 to check that the dilution factor for the treated mine water is being met.

1.4 Limitations

This Vista Gold Australia Retention Pond 3 Ecotoxicological Report ("Report"):

1. *has been prepared by GHD Pty Ltd ("GHD") for Vista Gold Australia Pty Ltd (Vista Gold) and the NT EPA;*
2. *may only be used and relied on by Vista Gold and the NT EPA;*
3. *must not be copied to, used by, or relied on by any person other than Vista Gold without the prior written consent of Vista Gold;*
4. *may only be used for the purpose of addressing WDL 178-2 requirements (and must not be used for any other purpose).*

GHD and its servants, employees and officers otherwise expressly disclaim responsibility to any person other than Vista Gold arising from or in connection with this Report.

To the maximum extent permitted by law, all implied warranties and conditions in relation to the services provided by GHD and the Report are excluded unless they are expressly stated to apply in this Report.

The services undertaken by GHD in connection with preparing this Report were limited to those specifically detailed in section 1.3 of this Report.

The opinions, conclusions and any recommendations in this Report are based on assumptions made by GHD when undertaking services and preparing the Report ("Assumptions"), including (but not limited to) those specified in section 1.5 below.

GHD expressly disclaims responsibility for any error in, or omission from, this Report arising from or in connection with any of the Assumptions being incorrect.

Subject to the paragraphs in this section of the Report, the opinions, conclusions and any recommendations in this Report are based on conditions encountered and information reviewed at the time of preparation and may be relied on until 6 months, after which time, GHD expressly disclaims responsibility for any error in, or omission from, this Report arising from or in connection with those opinions, conclusions and any recommendations.

1.5 Assumptions

The assumptions upon which this report is based are:

- The treated mine water tested for DTA was representative of the RP3 water at the time of testing; and
- SW2 water used in the DTA was representative of late wet season Edith River water.

2. Direct Toxicity Assessment Methodology

2.1 Direct Toxicity Assessment

Direct Toxicity Assessment (DTA) provides an integrated measure of the toxicity of chemicals within a complex mixture, and accounts for interactions between compounds which may be additive, synergistic or ameliorative. DTA provides a better representation of natural environmental conditions than single chemical testing, and is therefore recommended by ANZECC & ARMCANZ (2000) for assessing the environmental impact from complex effluents.

The Mt Todd DTA program was designed in line with the recommendations contained in ANZECC & ARMCANZ (2000). ANZECC & ARMCANZ (2000) states that a DTA program should address the following design considerations:

- Test species selection
- Receiving water selection
- Nature of contaminant(s)
- Test methods
- Statistical considerations.

2.1.1 Selected Species

ANZECC & ARMCANZ (2000) "Minimum requirements for DTA" (section 8.3.6.8) recommend that toxicity data from between three and five species representing at least four different taxonomic groups (where five species are used) is required for effluent DTA. Further information on species used in the DTAs conducted for Mt Todd discharge to the Edith River is shown in Table 1 below.

Often, due to laboratory issues, not all tests listed in Table 1 are available at the time of testing. DTAs conducted on RP1, RP7 and RP3 Pilot Trial used a maximum of seven species to provide confidence in the species sensitivity distribution calculation.

Tests were selected adhering to the following criteria:

- a minimum of five bioassays covering at least four taxa
- preferably chronic endpoints to avoid the used of application factors
- locally occurring species or species representative of the ecosystem
- dilution water sampled from SW2 (upstream site)
- sufficient test dilutions to obtain a statistically valid EC10 (i.e. tight 95% confidence limits)
- bioassay suite sensitive to a broad range of contaminants.

The bioassays were conducted in two laboratories because not all bioassays are available from one laboratory. The cladoceran (*Moinodaphnia macleayi*) is the most sensitive bioassay species and is only able to be conducted by the ERISS Laboratory in Darwin. All other bioassays are conducted by Ecotox Services Australasia located in Sydney as shown in Table 1. The fish larval development was not used in this suite of bioassays due to unavailability of suitable numbers and quality of fish eggs.

Table 1 Species used in the Ecotoxicological Assessment of Treated RP3 Discharge

Test Organism	Test Duration	Test Endpoint	Key Reference	Laboratory
Chlorella vulgaris (green alga)	72 hour (chronic)	Growth inhibition	USEPA Method 1003.0 (2002)	Ecotox Services
Lemna aequinoctialis (duckweed)	96 hour (chronic)	Growth (frond number)	OECD Method 221 (2006)	Ecotox Services
Moinodaphnia macleayi (water flea)	3 brood (chronic)	Reproduction	Riethmuller *et. al.* (2003)	ERISS
Hydra viridissima Pallas (green hydra)	96 hour (chronic)	Population growth	Riethmuller *et al.* (2003)	Ecotox Services
Chironomus tepperi (chironomid)	48 hour (acute)	Survival	USEPA (2002), OECD (2011)	Ecotox Services
Macrobrachium bullatum (shrimp)	96 hour (acute)	Survival	ESA SOP 123 (ESA 2012)	Ecotox Services

2.1.2 Concentrations Tested

Edith River water sampled from the upstream site SW2 was used as the diluent to assess the toxicity of the treated RP3 mine water. A laboratory control was run concurrently with all tests to confirm that the natural diluent water was not confounding the bioassays (as can happen in many cases). All samples were serially diluted with the SW2 river water to achieve the required test concentrations. Concentrations differed between bioassays due to methodologies used and sensitivity of the species tested as determined by previous bioassays, as shown in Table 2.

Table 2 Concentrations of Treated RP3 used in Bioassays

Microalga % RP3	Duckweed % RP3	Cladoceran % RP3	Hydra % RP3	Chironomid % RP3	Shrimp % RP3
0	0	0	0	0	0
6.3	3.1	0.03125	6.3	6.3	6.3
12.5	6.1	0.0625	12.5	12.5	12.5
25	12.1	0.125	25	25	25
50	24.2	0.25	50	50	50
100	48.4	0.5	100	100	100
-	96.8	1.0	-	-	-
-	-	2.0	-	-	-
-	-	4.0	-	-	-
-	-	8.0	-	-	-

2.1.3 Dilution Factors

Dilution factors are derived from results of DTA using bioassays representative of the receiving ecosystem which incorporates all actions of the constituents of the effluent. These results are placed in the BurrliOZ (Campbell *et al.* 2000) statistics program for calculation of a species sensitivity distribution and the concentration of effluent is determined to protect 80% of the populations in the receiving water from a 10% decrease in growth and / or reproduction.

Concentrations of individual chemicals cannot be extrapolated from DTAs for use as trigger values. However, individual chemical concentrations can be used for monitoring purposes to ensure that the dilution factors are met at the appropriate monitoring site. If the dilution factors are not met, the investigation procedure shown in Figure 1 of the Vista Gold Discharge Plan (GHD 2013) will be followed.

2.2 Surface Water Chemistry

Samples from SW2 (diluent) and treated RP3 water were analysed for the suite of analytes shown in Table 3.

Table 3 Analytes for SW2 and Treated RP3

	Analytes
In-Situ	DO, temperature, EC, pH
Metals (total and dissolved i.e. 0.45 µm)	Al, Cd, Co, Cu, Cr, Fe, Pb, Mg, Mn, Hg, Ni, Zn
Others	NO3, PO4, SO4, bicarbonate, alkalinity, hardness, TDS, TSS, TS, Na, Cl, Ca, WAD cyanide, TOC and DOC

2.3 Sample Sites

RP3 and SW2 sampling locations are shown in Table 4. The treated RP3 water was sampled at 10 metres below the RP surface. This depth is the proposed pumping depth for discharge and provides treated water with the highest pH and lowest metal concentrations.

Table 4 Sampling Sites

Site Name	Site Description	Easting (UTM)	Northing	Latitude (degree, decimal)	Longitude
SW2	Edith River at bridge on Edith Falls Road	0189088	8431347	-14.1718	132.12
RP3	Batman Pit	0187055	8434993	-14.1388	132.1015

3. Results

3.1 Ecotoxicology Testing of RP3 Treated Water (in-situ)

The species used were appropriate for the site and the EC10 and LC50 results were of sufficient quality to be used in the BurrliOZ (Campbell *et al.,* 2000) program for species sensitivity distribution (SSD) calculations. Not all species that were used in the ERISS (2005) toxicity testing program were available at the time of testing.

A copy of the Ecotox Report by Ecotox Services, "*Toxicity Assessment of a Treated Water Sample, March 2013*" and the ERISS Test Report are located in Appendix A. A summary of the results is provided in Table 5.

The results of the site specific DTA testing performed by Ecotox Services used in this assessment do meet the laboratory NATA requirements for quality assurance/quality control (QA/QC) parameters. It must be noted however, that the majority of the reference toxicant results are in the lower 10% of the Cusum chart limits, indicating that the organisms used were more sensitive to the toxicants than is usual (Appendix A). This sensitivity means that the results presented here from this treated RP3 DTA are very conservative.

ERISS does not hold NATA accreditation, but does perform similar QA/QC on toxicity tests, as is required for NATA accreditation.

Table 5 Summary of Treated RP3 Ecotox Results

Test	EC/IC10 and LC10 (95% confidence limits) % Treated RP3	EC/IC50 and LC50 (95% confidence limits) % Treated RP3
Microalgal 72-hour growth inhibition	3.1*	>100
Duckweed 96-hour growth	24.2 *	75.4 **
Cladoceran 6-day reproduction	4.1 **	6.8 **
Hydra 96-hour growth	61.7 (51.6-66.7)	>100
Chironomid 48-hour survival	100	>100
Shrimp 96-hour survival	100	>100

**No observable effect concentration (NOEC)*
***95% confidence limits not reliable,*

The results show that the algae and cladoceran are the most sensitive species to the treated RP3 water. There was no acute toxicity observed from treated RP3 water.

The cladoceran is sensitive to the treated RP3 water, which may be a reflection of the toxicity of the treated RP3 water to their food source (i.e. the unicellular alga).

The chemistry results shown in Table 8 indicate that the toxicity observed in the algal and cladoceran bioassays could not be attributed to the presence of metals, as the majority of toxic metals were below detection limits and below the 95% species protection default trigger values.

3.2 Acute to Chronic Ratios (ACR)

Two of the bioassays used in this study were acute bioassays where survival was the end point. The chironomid and shrimp are acute bioassays and the LC50 results need to be adjusted so that they can be incorporated into the statistics to calculate the SSD. ANZECC & ARMCANZ (2000) recommend an acute to chronic factor of 10 to go from an acute LC50 to a chronic NOEC or EC10. This acute to chronic ratio (ACR) was applied to the chironomid and shrimp LC50 data to obtain a conservative chronic EC10.

3.3 Concentrations used to calculate the SSD

The acute bioassays required an ACR adjustment, applied to the LC50 values for the chironomid (LC50/10) and shrimp (LC50/10). The values used to calculate the SSD and dilution factors are shown in Table 6.

Table 6 Values used to Calculate SSD

Bioassay	EC10 % RP3
Microalgal 72-hour growth inhibition	3.1
Duckweed 96-hour growth inhibition	24.2
Cladoceran 6-day reproduction	4.1
Hydra 96-hour growth	61.7
Chironomid 48-hour survival	10
Shrimp 96-hour survival	10

3.4 Species Protection Values

The EC10 and LC50/10 values from Table 6 were input into the BurrliOZ (Campbell *et al*., 2000) statistics program for calculation of the 80% species protection value using the SSD. The calculated value and the dilution factor required to reach the concentration are provided in Table 7.

Table 7 Species Protection Values

Species Protection Level	Concentration of Treated RP3 (%)	Dilution Factor
80%	5.1	1:20

3.5 Dilution Factor

The 80% species protection was selected as appropriate to provide a level of protection for the Edith River from the discharge of treated RP3 water over the dewatering period as required by the WDL 178-2. A dilution factor of 1:20 for the RP3 treated water (as at time of testing, 21/03/13) discharged into the Edith River will provide 80% of the species with protection from a 10% decrease in reproduction or growth (Table 7). The concentrations of chemicals at this dilution are shown in Table 8.

3.6 80% Species Protection Monitoring Values

The chemistry of the RP3 treated water at the 1:20 dilution is shown in Table 8. The complete chemistry results are located in Appendix B.

The levels of the 80% species protection trigger values for copper and zinc have been selected as monitoring values to be met to ensure that the dilution of the treated RP3 water is at 1:20 to meet the requirements of WDL 178-2 as the concentrations of other metals and metalloids in

the discharge will be below the 95% species protection trigger values at SW4 (with the exception of magnesium) and also below the laboratory detection limits in most cases.

Magnesium is above the designated trigger value for environmental protection of the Edith River, however, the presence of calcium in the treated RP3 water will ameliorate any toxicity threat posed by the elevated magnesium concentrations, as van Dam *et al*. (2010) have shown that elevated calcium reduced the toxicity of magnesium to three tropical freshwater species.

The concentrations of copper at 2.5 µg/L and zinc at 31 µg/L (80% species protection default trigger values) will be met at SW4 to ensure environmental protection of the Edith River. If this monitoring value is not met then action is triggered as shown in Figure 1 of the Discharge Plan (GHD 2013).

Table 8 Dilution Rates of RP3 Treated Water (in-situ) to Meet 80 % SSD Dilution (1:20)

Analytes (metals 0.45 µm)	SW2 13/03/13	RP3 Treated Water 21/03/13	Conc. in RP3 treated water at 1:20 to be met at SW4	ANZECC 80% species protection TVs/ISSTV	ANZECC 95% species protection TVs/ISSTV
pH	7.5	8.8	-	6-8	6-8
DO (%)	97.4	98.0	-	85-120	85-120
Conductivity (µS/cm)	19	2710	-	20-250	20-250
Mg (mg/L)	0.8	94	4.7	-	2.5*
Ca (mg/L)	0.5	490	24.5	-	-
SO_4 (mg/L)	<1	1,700	85	129	129
Al (µg/L)	29	<10	<10	150	149
Cd (µg/L)	<0.1	0.3	<0.1	0.8	0.2
Co (µg/L)	<1	2.0	<1	90	90
Cr (µg/L)	<1	2.0	<1	40	1.0
Cu (µg/L)	<1	<1.0	<1	2.5	1.4
Fe (µg/L)	270	<10	<10	300	300
Mn (µg/L)	<5	<5.0	<5	3,600	1,700
Ni (µg/L)	<1	13	<1	17	11
Pb (µg/L)	<1	<1.0	<1	9.4	3.4
Hg (µg/L)	<0.05	<0.05	<0.05	5.4	0.6
Zn (µg/L)	1	6.0	<1	31	8.0

*Note. Cells in green are below the ISSTVs and ANZECC & ARMCANZ (2000) default 95% trigger values and below the laboratory detection limits *van Dam et al (2010)*

Table 9 shows the Monitoring Values for each discharge point as of 21 March 2013.

Table 9 Monitoring Values for SW4 (March 2013)

Discharge	Chemical	Concentration (µg/L) 0.45 µm filtered
RP3 Treated Water	Cu	2.5
	Zn	31
RP1 Untreated Water	Cu	4.3
RP7 Untreated Water	Cu	4.2

RP3 treated water will be assessed for toxicity prior to the 2013/2014 wet season and a revised dilution factor and revised Monitoring Values will be calculated. RP1 and RP7 mine waters will also be assessed for toxicity prior to the 2013/2014 wet season and revised dilution factors and Monitoring Values will be calculated.

4. Conclusions and Recommendations

4.1 Conclusions

Ongoing treatment of water in RP3 has resulted in substantial improvements in water quality since treatment began in November 2012 (Table 10).

The results of this direct toxicity assessment on treated water from RP3 show that a conservative dilution factor of 1:20 will provide an 80% species protection level at the downstream monitoring point SW4 on the Edith River. That is, one unit of RP3 water is to be diluted with 20 units flowing down the Edith River to mitigate any adverse impact on organisms within the receiving ecosystem. All metal concentrations will be below the 95% species protection default trigger values at SW4 at this dilution.

Table 10 RP3 Chemistry (Top 15 metres)

Analyte Dissolved µg/L	20 Oct 12	23 Nov 12	20 Dec 12	17 Jan 13	14 Feb 13	21 Mar 13
Aluminium	57,000	31,000	1,700	270	<10	<10
Chromium	2	2	1	<1	<1	2
Copper	10,000	11,000	9,900	5,400	140	<1
Lead	210	190	160	86	<1	<1
Cadmium	140	140	140	130	45	0.3
Zinc	36,000	34,000	38,000	39,000	8,400	6

Treatment of RP3 is on-going and the water quality is constantly improving with elevated pH and reduced metal concentrations (Table 10). The dilution factor of 1: 20 calculated in this report will be applied until the results of the next DTA become available prior to discharging for the 2013/2014 wet season.

4.2 Recommendations

To meet the 80% species protection level required by WDL 178-2, based on ecotoxicological testing, it is recommended that a dilution factor of 1:20 be applied to the discharge of RP3 treated mine water, with the following Monitoring Values applied at SW4:

- Cu 2.5 µg/L
- Zn 31.0 µg/L

These monitoring values will apply until a further round of ecotoxicological testing is conducted to derive a new dilution factor and Monitoring Values prior to discharging in the 3013/2014 wet season.

5. References

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Appendices

Appendix A – Ecotox Reports

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Cladoceran Reproduction Test Report (1324D & 1333D)

Client: Vista Gold Pty Ltd.

Project: Ecotoxicological assessment of Mt Todd Gold Mine discharge waters

Test Performed: BTT-D, 3 brood reproduction test using *Moinodaphnia macleayi*

Samples Collected:	March 2013		
Samples Received:	14th March 2013 (SW2) 22nd March 2013 (Treated RP3 water)	**Tests Initiated:**	25th March 2013 and 20th April 2013
ERISS Sample ID.	**Sample Name**	**Sample Description**	
Reference water	Edith River (SW2)	Edith River upstream reference site	
Toxicant	Treated RP3 water (RP3)	Lime treated water from Retention Pond 3	

Comments: The test 1333D was a repeated test due to an unexpectedly low IC10 value. SW2 and treated RP3 waters were received and filtered on 14th March 2013. On the 22st March 2013, a new batch of Treated RP3 water arrived. This was filtered on 22nd March and used for both tests. All waters were stored at 4°C until use.

Sample Physico-Chemistry and Preparation:

Sample	**Physico-chemistry**			
	pH	**EC** [a]	**DO** [b]	**DOC** [c]
QA Magela Creek water control	5.8	10	98	3.3
Edith River – reference water	6.7 [e]	22 [e]	112.7 [e]	2.3
Mt Todd treated RP3 water	8.9	2670	93	-47.5 [d]

[a] EC = Electrical Conductivity (µS cm^{-1}) unless other units provided; [b] DO = Dissolved Oxygen (% saturation); [c] DOC = Dissolved organic carbon (mg L^{-1}); [d] Inorganic carbon = 47.8mg/L due to added carbonates. [e] Values based on water quality measurements from the start of test 1324D

Test Method: Cladoceran neonates (<6 h old) were exposed to a Magela Creek water QA control, a reference/control water (Edith River, SW2) and up to 9 concentrations of Mt Todd Mine treated RP3 water, diluted with the reference water, for a period of 144 h. All waters were filtered (3 µm pore size) prior to testing.

The test was completed when >80% of control cladocera had released their third brood offspring. Observations of the appearance and number of neonates produced by each cladoceran were recorded at 24 h intervals, after which the cladocera were transferred to fresh test solution containing food. The detailed test protocol is presented in Riethmuller et al. (2003).

Linear interpolation analysis was used to determine point estimates of Inhibitory Concentrations (ICs) that reduced neonate production by 10% and 50% (i.e. IC_{10} and IC_{50}) relative to the reference site control responses (CETIS v1.8.1.2).

Comments: A larger concentration range was achieved by reducing treatment replication from 10 to 5 per treatment. Control replication was not modified (i.e. 10 replicates). This allows greater characterisation of the concentration-response of the cladoceran.

Results [a]

	Test 1 (1324D)		**Test 2 (1333D)**	
Treated RP3 water (%)	**Mean neonate production ±**	**% of Reference**	**Mean neonate production ±**	**% of Reference**
QA Magela Creek water control	26.9 ± 3.5	82	31.5 ± 3.1	121
SW2 - Reference water	33.0 ± 1.4	100	26.1 ± 4.2	100
0.03	33.8 ± 0.9	102	Not tested	
0.06	34.4 ± 0.7	104	13.2 ± 6.8	50
0.13	24.3 ± 5.7	74	30.6 ± 2.7	117
0.25	26.6 ± 4.3	81	35.8 ± 1.2	137
0.5	35.8 ± 0.9	108	20.2 ± 7.5	77
1.0	20.0 ± 5.5	61	25.4 ± 6.9	97
2.0	28.0 ± 7.0	85	26.6 ± 6.0	101
4.0	28.0 ± 5.0	85	24.6 ± 6.5	94
8.0	24.2 ± 2.9	73	9.6 ± 3.9	36

Toxicity Estimates

Test Number	**IC10 (95% Confidence**	**IC50 (95% Confidence limits)**
Test 1 (1324D)	0.1 (0.07 – 1.28) % RP3	>8.0 (N/A) % RP3 water
Test 2 (1333D)	4.1 (NC [b] – 4.7) % RP3	6.8 (1.7 - NC) % RP3 water

[a] See Attachment A for test raw data and statistical analysis report. [b] Not calculable.

Comments

Test 1 (1324D)

There were no observed effects to individuals exposed to 0.03% and 0.06% treated RP3 water. Individuals exposed to 0.13 and 0.25% treated RP3 produced 36 and 19% less neonates compared to the SW2 control, respectively. Individuals exposed to 0.5% treated RP3 waters produced slightly more neonates compared to the control. All individuals exposed to ≥1.0% treated RP3 water produced less than the controls.

The variability in the results were due to delayed reproduction where exposed groups did not produce all of their 3rd brood before test termination (i.e. when ≥80% control cladocera had released their third brood offspring). Despite the reduction in brood number, individuals exposed to 4.0% treated RP3 water appeared to be morphologically healthy. Individuals exposed to 8.0% treated RP3 water also appeared healthy in the early stages of the test, having produced reasonable of neonates in the first and second broods. However, during the final 48 h of testing, one individual died and all others were immobile and appeared to be effected by oedema and haemorrhaging. Reproductive delay was clearly evident in this treatment with only one individual producing a 3rd brood. All other individuals had empty brood pouches therefore were considered unlikely to produce a third brood even if given additional time to do so. This severe adverse response provides evidence that the delayed reproduction detected between the 0.13 and 4.0% treatments was a true effect.

Test 2 (1333D)

An repeat test was conducted but due to the extended age of the SW2 water, there was >20% mortality in the SW2 control water. There were also random deaths throughout the treatments, which resulted in the 0.06% treatments producing 50% less neonates compared to the SW2 control and the 0.13 and 0.25% treatments producing more neonates than the SW2 control. However, there was a clear reduction in the health and reproduction of the individuals exposed to 8% treated RP3 water.

		Test 1 (1324D)			**Test 2 (1333D)**		
Quality Control *	**Criterion**	**NMC W**	**SW2**	**Criterion Met for SW2?**	**NMC W**	**SW2**	**Criterion Met for SW2?**
Mean # neonates in	>30	26.9	33	No	31.5	26.1	Yes
Control survival	>80%	90%	90%	Yes	80%	70%	No
pH	<1 unit	<1	1.6	No	<1	1.5	No
DO (throughout test)	>70%	>70%	>70%	Yes	>70%	>70%	Yes
EC	<10% or < 3 µS cm^{-1}	<10%	<10%	Yes	<10%	<10%	Yes
Reference toxicant EC50 (µg L^{-1} U)		±2 SD of running mean ie. 82±145			171		Yes

Comments

1324D

The pH in SW2 control water changed by > 1 unit over a 24 h period for both tests. This may have been due to the physico-chemical nature of the water possibly contributing to algal growth. The green alga, *Chlorella* sp., is a standard food and may be the source of pH increase because it removes carbon dioxide from the water. The pH change of the NMCW control was within the acceptable criteria of <1 unit. In test 1324D, NMCW control produced less than the acceptability criterion of >30 neonates but the SW2 reference water reached the criterion for both survival and neonate number.

1333D

In test 1333D, SW2 control did not meet the acceptability criterion of >30 neonates or survival of >80% of the organisms. This was likely due to the age of the water, which may have affected water quality. There was also pH increase of 1.5 pH units in the SW2 control, which is over the acceptability criteria. but replicated the result in the first test. NMCW QC control for this test met the criterion.

* See Attachment B for detailed test QC physico-chemical data.

References:

Riethmuller N, Camilleri C, Franklin N, Hogan AC, King A, Koch A, Markich SJ, Turley C & van Dam R 2003. *Ecotoxicological testing protocols for Australian tropical freshwater ecosystems*. Supervising Scientist Report 173, Supervising Scientist, Darwin NT. http://www.environment.gov.au/ssd/publications/ssr/pubs/ssr173-print-quality.pdf

Test carried out by:	Kim Cheng and Alicia Hogan
Test supervised by:	Rick van Dam and Andrew Harford
Test report prepared by:	Kim Cheng
	Professional Officer (ph: 08 8920 1361)
Test report authorised by:	Andrew Harford
	A/g Program Leader (ph: 08 8920 1175)
Date:	17/05/13

Attachment A1 – Test raw data and statistical analysis report (1324D)

CETIS Analytical Report

Report Date:	02 Apr-13 09:07 (p 1 of 2)
Test Code:	1324D | 15-5743-7365

Cladoceran Reproduction Test — **eriss ecotoxicology lab**

Analysis ID:	20-1744-2922	**Endpoint:**	Total neonates	**CETIS Version:**	CETISv1.8.7
Analyzed:	02 Apr-13 9:05	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Batch ID:	09-5414-2342	**Test Type:**	Cladoceran reproduction	**Analyst:**	Kim Cheng
Start Date:	25 Mar-13	**Protocol:**	Clad (chronic) eriss tropical freshwater	**Diluent:**	Upstream of Discharge
Ending Date:	31 Mar-13	**Species:**	Moinodaphnia macleayi	**Brine:**	Not Applicable
Duration:	6d 0h	**Source:**	In-House Culture	**Age:**	

Sample ID:	09-2982-9698	**Code:**	1324D	**Client:**	Vista Gold
Sample Date:	02 Apr-13 08:53	**Material:**	RP3	**Project:**	Mt Todd
Receive Date:	29 Mar-13	**Source:**	RP3		
Sample Age:	NA	**Station:**			

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	1365825	200	Yes	Two-Point Interpolation

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Extreme Value	Grubbs Extreme Value	3.586	3.159	0.0071	Outlier Detected

Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
IC5	0.08383	0.0587	0.7576	1193	132	1704
IC10	0.1056	0.07498	1.151	947	86.85	1334
IC15	0.5257	N/A	6.517	190.2	15.35	NA
IC20	0.7492	N/A	N/A	133.5	NA	NA
IC25	4.087	N/A	N/A	24.47	NA	NA
IC40	>8	N/A	N/A	<12.5	NA	NA
IC50	>8	N/A	N/A	<12.5	NA	NA

Total neonates Summary

			Calculated Variate						
C-%	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Upstream Contro	10	33	22	37	1.382	4.372	13.25%	0.0%
0.03125		5	33.8	32	37	0.8602	1.924	5.69%	-2.42%
0.0625		5	34.4	32	36	0.6782	1.517	4.41%	-4.24%
0.125		4	24.25	12	37	5.662	11.32	46.7%	26.52%
0.25		5	26.6	18	37	4.297	9.607	36.12%	19.39%
0.5		5	35.8	33	38	0.8602	1.924	5.37%	-8.49%
1		5	20	0	34	5.523	12.35	61.75%	39.39%
2		5	28	0	37	7.029	15.72	56.13%	15.15%
4		5	28	8	34	5.03	11.25	40.17%	15.15%
8		5	24.2	19	35	2.853	6.38	26.36%	26.67%

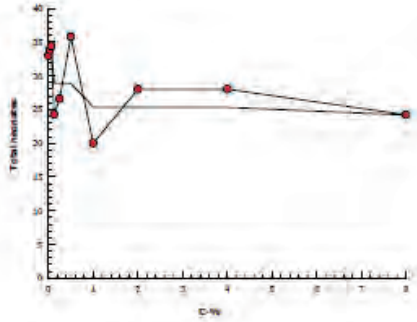
Total neonates Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Upstream Control	31	35	36	22	36	35	34	37	33	31
0.03125		33	33	37	34	32					
0.0625		32	35	36	35	34					
0.125		12	30	18	37						
0.25		37	18	19	22	37					
0.5		36	38	33	37	35					
1		22	21	34	0	23					
2		35	0	33	35	37					
4		33	31	8	34	34					
8		23	24	35	19	20					

Cladoceran Reproduction Test — eriss ecotoxicology lab

Analysis ID:	20-1744-2922	**Endpoint:**	Total neonates	**CETIS Version:**	CETISv1.8.7
Analyzed:	02 Apr-13 9:05	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Graphics



Attachment A2 – Test raw data and statistical analysis report (1333D)

CETIS Analytical Report

Report Date:	02 May-13 11:52 (p 1 of 2)
Test Code:	1333D | 06-5138-4157

Cladoceran Reproduction Test — **eriss ecotoxicology lab**

Analysis ID:	07-9367-4409	**Endpoint:**	Total neonates	**CETIS Version:**	CETISv1.8.7
Analyzed:	02 May-13 11:51	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Batch ID:	02-6379-8349	**Test Type:**	Cladoceran reproduction	**Analyst:**	Andrew J Harford
Start Date:	20 Apr-13 13:30	**Protocol:**	Clad (chronic) eriss tropical freshwater	**Diluent:**	Upstream of Discharge
Ending Date:	26 Apr-13 13:30	**Species:**	Moinodaphnia macleayi	**Brine:**	Not Applicable
Duration:	6d 0h	**Source:**	In-House Culture	**Age:**	

Sample ID:	09-4476-6604	**Code:**	384FFE8C	**Client:**	Vista Gold
Sample Date:	22 Mar-13 16:17	**Material:**	RP3	**Project:**	Mt Todd
Receive Date:	25 Mar-13 09:00	**Source:**	RP3		
Sample Age:	28d 21h	**Station:**			

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	1921000	200	Yes	Two-Point Interpolation

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Extreme Value	Grubbs Extreme Value	2.236	3.128	1.0000	No Outliers Detected

Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
IC5	0.3929	N/A	6.147	254.5	16.27	NA
IC10	4.085	N/A	4.696	24.48	21.3	NA
IC15	4.363	N/A	5.043	22.92	19.83	NA
IC20	4.656	N/A	5.412	21.48	18.48	NA
IC25	4.964	N/A	6.156	20.14	16.24	NA
IC40	5.996	0.6428	N/A	16.68	NA	155.6
IC50	6.782	1.722	N/A	14.75	NA	58.06

Total neonates Summary

			Calculated Variate						
C-%	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Upstream Contro	10	26.1	0	40	4.244	13.42	51.42%	0.0%
0.0625		5	13.2	0	31	6.793	15.19	115.1%	49.43%
0.125		5	30.6	21	35	2.731	6.107	19.96%	-17.24%
0.25		5	35.8	33	39	1.2	2.683	7.5%	-37.16%
0.5		5	20.2	0	36	7.539	16.86	83.46%	22.61%
1		5	25.4	3	40	6.925	15.49	60.97%	2.68%
2		5	26.6	6	36	5.98	13.37	50.27%	-1.92%
4		5	24.6	6	42	6.462	14.45	58.74%	5.75%
8		5	9.6	0	19	3.932	8.792	91.58%	63.22%

Total neonates Detail

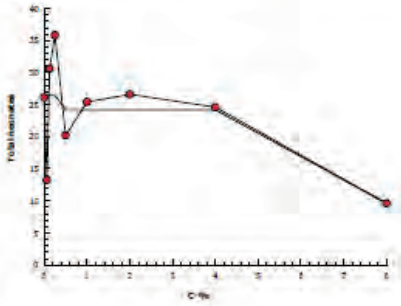
C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Upstream Control	40	20	30	35	0	37	20	37	33	9
0.0625		0	31	0	7	28					
0.125		35	21	28	34	35					
0.25		39	33	37	33	37					
0.5		32	4	29	0	36					
1		32	3	16	36	40					
2		36	20	35	6	36					
4		18	21	42	6	36					
8		0	19	2	9	18					

000-428-181-1 CETIS™ v1.8.7.4 Analyst:_____ QA:_____

Cladoceran Reproduction Test — eriss ecotoxicology lab

Analysis ID:	07-9367-4409	**Endpoint:**	Total neonates	**CETIS Version:**	CETISv1.8.7
Analyzed:	02 May-13 11:51	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Graphics



Attachment B1 - Physico-chemical measurements of the test solutions for test 1324D

Treatment (%)		MCW		SW2		0.03125		0.0625		0.125		0.25		0.5		1.0		2.0		4.0		8.0	
Parameter		**0 h**	**24 h**	**0 h**	**24 h**	**0 h**	**24 h**	**0 h**	**24 h**	**0 h**	**24 h**	**0 h**	**24 h**	**0 h**	**24 h**	**0 h**	**24 h**	**0 h**	**24 h**	**0 h**	**24 h**	**0 h**	**24 h**
Day 0	pH	6.05	6.63	6.7	8.16	6.71	7.93	6.73	7.89	6.77	8.01	6.77	7.96	6.78	8.17	6.84	8.24	6.85	8.34	6.99	8.23	7.11	8.54
	EC (µS cm^{-1})	12	12	21	21	23	21	23	23	26	27	32	33	44	44	65	65	107	106	185	184	338	339
	DO (%)	110.7	94.7	112.7	95.9	109.2	94.4	108.1	92.2	107.9	92	103.9	93.6	104.3	95.3	101	94.6	106	94.4	102	94.3	97.7	95
	Temp (°C)	22.5	21.4	22.3	21.4	22.2	21.4	22.1	21.3	21.7	21.2	21.6	21.5	21.3	21.5	21.1	21.4	21	21.3	20.8	21.2	20.8	21.2
Day 1	pH	5.82	6.65	6.82	8.27	6.78	7.99	6.81	8.19	6.81	8.83	6.84	8.27	6.84	8.37	6.88	8.34	6.92	8.47	7.01	8.37	7.06	8.49
	EC (µS cm^{-1})	13	12	21	21	23	22	25	24	28	27	33	32	44	45	66	66	107	108	186	185	335	339
	DO (%)	103.5	94	106.7	97.7	106.3	96.2	104.5	93	104.6	95	104.4	97.6	102.7	98.6	102.8	98.5	102.4	94.1	104	89.2	105.6	99
	Temp (°C)	21.8	24.3	21.7	24.6	21.7	24.4	21.7	24.4	21.7	24.3	21.1	23.8	20.6	23.9	20.6	23.9	20.5	23.5	20.3	23.4	20.3	23.1
Day 2	pH	6.07	6.39	6.89	7.54	6.85	7.65	6.75	7.55	6.68	7.84	6.86	7.72	6.86	7.66	6.89	7.87	6.92	7.75	6.97	7.95	7.06	7.87
	EC (µS cm^{-1})	13	12	22	21	23	22	24	24	27	27	33	32	44	45	66	66	108	109	185	189	333	342
	DO (%)	102.1	92.4	103.1	96.2	105.3	95.8	107.3	95.6	105.3	91.3	106.3	97.5	107.1	95.9	103.5	97.4	106.6	101.9	107.4	95.6	100.3	95.1
	Temp (°C)	23	23.3	22	23.6	21.4	23.1	21.3	24.4	21	22.8	21	22.5	20.8	22.7	20.6	22.5	20.6	22.4	20.5	22	20.6	21.5
Day 3	pH	6.2	6.42	6.91	7.66	6.86	7.76	6.88	7.55	6.88	8.02	6.92	7.76	6.97	8.05	7	7.75	7	8.36	7.1	8.11	7.18	8.19
	EC (µS cm^{-1})	14	11	22	21	24	22	24	24	27	27	32	32	45	45	65	67	105	109	186	189	336	341
	DO (%)	103.9	94.3	101.1	98.5	103.6	96	103.7	95.6	101.6	98.9	104.3	99.7	98.9	99.1	99.3	100.1	100.3	103.8	101.5	100.3	99.7	98.9
	Temp (°C)	24.2	22.9	23.9	23.1	22.5	23	22.6	21.3	22.5	23.1	22	23.5	22	23.8	21.9	23.6	21.6	23.3	21.4	22.9	21.3	23
Day 4	pH	5.89	6.33	6.62	7.2	6.69	7.22	6.74	7.49	6.76	7.29	6.71	7.28	6.81	7.33	6.85	7.35	6.9	7.51	6.91	7.51	6.94	7.49
	EC (µS cm^{-1})	13	12	21	21	23	22	24	23	28	28	32	33	45	45	66	67	108	109	186	189	336	349
	DO (%)	116.2	90.3	118.6	92.1	121.2	92.8	119.3	96.1	117.6	92.5	117.5	91.2	112.5	91.8	109.1	87.2	117	89.9	118.9	89.1	111.6	86.7
	Temp (°C)	22.9	22.2	22.8	22.1	22.9	21.8	22.7	22.9	22.6	22.6	22.6	22.2	22	22.2	21.7	21.9	21.3	21.8	21.1	21.4	21.3	21.2
Day 5	pH	5.99	6.17	6.87	7.09	6.83	7.09	6.84	7.22	6.85	7.15	6.87	7.13	6.95	7.14	6.98	7.12	7.07	7.27	7.09	7.23	7.18	7.39
	EC (µS cm^{-1})	13	12	22	22	23	22	25	24	27	27	32	32	45	45	67	66	107	109	186	188	336	342
	DO (%)	108.2	90.3	110.9	91.7	110.7	91.7	111.6	89.7	104.6	93.1	103.9	93.6	101.5	88.9	96.9	90.4	96.8	90.6	98.8	86.2	96.6	88.3
	Temp (°C)	25.6	23.8	25.4	24	24.7	24	24.9	21.8	24.8	23.6	23.3	23.7	22.1	23.5	21.6	23.3	21	22.3	20.8	23.1	20.6	23.1

Attachment B2 - Physico-chemical measurements of the test solutions for test 1333D

Treatment (%)		MCW		SW2		0.0625		0.125		0.25		0.5		1.0		2.0		4.0		8.0	
Parameter		0 h	24 h	0 h	24 h	0 h	24 h	0 h	24 h	0 h	24 h	0 h	24 h	0 h	24 h	0 h	24 h	0 h	24 h	0 h	24 h
Day 0	pH	6.4	7.7	6.8	8.5	6.7	8.4	6.8	8.3	6.8	8.4	6.8	8.4	6.8	8.4	6.9	8.6	6.9	8.6	7.0	8.7
	EC (µS cm-1)	19	17	22	22	26	24	28	27	33	33	45	44	66	67	108	110	191	191	340	337
	DO (%)	109.1	95.2	106.3	98.0	111.1	99.9	107.0	92.8	105.5	98.0	110.0	97.5	111.5	98.9	109.3	97.2	107.4	100.5	106.9	104.2
	Temp (°C)	23.9	23.5	23.8	23.7	23.8	23.9	23.8	23.7	23.6	23.6	23.0	23.3	23.0	23.5	22.9	23.6	22.8	23.7	22.6	23.5
Day 1	pH	6.4	8.0	6.8	8.6	6.8	8.5	6.9	8.4	6.9	8.6	6.9	8.5	6.9	8.7	7.0	8.7	7.0	8.8	7.2	8.8
	EC (µS cm-1)	18	18	22	21	24	24	26	26	33	33	45	44	66	67	109	110	189	188	335	338
	DO (%)	105.5	97.3	111.2	101.5	111.6	99.8	107.0	97.2	106.3	101.2	106.0	102.4	112.4	105.1	110.7	104.7	111.5	102.6	106.2	101.9
	Temp (°C)	23.5	23.8	23.0	24.4	22.9	24.1	22.8	23.6	22.5	24.8	23.7	25.0	23.4	24.7	22.8	24.6	22.5	23.9	22.4	23.9
Day 2	pH	6.6	7.8	7.0	8.3	7.0	8.2	7.0	8.0	7.0	8.2	7.0	8.0	7.0	7.9	7.1	8.0	7.1	8.1	7.2	8.2
	EC (µS cm-1)	18	16	21	21	25	25	27	26	33	32	44	44	66	68	110	110	189	191	336	340
	DO (%)	108.1	101.7	105.7	100.0	108.2	100.7	105.5	102.2	107.7	101.9	102.4	100.4	107.9	99.4	101.5	99.9	104.4	98.2	102.2	99.9
	Temp (°C)	24.0	25.8	23.4	26.1	22.6	26.1	22.5	25.9	22.4	25.9	22.1	25.3	21.8	25.1	21.5	24.8	21.5	24.6	21.4	24.3
Day 3	pH	6.9	7.7	7.0	8.4	7.1	8.5	7.1	8.5	7.1	8.5	7.0	8.5	6.8	8.5	7.3	8.6	7.2	8.6	7.3	0.0
	EC (µS cm-1)	17	16	21	21	25	24	27	27	33	33	44	45	67	66	109	109	189	190	335	337
	DO (%)	109.4	92.0	118.1	95.1	113.3	98.0	117.3	91.9	113.2	96.7	109.5	94.3	111.8	93.0	100.6	96.9	111.3	97.0	110.2	93.0
	Temp (°C)	27.3	21.2	27.3	21.4	26.4	21.0	26.4	21.0	26.1	21.4	25.8	21.2	25.6	21.1	25.3	21.0	25.1	21.0	24.7	21.0
Day 4	pH	6.6	7.0	6.9	7.6	6.9	7.9	7.0	7.4	7.0	7.6	7.0	7.6	7.0	7.5	7.1	7.6	7.1	7.6	7.2	8.2
	EC (µS cm-1)	18	15	21	21	25	25	27	26	33	33	44	44	67	67	110	111	190	194	336	342
	DO (%)	104.9	89.3	108.3	97.1	104.6	94.2	109.7	94.5	107.1	90.9	101.0	92.7	106.3	93.8	106.2	95.9	107.4	94.9	106.1	93.5
	Temp (°C)	24.9	23.1	25.3	23.4	25.3	22.8	25.1	22.9	24.9	22.7	24.5	22.4	24.4	22.6	24.0	22.3	23.9	21.9	23.3	21.6
Day 5	pH	6.7	7.0	6.9	8.0	7.1	8.0	6.9	8.2	7.0	8.3	7.1	8.2	6.9	8.5	7.3	8.6	7.2	8.5	7.1	8.7
	EC (µS cm-1)	16	17	21	21	24	24	27	27	33	33	44	44	66	68	108	110	186	190	334	338
	DO (%)	101.7	103.6	101.5	109.5	101.5	107.3	103.9	111.6	101.1	108.8	99.5	109.1	107.3	108.8	102.8	109.8	106.9	107.6	102.4	108.7
	Temp (°C)	22.9	23.6	23.1	23.9	22.8	24.1	22.6	23.9	21.8	23.0	22.1	23.0	22.1	22.4	21.9	22.6	21.7	22.2	21.1	22.2

Attachment C - Metal and major ion analyses of QA/QC waters

		Sample ID			
Elements	**Units**	**1324D P. Blk**	**1324D Blk**	**1333D P. Blk**	**1333D Blk**
Aluminium	µg L^{-1}	<0.5	0.24	0.78	<0.1
Cadmium	µg L^{-1}	<0.1	<0.02	<0.02	<0.02
Cobalt	µg L^{-1}	<0.02	<0.01	<0.01	<0.01
Chromium	µg L^{-1}	<0.01	<0.1	<0.1	<0.1
Copper	µg L^{-1}	<0.1	0.082	0.099	0.083
Iron	µg L^{-1}	0.083	<1	<1	<1
Manganese	µg L^{-1}	<1	0.012	<0.01	<0.01
Nickel	µg L^{-1}	<0.01	0.15	0.047	0.043
Lead	µg L^{-1}	0.16	<0.01	0.073	<0.01
Selenium	µg L^{-1}	<0.01	<0.2	<0.2	<0.2
Uranium	µg L^{-1}	<0.2	0.001	0.001	<0.001
Zinc	µg L^{-1}	<0.001	<0.1	<0.1	<0.1
Sodium	mg L^{-1}	<0.1	<0.1	<0.1	<0.1
Calcium	mg L^{-1}	<0.1	<0.1	<0.1	<0.1
Magnesium	µm L^{-1}	<0.1	<0.1	<0.1	<0.1
Sulphate, SO4	mg L^{-1}L	<0.1	<0.5	<0.5	<0.5

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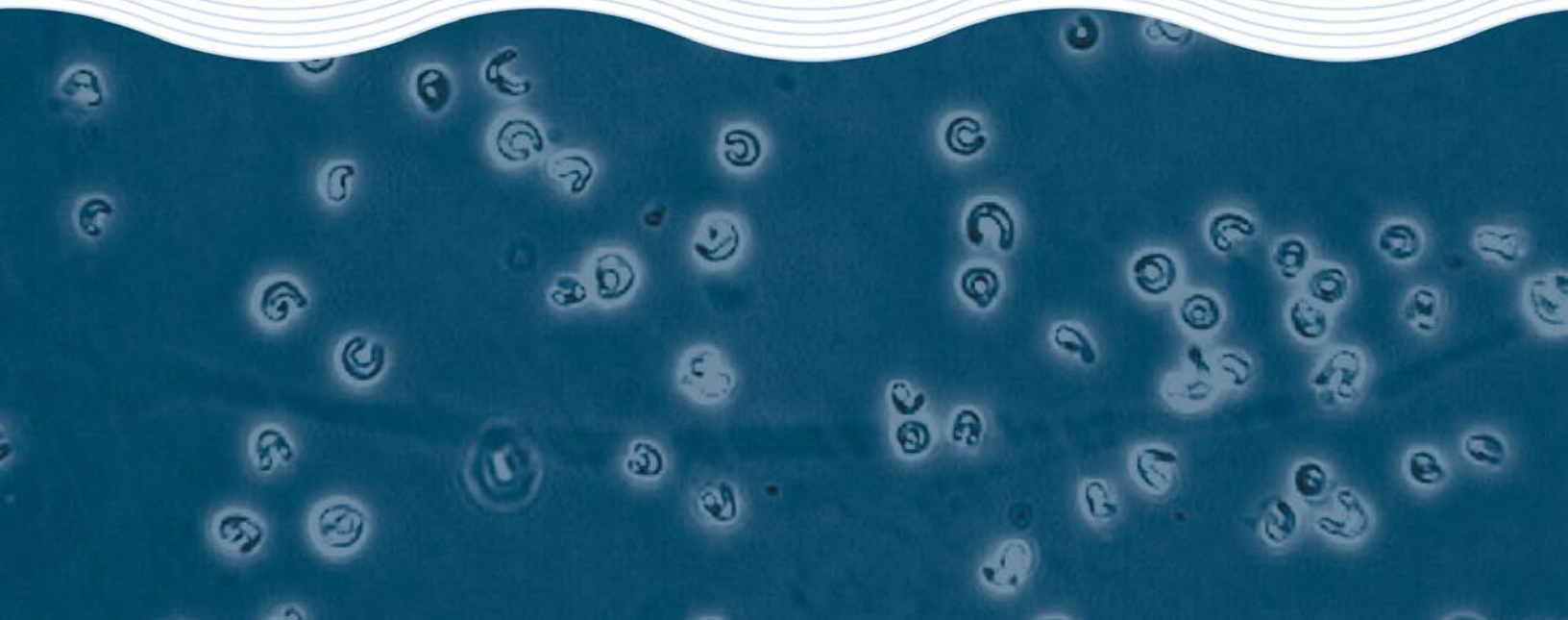
SERVICES AUSTRALASIA

Toxicity Assessment of a Treated Water Sample

Vista Gold Australia Pty Ltd

Test Report

March 2013



Toxicity Assessment of a Treated Water Sample

Vista Gold Australia Pty Ltd

Test Report

March 2013

ECOTOX Services Australasia Pty Ltd ABN>45 094 714 904
unit 27/2 chaplin drive lane cove nsw 2066 T>61 2 9420 9481 F>61 2 9420 9484 W>www.ecotox.com.au

Toxicity Test Report: TR1022/1

This document is issued in accordance with NATA's accreditation requirements

Client:	Vista Gold Pty Ltd PO Box 1616 Katherine NT 0851	**ESA Job #:** **Date Sampled:** **Date Received:**	PR1022 Not supplied 14 and 23 March 2013
Attention:	Andrew Sawicki	**Sampled By:**	Client
Client Ref:	Not supplied	**ESA Quote #:**	PL1022_q01

Lab ID No.:	Sample Name:	Sample Description:
5921	SW2	Aqueous sample, pH 7.4, conductivity 20.1 µS/cm, total ammonia <2.0mg/L*. Sample received at room temperature in apparent good condition.
5929	RP3	Aqueous sample, pH 9.0, conductivity 2690µS/cm, total ammonia <2.0mg/L*. Sample received at room temperature in apparent good condition.

*Ammonia analysis is not covered by Ecotox Services Australasia's scope of accreditation

Test Performed:	72-hr microalgal growth inhibition test using the green alga *Chlorella vulgaris*
Test Protocol:	ESA SOP 103 (ESA 2011), based on USEPA (2002)
Test Temperature:	The test was performed at 29±1°C.
Deviations from Protocol:	Nil
Comments on Solution Preparation:	The samples were filtered to 0.45µm prior to use. Sample RP3 was serially diluted with sample SW2 to achieve the test concentrations. A USEPA control and a diluent control (SW2) were tested concurrently with the sample.
Source of Test Organisms:	ESA Laboratory culture, originally sourced from CSIRO Microalgal Supply Service, TAS
Test Initiated:	25 March 2013 at 1430h

Sample 5929 : *RP3*		*Vacant*	*Vacant*
Concentration (%)	**Cell Yield x10^4 cells/mL (Mean ± SD)**		
USEPA Control	107.2 ± 5.0		
Diluent Control	102.5 ± 8.1		
0.8	85.6 ± 7.1 *		
1.6	86.8 ± 4.9 *		
3.1	89.9 ± 12.5		
6.3	69.3 ± 14.6 *		
12.5	70.3 ± 6.5 *		
25	62.3 ± 7.5 *		
50	65.9 ± 6.0 *		
100	75.1 ± 4.1 *		
72-hr IC10 = <0.8% **72-hr IC50 = >100%** **NOEC = 3.1%** **LOEC = 6.3%**			

*Significantly lower cell yield compared with the Diluent Control (Dunnett's Test, 1-tailed, P=0.05)

Toxicity Test Report: TR1022/1

QA/QC Parameter	Criterion	This Test	Criterion met?
Control mean cell density	≥16.0x10^4 cells/mL	108.2x10^4 cells/mL	Yes
Control coefficient of variation	<20%	4.9%	Yes
Reference Toxicant within cusum chart limits	0.3-3.4g KCl/L	1.1g KCl/L	Yes

Test Report Authorised by: Dr Rick Krassoi, Director on 9 May 2013

Results are based on the samples in the condition as received by ESA.

NATA Accredited Laboratory Number: 14709

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Citations:

ESA (2011) *ESA SOP 103 – Green Alga, Selenastrum capricornutum, Growth Test*. Issue No 9. Ecotox Services Australasia, Sydney, NSW.

USEPA (2002) *Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms*. Fourth Edition. EPA-821-R-02-013. United States Environmental Protection Agency, Office of Research and Development, Washington DC, USA,

Toxicity Test Report: TR1022/2

This document is issued in accordance with NATA's accreditation requirements

Client:	Vista Gold Pty Ltd PO Box 1616 Katherine NT 0851	**ESA Job #:** **Date Sampled:** **Date Received:**	PR1022 Not supplied 14 and 23 March 2013
Attention:	Andrew Sawicki	**Sampled By:**	Client
Client Ref:	Not supplied	**ESA Quote #:**	PL1022_q01

Lab ID No.:	**Sample Name:**	**Sample Description:**
5921	SW2	Aqueous sample, pH 7.4, conductivity 20.1 µS/cm, total ammonia <2.0mg/L*. Sample received at room temperature in apparent good condition.
5929	RP3	Aqueous sample, pH 9.0, conductivity 2690µS/cm, total ammonia <2.0mg/L*. Sample received at room temperature in apparent good condition.

*Ammonia analysis is not covered by Ecotox Services Australasia's scope of accreditation

Test Performed:	96-hr Growth inhibition of the freshwater aquatic duckweed *Lemna aequinoctialis*
Test Protocol:	ESA SOP 112 (ESA 2011), based on OECD method 221 (2006)
Test Temperature:	The test was performed at 29±1°C.
Deviations from Protocol:	The test was extended to 120 days
Comments on Solution Preparation:	Sample RP3 was serially diluted with sample SW2 to achieve the test concentrations. A SIS (media) control and a diluent control (SW2) were tested concurrently with the sample.
Source of Test Organisms:	ESA Laboratory culture
Test Initiated:	28 March 2013 at 1445h

Sample 5929: *RP3*		*Vacant*	*Vacant*
Concentration (%)	**Specific Growth Rate (Mean ± SD)**		
SIS Control	0.25 ± 0.07		
Diluent Control	0.26 ± 0.02		
3.1	0.24 ± 0.01		
6.1	0.24 ± 0.02		
12.1	0.23 ± 0.03		
24.2	0.23 ± 0.03		
48.4	0.17 ± 0.07 *		
96.8	0.10 ± 0.07 *		
120-hr IC10 = 10.2%**			
120-hr IC50 = 75.4%**			
NOEC = 24.2%			
LOEC = 48.4%			

*Significantly lower specific growth rate compared with the Diluent Control (Steel's Many-One Rank Test, 1-tailed, P=0.05)
**95% confidence limits not reliable

Toxicity Test Report: TR1022/2

QA/QC Parameter	Criterion	This Test	Criterion met?
Control frond doubling time	<3 days	2.7 days	Yes
Reference Toxicant within cusum chart limits	7.7-61.0 $MgSO_4$/L	12.7mg $MgSO_4$/L	Yes

Test Report Authorised by: Dr Rick Krassoi, Director on 9 May 2013

Results are based on the samples in the condition as received by ESA.

NATA Accredited Laboratory Number: 14709

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Citations:

ESA (2011) *SOP 112 – Duckweed Growth Inhibition Test.* Issue No. 3. Ecotox Services Australasia, Sydney NSW

OECD (2006) *Lemna sp*. Growth Inhibition Test. Method 221. OECD Guideline for the Testing of Chemicals. Organisation for Economic Cooperation and Development, Paris

Toxicity Test Report: TR1022/3

This document is issued in accordance with NATA's accreditation requirements

Client:	Vista Gold Pty Ltd PO Box 1616 Katherine NT 0851	**ESA Job #:** **Date Sampled:** **Date Received:**	PR1022 Not supplied 14 and 23 March 2013
Attention:	Andrew Sawicki	**Sampled By:**	Client
Client Ref:	Not supplied	**ESA Quote #:**	PL1022_q01

Lab ID No.:	Sample Name:	Sample Description:
5921	SW2	Aqueous sample, pH 7.4, conductivity 20.1 µS/cm, total ammonia <2.0mg/L*. Sample received at room temperature in apparent good condition.
5929	RP3	Aqueous sample, pH 9.0, conductivity 2690µS/cm, total ammonia <2.0mg/L*. Sample received at room temperature in apparent good condition.

*Ammonia analysis is not covered by Ecotox Services Australasia's scope of accreditation

Test Performed:	48-hr acute (survival) toxicity test using the freshwater chironomid *Chironomus tepperi*
Test Protocol:	ESA SOP 121 (ESA 2012), based on OECD (2011) USEPA (2002) and Bailey *et al*. (2000)
Test Temperature:	The test was performed at 25±1°C.
Deviations from Protocol:	Nil
Comments on Solution Preparation:	Sample RP3 was serially diluted with sample SW2 to achieve the test concentrations. A Dilute Mineral Water (DMW) control and a diluent control were tested concurrently with the sample.
Source of Test Organisms:	ESA Laboratory culture
Age of Test Organisms:	8 days
Test Initiated:	27 March 2013 at 1130h

Sample 5929: *RP3*		*Vacant*	*Vacant*
Concentration (%)	**% Survival (Mean ± SD)**		
DMW Control	100 ± 0.0		
Diluent Control	100 ± 0.0		
6.3	100 ± 0.0		
12.5	95.0 ± 10.0		
25	95.0 ± 10.0		
50	100 ± 0.0		
100	95.0 ± 10.0		
48-hr EC10 = >100% **48-hr EC50 = >100%** **NOEC = 100%** **LOEC = >100%**			

QA/QC Parameter	Criterion	This Test	Criterion met?
Control mean % survival	≥85.0%	100%	Yes
Reference Toxicant within cusum chart limits	18.0-3218.1µg Cu/L	260.5µg Cu/L	Yes

Toxicity Test Report: TR1022/3

Test Report Authorised by: Dr Rick Krassoi, Director on 9 May 2013

Results are based on the samples in the condition as received by ESA.

NATA Accredited Laboratory Number: 14709

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Citations:

Bailey, H.C., Krassoi, R., Elphick, J.R., Mulhall, A., Hunt, 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* 19:88-93.

ESA (2012) *SOP 121 – Acute toxicity test using Chironomus tepperi*. Issue No. 1. Ecotox Services Australasia, Sydney, New South Wales.

OECD (2011) OECD Guideline for the Testing of Chemicals. Test Guideline 235: *Chironomus sp*, Acute Immobilisation Test.

USEPA (2002) *Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms. 4th Ed.* United States Environmental Protection Agency, Office of Water, Washington DC.

Toxicity Test Report: TR1022/4

This document is issued in accordance with NATA's accreditation requirements

Client:	Vista Gold Pty Ltd PO Box 1616 Katherine NT 0851	**ESA Job #:** **Date Sampled:** **Date Received:**	PR01022 Not supplied 14 and 23 March 2013
Attention:	Andrew Sawicki	**Sampled By:**	Client
Client Ref:	Not supplied	**ESA Quote #:**	PL1022_q01

Lab ID No.:	Sample Name:	Sample Description:
5921	SW2	Sample RP3 was serially diluted with sample SW2 to achieve the test concentrations. A USEPA control and a diluent control (SW2) were tested concurrently with the sample.
5929	RP3	Sample RP3 was serially diluted with sample SW2 to achieve the test concentrations. A USEPA control and a diluent control (SW2) were tested concurrently with the sample.

*Ammonia analysis is not covered by Ecotox Services Australasia's scope of accreditation

Test Performed:	96-hr acute toxicity test using the freshwater hydra *hydra viridissima*
Test Protocol:	ESA SOP 125 (2012), based on Riethmuller et al. (2003)
Test Temperature:	The test was performed at 27±1°C.
Deviations from Protocol:	Nil
Comments on Solution Preparation:	Sample RP3 was serially diluted with sample SW2 to achieve the test concentrations. A Laboratory Water control (2.5% mineral water) and a diluent control (SW2) were tested concurrently with the sample.
Source of Test Organisms:	ESA Laboratory culture
Test Initiated:	28 March 2013 at 1330h

Sample 5929: *RP3*

Concentration (%)	Population Growth Rate (Mean ± SD)			
Laboratory Water Control	0.40	±	0.01	
Diluent Control	0.41	±	0.01	
3.1	0.40	±	0.02	
6.3	0.40	±	0.01	
12.5	0.40	±	0.01	
25	0.41	±	0.01	
50	0.40	±	0.02	
100	0.26	±	0.02	*

96-hr IC10 = 61.7 (51.6-66.7)%
96-hr IC50 = >100%
NOEC = 50%
LOEC = 100%

Vacant

*Significantly lower population growth rate compared with the Diluent Control (Dunnett's Test, 1-tailed, P=0.05)

QA/QC Parameter	Criterion	This Test	Criterion met?
Control mean population growth rate	≥0.25	0.40	Yes
Reference Toxicant within cusum chart limits	1.2-15.6µg Cu/L	2.5µg Cu/L	n/a

Toxicity Test Report: TR1022/4

Test Report Authorised by: Dr Rick Krassoi, Director on 9 May 2013

Results are based on the samples in the condition as received by ESA.

NATA Accredited Laboratory Number: 14709

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Citations:

ESA (2012) SOP 125 –*Hydra Population Growt Test.* Issue No 1. Ecotox Services Australasia, Sydney, NSW

Riethmuller N, Camilleri C, Franklin N, Hogan A, King A, Koch A, Markich SJ, Turley C and van Dam R (2003). Green Hydra Population Growth Test. In: *Ecotoxicological testing protocols for Australian tropical freshwater ecosystems*. Supervising Scientist Report 173, Supervising Scientist, Darwin NT.

Toxicity Test Report: TR1022/5

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Client:	Vista Gold Pty Ltd PO Box 1616 Katherine NT 0851 Andrew Sawicki	**ESA Job #:** **Date Sampled:** **Date Received:** **Sampled By:**	PR1022 Not supplied 14 and 23 March 2013 Client
Attention:	Not supplied	**ESA Quote #:**	PL1022_q01

Lab ID No.:	Sample Name:	Sample Description:
5921	SW2	Aqueous sample, pH 7.4, conductivity 20.1 µS/cm, total ammonia <2.0mg/L*. Sample received at room temperature in apparent good condition.
5929	RP3	Aqueous sample, pH 9.0, conductivity 2690µS/cm, total ammonia <2.0mg/L*. Sample received at room temperature in apparent good condition.

*Ammonia analysis is not covered by Ecotox Services Australasia's scope of accreditation

Test Performed:	96-hr acute survival test using the freshwater shrimp *Macrobrachium bullatum*
Test Protocol:	ESA SOP 123 (ESA 2012)
Test Temperature:	The test was performed at 25±1°C.
Deviations from Protocol:	Nil
Comments on Solution Preparation:	Sample RP3 was serially diluted with sample SW2 to achieve the test concentrations. A dilute mineral water (DMW) control and a diluent control (SW2) were tested concurrently with the sample.
Source of Test Organisms:	Hatchery reared, Northern Territory
Test Initiated:	4 April 2013 at 1400h

Sample 5929: *RP3*		*Vacant*	*Vacant*
Concentration (%)	**% Un-affected (Mean ± SD)**		
DMW Control	100 ± 0.0		
Diluent Control	100 ± 0.0		
6.3	85.0 ± 19.2		
12.5	100 ± 0.0		
25	95.0 ± 10.0		
50	100 ± 0.0		
100	90.0 ± 11.6		
96-hr EC10 = >100% **96-hr EC50 = >100%** **NOEC = 100%** **LOEC = >100%**			

QA/QC Parameter	Criterion	This Test	Criterion met?
Control mean % un-affected	≥90.0%	100%	Yes
Reference Toxicant within cusum chart limits	26.2-284.6µg Cu/L	137.6µg Cu/L	Yes

Toxicity Test Report: TR1022/5

Test Report Authorised by: Dr Rick Krassoi, Director on 9 May 2013

Results are based on the samples in the condition as received by ESA.

NATA Accredited Laboratory Number: 14709

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Citations:

ESA (2012) SOP 123 –*Acute Toxicity Test Using Freshwater Shrimp.* Issue No 2. Ecotox Services Australasia, Sydney, NSW

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Statistical Printouts for the *Chlorella* Growth Inhibition Tests

Microalgal Cell Yield-Cell Yield

Start Date:	16/04/2013 17:30	Test ID:	PR1022/01	Sample ID:	RP3
End Date:	19/04/2013 16:00	Lab ID:	5929	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 103	Test Species:	CV-Chlorella vulgaris
Comments:					

Conc-%	1	2	3	4
USEPA Control	24.683	24.683	23.183	23.683
Diluent Control	26.683	26.683	25.183	25.183
0.8	24.183	26.183	22.683	27.183
1.6	20.683	19.683	16.183	19.183
3.1	19.683	14.183	18.183	21.683
6.3	19.683	17.683	14.183	15.683
12.5	9.683	9.683	7.183	6.683
25	10.683	8.183	10.683	10.183
50	9.683	13.183	11.183	12.183
100	8.683	9.683	9.183	9.183

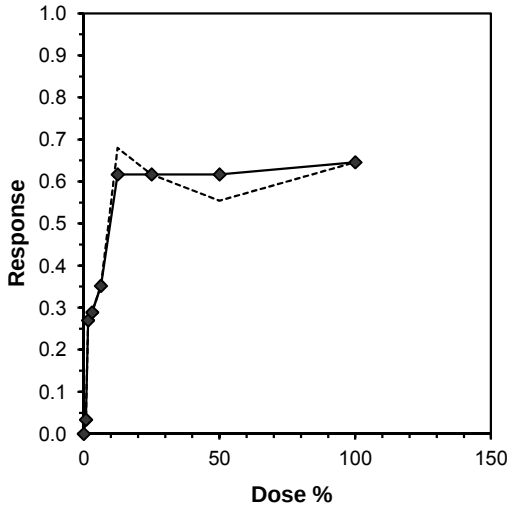
			Transform: Untransformed						1-Tailed		Isotonic	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
USEPA Control	24.058	0.9277	24.058	23.183	24.683	3.117	4					
Diluent Control	25.933	1.0000	25.933	25.183	26.683	3.339	4	*			25.933	1.0000
0.8	25.058	0.9663	25.058	22.683	27.183	8.044	4	0.669	2.513	3.285	25.058	0.9663
*1.6	18.933	0.7301	18.933	16.183	20.683	10.228	4	5.356	2.513	3.285	18.933	0.7301
*3.1	18.433	0.7108	18.433	14.183	21.683	17.227	4	5.738	2.513	3.285	18.433	0.7108
*6.3	16.808	0.6481	16.808	14.183	19.683	14.241	4	6.981	2.513	3.285	16.808	0.6481
*12.5	8.308	0.3204	8.308	6.683	9.683	19.268	4	13.485	2.513	3.285	9.933	0.3830
*25	9.933	0.3830	9.933	8.183	10.683	11.983	4	12.241	2.513	3.285	9.933	0.3830
*50	11.558	0.4457	11.558	9.683	13.183	12.918	4	10.998	2.513	3.285	9.933	0.3830
*100	9.183	0.3541	9.183	8.683	9.683	4.446	4	12.815	2.513	3.285	9.183	0.3541

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.05)	0.979796	0.935	-0.42949	0.270188
Bartlett's Test indicates equal variances (p = 0.18)	11.39297	20.09023		
The control means are significantly different (p = 0.02)	3.273268	2.446912		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	0.8	1.6	1.131371	125	3.285008	0.126672	179.0313	3.416667	1.9E-14	8, 27
Treatments vs Diluent Control										

Linear Interpolation (200 Resamples)

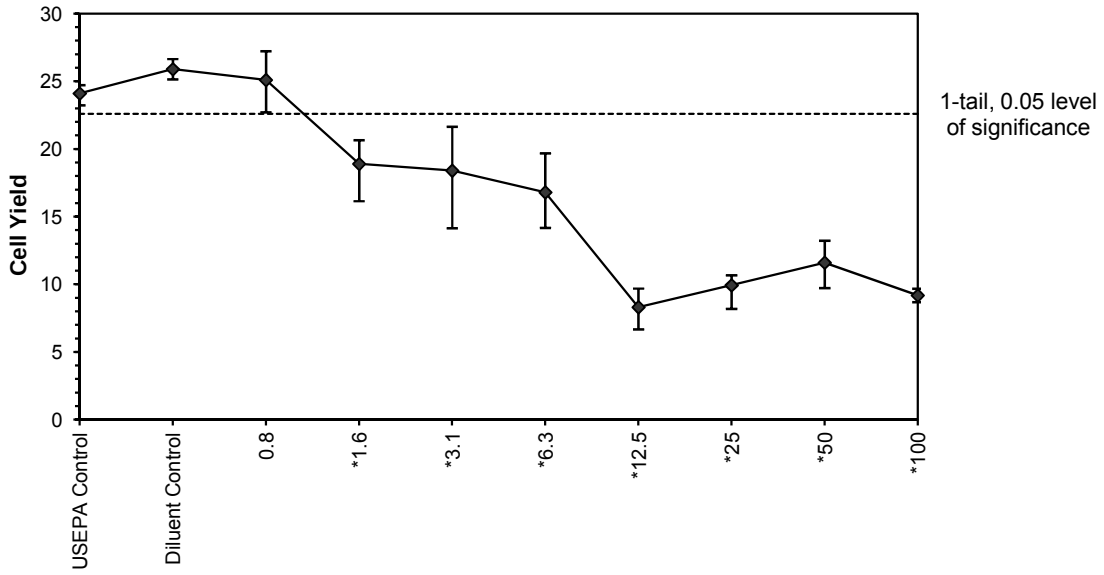
Point	%	SD	95% CL(Exp)		Skew
IC05	0.8551	0.1659	0.1602	1.0349	-1.0484
IC10	1.0244	0.0944	0.6969	1.2213	-1.1385
IC15	1.1938	0.0844	0.9130	1.4431	-0.6806
IC20	1.3632	0.0888	1.0817	1.6596	-0.2356
IC25	1.5325	0.7487	1.2808	5.5072	2.0585
IC40	7.4257	0.8772	4.0182	9.2327	-1.1609
IC50	9.7644	0.5134	7.7273	10.9629	-0.7414



Microalgal Cell Yield-Cell Yield

Start Date:	16/04/2013 17:30	Test ID:	PR1022/01	Sample ID:	RP3
End Date:	19/04/2013 16:00	Lab ID:	5929	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 103	Test Species:	CV-Chlorella vulgaris
Comments:					

Dose-Response Plot



Microalgal Cell Yield-Cell Yield

Start Date:	16/04/2013 17:30	Test ID:	PR1022/01	Sample ID:	RP3
End Date:	19/04/2013 16:00	Lab ID:	5929	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 103	Test Species:	CV-Chlorella vulgaris
Comments:					

		Auxiliary Data Summary					
Conc-%	**Parameter**	**Mean**	**Min**	**Max**	**SD**	**CV%**	**N**
USEPA Control	Cell Yield	24.06	23.18	24.68	0.75	3.60	4
Diluent Control		25.93	25.18	26.68	0.87	3.59	4
0.8		25.06	22.68	27.18	2.02	5.67	4
1.6		18.93	16.18	20.68	1.94	7.35	4
3.1		18.43	14.18	21.68	3.18	9.67	4
6.3		16.81	14.18	19.68	2.39	9.20	4
12.5		8.31	6.68	9.68	1.60	15.23	4
25		9.93	8.18	10.68	1.19	10.98	4
50		11.56	9.68	13.18	1.49	10.57	4
100		9.18	8.68	9.68	0.41	6.96	4
USEPA Control	pH	7.50	7.50	7.50	0.00	0.00	1
Diluent Control		6.60	6.60	6.60	0.00	0.00	1
0.8		6.60	6.60	6.60	0.00	0.00	1
1.6		6.60	6.60	6.60	0.00	0.00	1
3.1		6.60	6.60	6.60	0.00	0.00	1
6.3		6.50	6.50	6.50	0.00	0.00	1
12.5		6.50	6.50	6.50	0.00	0.00	1
25		6.50	6.50	6.50	0.00	0.00	1
50		6.70	6.70	6.70	0.00	0.00	1
100		6.70	6.70	6.70	0.00	0.00	1
USEPA Control	Conductivity uS/cm	96.50	96.50	96.50	0.00	0.00	1
Diluent Control		113.00	113.00	113.00	0.00	0.00	1
0.8		137.20	137.20	137.20	0.00	0.00	1
1.6		162.40	162.40	162.40	0.00	0.00	1
3.1		212.00	212.00	212.00	0.00	0.00	1
6.3		315.00	315.00	315.00	0.00	0.00	1
12.5		513.00	513.00	513.00	0.00	0.00	1
25		877.00	877.00	877.00	0.00	0.00	1
50		1548.00	1548.00	1548.00	0.00	0.00	1
100		2800.00	2800.00	2800.00	0.00	0.00	1

Microalgal Cell Yield-Cell Yield

Start Date:	25/03/2013 14:30	Test ID:	PR1022/01	Sample ID:	RP3
End Date:	28/03/2013 15:30	Lab ID:	5929	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 103	Test Species:	CV-Chlorella vulgaris
Comments:					

Conc-%	1	2	3	4
USEPA Control	114.00	103.20	108.00	103.60
Diluent Control	94.80	96.40	110.80	108.00
0.8	80.40	80.80	95.60	85.60
1.6	90.80	84.80	90.80	80.80
3.1	107.60	82.00	89.60	80.40
6.3	72.40	69.60	50.00	85.20
12.5	75.20	76.00	62.40	67.60
25	54.00	70.80	66.00	58.40
50	70.00	66.80	69.60	57.20
100	73.20	76.00	70.80	80.40

			Transform: Untransformed						1-Tailed		Isotonic	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
USEPA Control	107.20	1.0459	107.20	103.20	114.00	4.690	4					
Diluent Control	102.50	1.0000	102.50	94.80	110.80	7.878	4	*			102.50	1.0000
*0.8	85.60	0.8351	85.60	80.40	95.60	8.263	4	2.796	2.513	15.19	87.44	0.8530
*1.6	86.80	0.8468	86.80	80.80	90.80	5.644	4	2.597	2.513	15.19	87.44	0.8530
3.1	89.90	0.8771	89.90	80.40	107.60	13.864	4	2.085	2.513	15.19	87.44	0.8530
*6.3	69.30	0.6761	69.30	50.00	85.20	20.993	4	5.493	2.513	15.19	69.80	0.6810
*12.5	70.30	0.6859	70.30	62.40	76.00	9.226	4	5.327	2.513	15.19	69.80	0.6810
*25	62.30	0.6078	62.30	54.00	70.80	12.084	4	6.651	2.513	15.19	67.77	0.6611
*50	65.90	0.6429	65.90	57.20	70.00	9.062	4	6.055	2.513	15.19	67.77	0.6611
*100	75.10	0.7327	75.10	70.80	80.40	5.490	4	4.533	2.513	15.19	67.77	0.6611

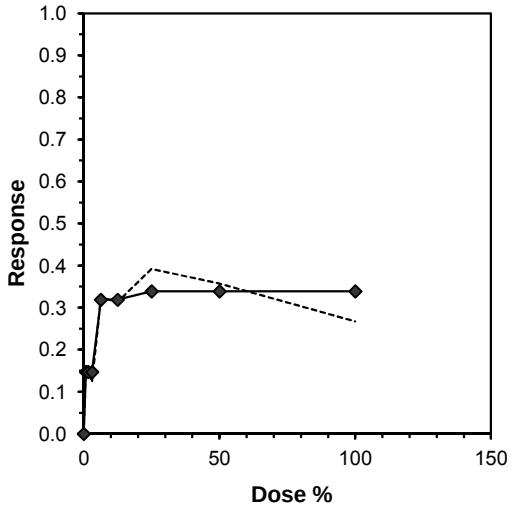
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.05)	0.977411	0.935	0.079448	0.566161
Bartlett's Test indicates equal variances (p = 0.49)	7.397374	20.09023		
The control means are not significantly different (p = 0.36)	0.988142	2.446912		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	3.1	6.3	4.419276	32.25806	15.19206	0.148211	704.87	73.07407	3.3E-06	8, 27
Treatments vs Diluent Control										

Linear Interpolation (200 Resamples)

Point	%	SD	95% CL(Exp)		Skew
IC05*	0.2721	0.3224	0.1516	0.9000	8.0882
IC10*	0.5443	0.8725	0.3031	5.2002	2.5438
IC15	3.1560	1.4812	0.0000	5.1735	-0.0361
IC20	4.0861	1.2143	0.0000	7.3545	-0.5704
IC25	5.0162	1.6677	3.1691	13.2260	2.1956
IC40	>100				
IC50	>100				

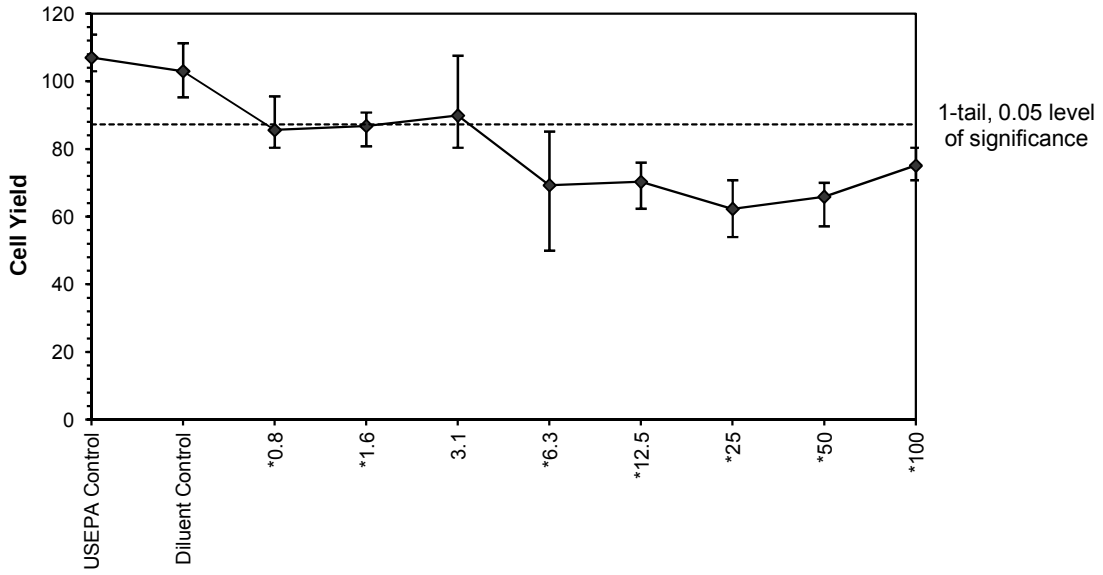
* indicates IC estimate less than the lowest concentration



Microalgal Cell Yield-Cell Yield

Start Date:	25/03/2013 14:30	Test ID:	PR1022/01	Sample ID:	RP3
End Date:	28/03/2013 15:30	Lab ID:	5929	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 103	Test Species:	CV-Chlorella vulgaris
Comments:					

Dose-Response Plot



 ToxCalc v5.0.23 Reviewed by:_____

Microalgal Cell Yield-Cell Yield

Start Date:	25/03/2013 14:30	Test ID:	PR1022/01	Sample ID:	RP3
End Date:	28/03/2013 15:30	Lab ID:	5929	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 103	Test Species:	CV-Chlorella vulgaris
Comments:					

		Auxiliary Data Summary					
Conc-%	**Parameter**	**Mean**	**Min**	**Max**	**SD**	**CV%**	**N**
USEPA Control	Cell Yield	107.20	103.20	114.00	5.03	2.09	4
Diluent Control		102.50	94.80	110.80	8.08	2.77	4
0.8		85.60	80.40	95.60	7.07	3.11	4
1.6		86.80	80.80	90.80	4.90	2.55	4
3.1		89.90	80.40	107.60	12.46	3.93	4
6.3		69.30	50.00	85.20	14.55	5.50	4
12.5		70.30	62.40	76.00	6.49	3.62	4
25		62.30	54.00	70.80	7.53	4.40	4
50		65.90	57.20	70.00	5.97	3.71	4
100		75.10	70.80	80.40	4.12	2.70	4
USEPA Control	pH	7.50	7.50	7.50	0.00	0.00	1
Diluent Control		7.40	7.40	7.40	0.00	0.00	1
0.8		7.40	7.40	7.40	0.00	0.00	1
1.6		7.30	7.30	7.30	0.00	0.00	1
3.1		7.40	7.40	7.40	0.00	0.00	1
6.3		7.40	7.40	7.40	0.00	0.00	1
12.5		7.50	7.50	7.50	0.00	0.00	1
25		7.50	7.50	7.50	0.00	0.00	1
50		7.80	7.80	7.80	0.00	0.00	1
100		8.50	8.50	8.50	0.00	0.00	1
USEPA Control	Conductivity uS/cm	95.00	95.00	95.00	0.00	0.00	1
Diluent Control		115.80	115.80	115.80	0.00	0.00	1
0.8		163.60	163.60	163.60	0.00	0.00	1
1.6		210.00	210.00	210.00	0.00	0.00	1
3.1		296.00	296.00	296.00	0.00	0.00	1
6.3		454.00	454.00	454.00	0.00	0.00	1
12.5		538.00	538.00	538.00	0.00	0.00	1
25		867.00	867.00	867.00	0.00	0.00	1
50		1661.00	1661.00	1661.00	0.00	0.00	1
100		2820.00	2820.00	2820.00	0.00	0.00	1

Statistical Printouts for the Duckweed Growth Inhibition Tests

Duckweed Growth Inhibtion Test-Specific Growth Rate

Start Date:	28/03/2013 14:45	Test ID:	PR1022/2	Sample ID:	RP3
End Date:	2/04/2013 10:00	Lab ID:	5922	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 112	Test Species:	LA-Lemna aequinoctialis
Comments:					

Conc-%	1	2	3	4
CAAC Control	0.2464	0.3486	0.1889	0.2290
Diluent Control	0.2624	0.2624	0.2773	0.2290
3.1	0.2197	0.2379	0.2379	0.2464
6.1	0.2546	0.2624	0.2197	0.2379
12.1	0.2624	0.1997	0.1997	0.2464
24.2	0.2290	0.2464	0.1889	0.2546
48.4	0.0713	0.1997	0.2197	0.1775
96.8	0.0000	0.0904	0.1653	0.1386

			Transform: Untransformed					Rank	1-Tailed	Isotonic	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Mean	N-Mean
CAAC Control	0.2532	0.9823	0.2532	0.1889	0.3486	26.846	4				
Diluent Control	0.2578	1.0000	0.2578	0.2290	0.2773	7.917	4	*		0.2578	1.0000
3.1	0.2355	0.9135	0.2355	0.2197	0.2464	4.779	4	13.00	10.00	0.2396	0.9294
6.1	0.2437	0.9452	0.2437	0.2197	0.2624	7.780	4	14.00	10.00	0.2396	0.9294
12.1	0.2271	0.8808	0.2271	0.1997	0.2624	14.210	4	13.00	10.00	0.2284	0.8860
24.2	0.2297	0.8912	0.2297	0.1889	0.2546	12.728	4	12.50	10.00	0.2284	0.8860
*48.4	0.1671	0.6480	0.1671	0.0713	0.2197	39.572	4	10.00	10.00	0.1671	0.6480
*96.8	0.0986	0.3824	0.0986	0.0000	0.1653	73.715	4	10.00	10.00	0.0986	0.3824

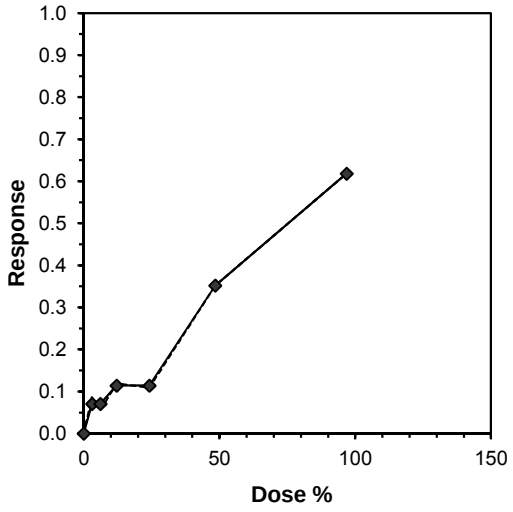
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.05)	0.918283	0.924	-1.05017	1.884819
Bartlett's Test indicates equal variances (p = 0.04)	13.23951	16.81189		
The control means are not significantly different (p = 0.90)	0.128332	2.446912		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	24.2	48.4	34.22397	4.132231
Treatments vs Diluent Control				

Linear Interpolation (200 Resamples)

Point	%	SD	95% CL(Exp)		Skew
IC05*	2.194	6.318	0.671	38.175	2.1205
IC10	10.163	9.623	0.000	42.883	0.4692
IC15	27.861	8.658	0.000	52.718	-0.0754
IC20	32.946	7.951	14.790	63.069	0.9616
IC25	38.030	8.624	21.576	73.158	0.9201
IC40	57.153				
IC50	75.377				

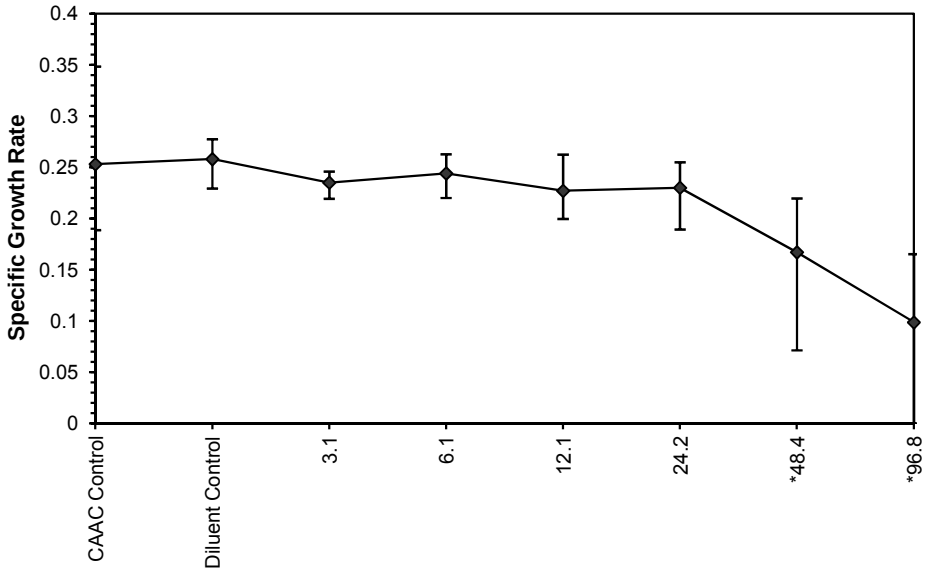
* indicates IC estimate less than the lowest concentration



Duckweed Growth Inhibtion Test-Specific Growth Rate

Start Date:	28/03/2013 14:45	Test ID:	PR1022/2	Sample ID:	RP3
End Date:	2/04/2013 10:00	Lab ID:	5922	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 112	Test Species:	LA-Lemna aequinoctialis
Comments:					

Dose-Response Plot



Duckweed Growth Inhibtion Test-Specific Growth Rate

Start Date:	28/03/2013 14:45	Test ID:	PR1022/2	Sample ID:	RP3
End Date:	2/04/2013 10:00	Lab ID:	5922	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 112	Test Species:	LA-Lemna aequinoctialis
Comments:					

		Auxiliary Data Summary					
Conc-%	**Parameter**	**Mean**	**Min**	**Max**	**SD**	**CV%**	**N**
CAAC Control	Specific Growth Rate	0.25	0.19	0.35	0.07	102.96	4
Diluent Control		0.26	0.23	0.28	0.02	55.42	4
3.1		0.24	0.22	0.25	0.01	45.05	4
6.1		0.24	0.22	0.26	0.02	56.51	4
12.1		0.23	0.20	0.26	0.03	79.11	4
24.2		0.23	0.19	0.25	0.03	74.43	4
48.4		0.17	0.07	0.22	0.07	153.91	4
96.8		0.10	0.00	0.17	0.07	273.44	4
CAAC Control	pH	5.90	5.90	5.90	0.00	0.00	1
Diluent Control		6.70	6.70	6.70	0.00	0.00	1
3.1		6.80	6.80	6.80	0.00	0.00	1
6.1		6.90	6.90	6.90	0.00	0.00	1
12.1		6.90	6.90	6.90	0.00	0.00	1
24.2		7.10	7.10	7.10	0.00	0.00	1
48.4		7.30	7.30	7.30	0.00	0.00	1
96.8		8.30	8.30	8.30	0.00	0.00	1
CAAC Control	Cond uS/cm	35.80	35.80	35.80	0.00	0.00	1
Diluent Control		52.80	52.80	52.80	0.00	0.00	1
3.1		189.80	189.80	189.80	0.00	0.00	1
6.1		311.00	311.00	311.00	0.00	0.00	1
12.1		532.00	532.00	532.00	0.00	0.00	1
24.2		920.00	920.00	920.00	0.00	0.00	1
48.4		1576.00	1576.00	1576.00	0.00	0.00	1
96.8		2750.00	2750.00	2750.00	0.00	0.00	1

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Statistical Printouts for the Acute Test with *Chironomus tepperi*

Chironomid Acute Toxicity Test-48hr Survival

Start Date:	27/03/2013 11:30	Test ID:	PR1022/02	Sample ID:	RP3
End Date:	29/03/2013 11:30	Lab ID:	5929	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 121	Test Species:	CT-Chironomus tepperi
Comments:					

Conc-%	1	2	3	4
DMW Control	1.0000	1.0000	1.0000	1.0000
t Control (SW2)	1.0000	1.0000	1.0000	1.0000
6.3	1.0000	1.0000	1.0000	1.0000
12.5	0.8000	1.0000	1.0000	1.0000
25	0.8000	1.0000	1.0000	1.0000
50	1.0000	1.0000	1.0000	1.0000
100	1.0000	1.0000	0.8000	1.0000

			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
DMW Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4				
t Control (SW2)	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	*		0	20
6.3	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
12.5	0.9500	0.9500	1.2857	1.1071	1.3453	9.261	4	16.00	10.00	1	20
25	0.9500	0.9500	1.2857	1.1071	1.3453	9.261	4	16.00	10.00	1	20
50	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
100	0.9500	0.9500	1.2857	1.1071	1.3453	9.261	4	16.00	10.00	1	20

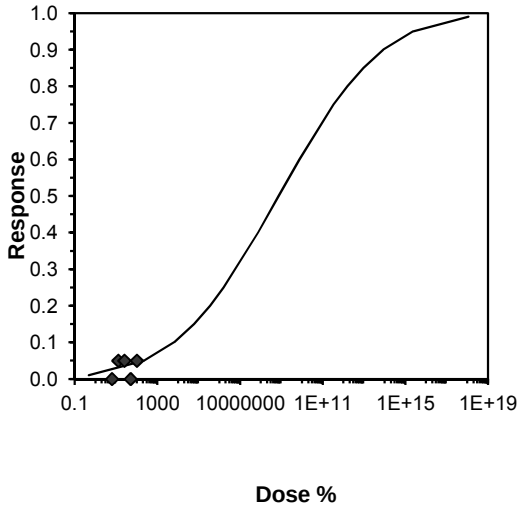
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.05)	0.668306	0.916	-1.74394	2.373016
Equality of variance cannot be confirmed				
The control means are not significantly different (p = 1.00)	0	2.446912		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	100	>100		1
Treatments vs Diluent Control (SW2)				

Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	0.253136	0.599134	-0.92117	1.427438	0	2.023721	7.814728	0.57	8.871225	3.950452	4
Intercept	2.754377	0.915847	0.959316	4.549437							
TSCR											

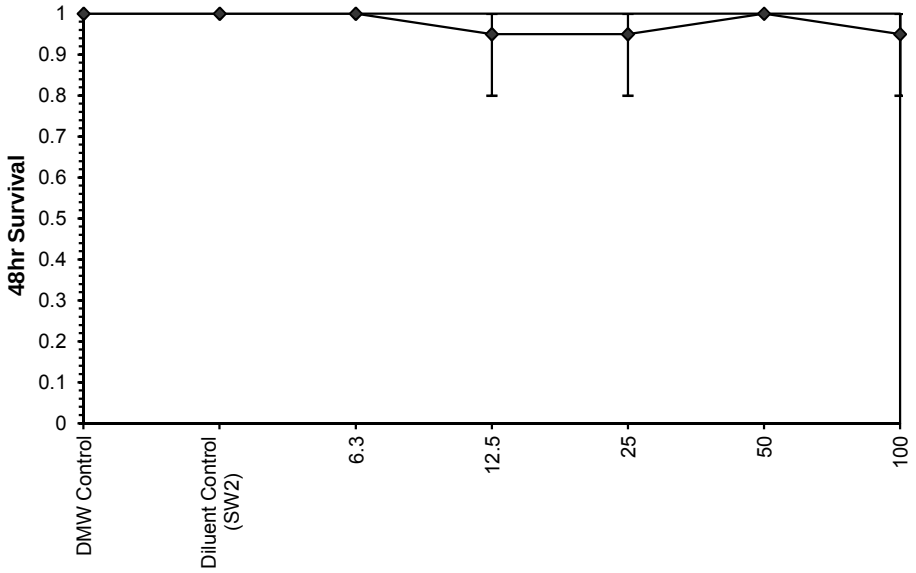
Point	Probits	%	95% Fiducial Limits
EC01	2.674	0.479845	
EC05	3.355	236.2168	
EC10	3.718	6434.549	
EC15	3.964	59819.91	
EC20	4.158	351918.1	
EC25	4.326	1609483	
EC40	4.747	74197638	
EC50	5.000	7.43E+08	
EC60	5.253	7.45E+09	
EC75	5.674	3.43E+11	
EC80	5.842	1.57E+12	
EC85	6.036	9.24E+12	
EC90	6.282	8.59E+13	
EC95	6.645	2.34E+15	
EC99	7.326	1.15E+18	



Chironomid Acute Toxicity Test-48hr Survival

Start Date:	27/03/2013 11:30	Test ID:	PR1022/02	Sample ID:	RP3
End Date:	29/03/2013 11:30	Lab ID:	5929	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 121	Test Species:	CT-Chironomus tepperi
Comments:					

Dose-Response Plot



Chironomid Acute Toxicity Test-48hr Survival

Start Date:	27/03/2013 11:30	Test ID:	PR1022/02	Sample ID:	RP3
End Date:	29/03/2013 11:30	Lab ID:	5929	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 121	Test Species:	CT-Chironomus tepperi
Comments:					

		Auxiliary Data Summary					
Conc-%	**Parameter**	**Mean**	**Min**	**Max**	**SD**	**CV%**	**N**
DMW Control	% Survival	100.00	100.00	100.00	0.00	0.00	4
t Control (SW2)		100.00	100.00	100.00	0.00	0.00	4
6.3		100.00	100.00	100.00	0.00	0.00	4
12.5		95.00	80.00	100.00	10.00	3.33	4
25		95.00	80.00	100.00	10.00	3.33	4
50		100.00	100.00	100.00	0.00	0.00	4
100		95.00	80.00	100.00	10.00	3.33	4
DMW Control	pH	8.00	8.00	8.00	0.00	0.00	1
t Control (SW2)		7.40	7.40	7.40	0.00	0.00	1
6.3		7.00	7.00	7.00	0.00	0.00	1
12.5		6.90	6.90	6.90	0.00	0.00	1
25		6.80	6.80	6.80	0.00	0.00	1
50		7.90	7.90	7.90	0.00	0.00	1
100		9.00	9.00	9.00	0.00	0.00	1
DMW Control	Cond uS/cm	167.30	167.30	167.30	0.00	0.00	1
t Control (SW2)		20.10	20.10	20.10	0.00	0.00	1
6.3		287.00	287.00	287.00	0.00	0.00	1
12.5		508.00	508.00	508.00	0.00	0.00	1
25		884.00	884.00	884.00	0.00	0.00	1
50		1541.00	1541.00	1541.00	0.00	0.00	1
100		2690.00	2690.00	2690.00	0.00	0.00	1
DMW Control	DO %	96.80	96.80	96.80	0.00	0.00	1
t Control (SW2)		97.10	97.10	97.10	0.00	0.00	1
6.3		98.20	98.20	98.20	0.00	0.00	1
12.5		98.60	98.60	98.60	0.00	0.00	1
25		98.80	98.80	98.80	0.00	0.00	1
50		98.60	98.60	98.60	0.00	0.00	1
100		98.90	98.90	98.90	0.00	0.00	1

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Statistical Printouts for *Hydra* Population Growth Tests

Hydra Population Growth Test-Growth Rate

Start Date:	28/03/2013 13:30	Test ID:	PR1022/02	Sample ID:	RP3
End Date:	1/04/2013 11:30	Lab ID:	5929	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 125	Test Species:	HV-Hydra viridissima
Comments:					

Conc-%	1	2	3	4
Lab Control	0.4069	0.3835	0.3931	0.4156
Diluent Control	0.4024	0.4241	0.4113	0.3931
3.1	0.4024	0.4024	0.3835	0.4241
6.3	0.3978	0.3931	0.4156	0.4113
12.5	0.4024	0.4156	0.3883	0.4069
25	0.3978	0.4113	0.4113	0.4113
50	0.3931	0.4241	0.3931	0.3883
100	0.2894	0.2508	0.2590	0.2424

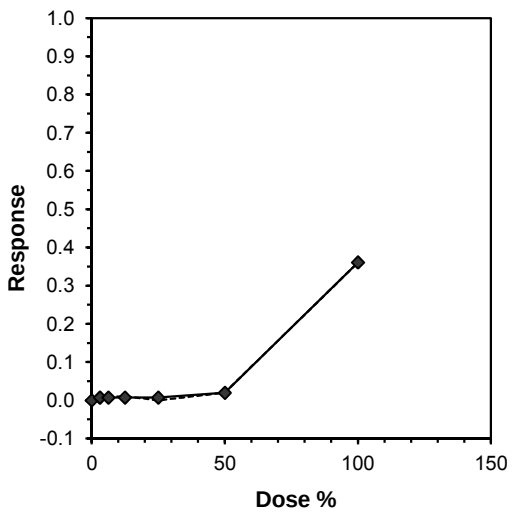
			Transform: Untransformed						1-Tailed		Isotonic	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
Lab Control	0.3998	0.9805	0.3998	0.3835	0.4156	3.572	4					
Diluent Control	0.4077	1.0000	0.4077	0.3931	0.4241	3.241	4	*			0.4077	1.0000
3.1	0.4031	0.9886	0.4031	0.3835	0.4241	4.120	4	0.459	2.451	0.0248	0.4047	0.9926
6.3	0.4044	0.9920	0.4044	0.3931	0.4156	2.653	4	0.323	2.451	0.0248	0.4047	0.9926
12.5	0.4033	0.9892	0.4033	0.3883	0.4156	2.826	4	0.437	2.451	0.0248	0.4047	0.9926
25	0.4079	1.0005	0.4079	0.3978	0.4113	1.657	4	-0.019	2.451	0.0248	0.4047	0.9926
50	0.3997	0.9802	0.3997	0.3883	0.4241	4.117	4	0.796	2.451	0.0248	0.3997	0.9802
*100	0.2604	0.6387	0.2604	0.2424	0.2894	7.865	4	14.570	2.451	0.0248	0.2604	0.6387

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.05)	0.955214	0.924	0.611284	-0.10941
Bartlett's Test indicates equal variances (p = 0.72)	3.690156	16.81189		
The control means are not significantly different (p = 0.45)	0.816567	2.446912		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	50	100	70.71068	2	0.024787	0.060794	0.011875	0.000204	5.4E-12	6, 21
Treatments vs Diluent Control										

Linear Interpolation (200 Resamples)

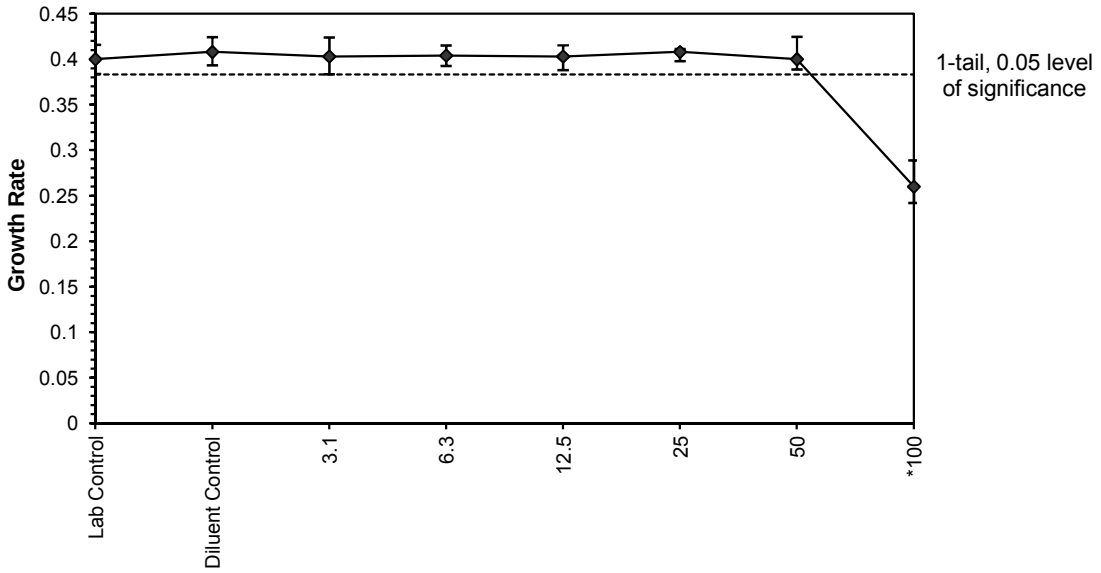
Point	%	SD	95% CL(Exp)		Skew
IC05	54.428	5.903	27.169	59.178	-4.1643
IC10	61.746	2.462	51.617	66.685	-0.4119
IC15	69.065	2.356	59.856	74.330	-0.2298
IC20	76.384	2.391	67.368	82.405	-0.0335
IC25	83.702	2.560	74.900	90.263	0.1413
IC40	>100				
IC50	>100				



Hydra Population Growth Test-Growth Rate

Start Date:	28/03/2013 13:30	Test ID:	PR1022/02	Sample ID:	RP3
End Date:	1/04/2013 11:30	Lab ID:	5929	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 125	Test Species:	HV-Hydra viridissima
Comments:					

Dose-Response Plot



 ToxCalc v5.0.23 Reviewed by:_____

Hydra Population Growth Test-Growth Rate

Start Date:	28/03/2013 13:30	Test ID:	PR1022/02	Sample ID:	RP3
End Date:	1/04/2013 11:30	Lab ID:	5929	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 125	Test Species:	HV-Hydra viridissima
Comments:					

		Auxiliary Data Summary					
Conc-%	**Parameter**	**Mean**	**Min**	**Max**	**SD**	**CV%**	**N**
Lab Control	Growth Rate	0.40	0.38	0.42	0.01	29.89	4
Diluent Control		0.41	0.39	0.42	0.01	28.20	4
3.1		0.40	0.38	0.42	0.02	31.97	4
6.3		0.40	0.39	0.42	0.01	25.61	4
12.5		0.40	0.39	0.42	0.01	26.47	4
25		0.41	0.40	0.41	0.01	20.15	4
50		0.40	0.39	0.42	0.02	32.10	4
100		0.26	0.24	0.29	0.02	54.96	4
Lab Control	Conductivity	31.40	31.40	31.40	0.00	0.00	1
Diluent Control		19.00	19.00	19.00	0.00	0.00	1
3.1		165.10	165.10	165.10	0.00	0.00	1
6.3		285.00	285.00	285.00	0.00	0.00	1
12.5		506.00	506.00	506.00	0.00	0.00	1
25		886.00	886.00	886.00	0.00	0.00	1
50		1557.00	1557.00	1557.00	0.00	0.00	1
100		2710.00	2710.00	2710.00	0.00	0.00	1
Lab Control	pH	7.50	7.50	7.50	0.00	0.00	1
Diluent Control		7.50	7.50	7.50	0.00	0.00	1
3.1		7.30	7.30	7.30	0.00	0.00	1
6.3		7.20	7.20	7.20	0.00	0.00	1
12.5		7.10	7.10	7.10	0.00	0.00	1
25		7.00	7.00	7.00	0.00	0.00	1
50		7.10	7.10	7.10	0.00	0.00	1
100		8.80	8.80	8.80	0.00	0.00	1
Lab Control	DO, % sat	97.40	97.40	97.40	0.00	0.00	1
Diluent Control		95.20	95.20	95.20	0.00	0.00	1
3.1		95.80	95.80	95.80	0.00	0.00	1
6.3		96.10	96.10	96.10	0.00	0.00	1
12.5		95.90	95.90	95.90	0.00	0.00	1
25		96.10	96.10	96.10	0.00	0.00	1
50		96.20	96.20	96.20	0.00	0.00	1
100		98.00	98.00	98.00	0.00	0.00	1

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Statistical Printouts for the Freshwater Shrimp Tests

Freshwater Shrimp Acute Toxicity Test-96 hr Survival

Start Date:	4/04/2013 14:00	Test ID:	PR1022/02	Sample ID:	RP3
End Date:	8/04/2013 14:00	Lab ID:	5929	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 123	Test Species:	MB-Macrobrachium bullatum
Comments:					

Conc-%	1	2	3	4
DMW Control	1.0000	1.0000	1.0000	1.0000
DIluent Control	1.0000	1.0000	1.0000	1.0000
6.3	1.0000	0.6000	0.8000	1.0000
12.5	1.0000	1.0000	1.0000	1.0000
25	1.0000	1.0000	1.0000	0.8000
50	1.0000	1.0000	1.0000	1.0000
100	1.0000	1.0000	0.8000	0.8000

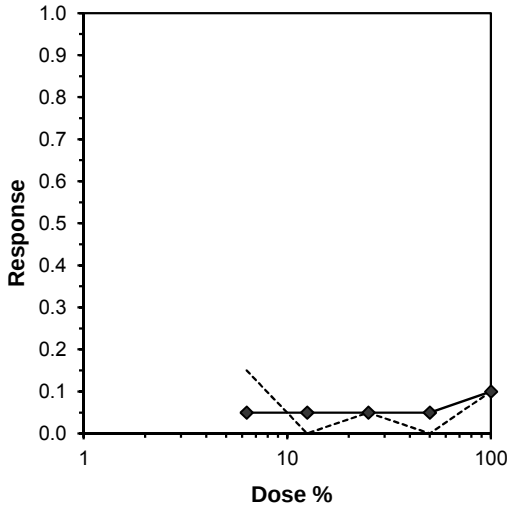
			Transform: Arcsin Square Root					Rank	1-Tailed	Isotonic	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Mean	N-Mean
DMW Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4				
DIluent Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	*		1.0000	1.0000
6.3	0.8500	0.8500	1.1709	0.8861	1.3453	18.840	4	14.00	10.00	0.9500	0.9500
12.5	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0.9500	0.9500
25	0.9500	0.9500	1.2857	1.1071	1.3453	9.261	4	16.00	10.00	0.9500	0.9500
50	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0.9500	0.9500
100	0.9000	0.9000	1.2262	1.1071	1.3453	11.212	4	14.00	10.00	0.9000	0.9000

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.05)	0.886449	0.916	-0.76869	1.684537
Equality of variance cannot be confirmed				
The control means are not significantly different (p = 1.00)	0	2.446912		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	100	>100		1
Treatments vs DIluent Control				

Log-Logit Interpolation (200 Resamples)

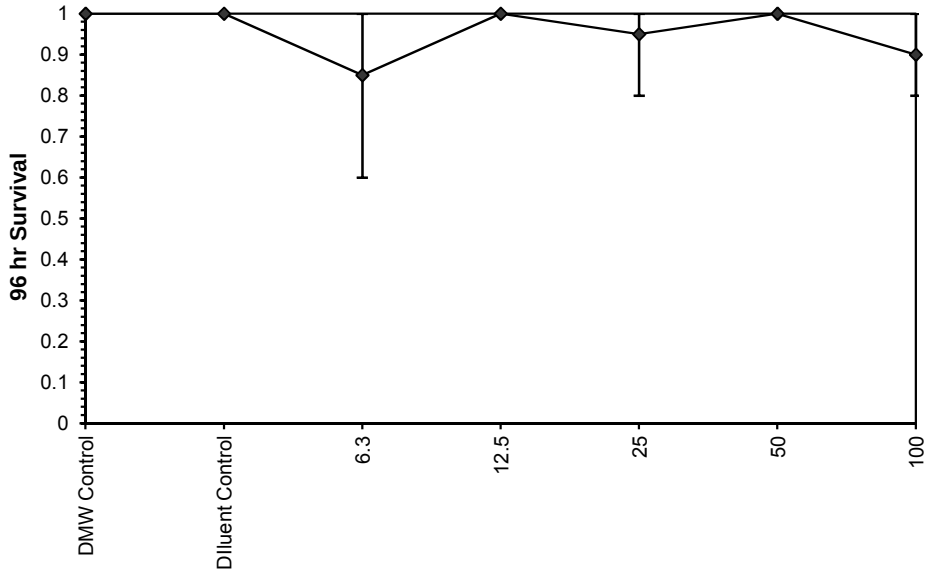
Point	%	SD	95% CL(Exp)	Skew
IC05	50.000			
IC10	>100			
IC15	>100			
IC20	>100			
IC25	>100			
IC40	>100			
IC50	>100			



Freshwater Shrimp Acute Toxicity Test-96 hr Survival

Start Date:	4/04/2013 14:00	Test ID:	PR1022/02	Sample ID:	RP3
End Date:	8/04/2013 14:00	Lab ID:	5929	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 123	Test Species:	MB-Macrobrachium bullatum
Comments:					

Dose-Response Plot



ToxCalc v5.0.23

Reviewed by:_____

Freshwater Shrimp Acute Toxicity Test-96 hr Survival

Start Date:	4/04/2013 14:00	Test ID:	PR1022/02	Sample ID:	RP3
End Date:	8/04/2013 14:00	Lab ID:	5929	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 123	Test Species:	MB-Macrobrachium bullatum
Comments:					

		Auxiliary Data Summary					
Conc-%	**Parameter**	**Mean**	**Min**	**Max**	**SD**	**CV%**	**N**
DMW Control	% Survival	100.00	100.00	100.00	0.00	0.00	4
DIluent Control		100.00	100.00	100.00	0.00	0.00	4
6.3		85.00	60.00	100.00	19.15	5.15	4
12.5		100.00	100.00	100.00	0.00	0.00	4
25		95.00	80.00	100.00	10.00	3.33	4
50		100.00	100.00	100.00	0.00	0.00	4
100		90.00	80.00	100.00	11.55	3.78	4
DMW Control	pH	8.20	8.20	8.20	0.00	0.00	1
DIluent Control		7.30	7.30	7.30	0.00	0.00	1
6.3		6.90	6.90	6.90	0.00	0.00	1
12.5		6.90	6.90	6.90	0.00	0.00	1
25		7.00	7.00	7.00	0.00	0.00	1
50		7.90	7.90	7.90	0.00	0.00	1
100		9.00	9.00	9.00	0.00	0.00	1
DMW Control	Cond uS/cm	169.70	169.70	169.70	0.00	0.00	1
DIluent Control		22.00	22.00	22.00	0.00	0.00	1
6.3		279.00	279.00	279.00	0.00	0.00	1
12.5		497.00	497.00	497.00	0.00	0.00	1
25		885.00	885.00	885.00	0.00	0.00	1
50		1555.00	1555.00	1555.00	0.00	0.00	1
100		2690.00	2690.00	2690.00	0.00	0.00	1
DMW Control	DO %	99.40	99.40	99.40	0.00	0.00	1
DIluent Control		95.10	95.10	95.10	0.00	0.00	1
6.3		97.10	97.10	97.10	0.00	0.00	1
12.5		99.30	99.30	99.30	0.00	0.00	1
25		98.70	98.70	98.70	0.00	0.00	1
50		100.90	100.90	100.90	0.00	0.00	1
100		113.90	113.90	113.90	0.00	0.00	1

Appendix B – Chemistry Reports

Reference	Description	Sample Description	Sample No.	Replicate	Date Sampled	Type of sample	Date prepared	Date analysed
Units							-	-
PQL								
Method								
87792	Mount Todd - SW2 & RP3 Treated Ecotox March 2	SW2	1	0	13/03/2013	Water	22/03/2013	22/03/2013
87792	Mount Todd - SW2 & RP3 Treated Ecotox March 2	SW2	1	1	13/03/2013	Water		
87792	Mount Todd - SW2 & RP3 Treated Ecotox March 2	RP3 treated	2	0	13/03/2013	Water	22/03/2013	22/03/2013

Reference	Total Suspended Solids	Total Dissolved Solids	Total Solids	Total Cyanide	Dissolved Organic Carbon	Total Organic Carbon	Nitrate as N in water
	@ 103-105°C	(grav)					
Units	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
PQL	5	5	5	0.004	1	1	0.005
Method	Inorg-019	Inorg-018	Inorg-041	Inorg-013	Inorg-079	Inorg-079	Inorg-055
87792	<5	31	31	<0.004	2	2	<0.005
87792							
87792	<5	1600	1600	<0.004	<1	<1	2.8

Reference	Phosphate as P in water	Date prepared	Date analysed	Aluminium-(0.45µm filtered)	Cadmium-(0.45µm filtered)	Cobalt-(0.45µm filtered)
Units	mg/L	-	-	µg/L	µg/L	µg/L
PQL	0.005			10	0.1	1
Method	Inorg-060			Metals-022 ICP-MS	Metals-022 ICP-MS	Metals-022 ICP-MS
87792	<0.005	22/03/2013	22/03/2013	29	<0.1	<1
87792						
87792	<0.005	22/03/2013	22/03/2013	<10	5.5	110

Reference	Chromium-(0.45µm filtered)	Copper-(0.45µm filtered)	Iron-(0.45µm filtered)	Lead-(0.45µm filtered)	Manganese-(0.45µm filtered)
Units	µg/L	µg/L	µg/L	µg/L	µg/L
PQL	1	1	10	1	5
Method	Metals-022 ICP-MS	Metals-022 ICP-MS	Metals-022 ICP-MS	Metals-022 ICP-MS	Metals-022 ICP-MS
87792	<1	<1	270	<1	<5
87792					
87792	1	<1	<10	<1	2200

Reference	Mercury-(0.45µm filtered)	Nickel-(0.45µm filtered)	Zinc-(0.45µm filtered)	Date prepared	Date analysed	Aluminium-Total	Cadmium-Total
Units	µg/L	µg/L	µg/L	-	-	µg/L	µg/L
PQL	0.05	1	1			10	0.1
Method	Metals-021 CV-AAS	Metals-022 ICP-MS	Metals-022 ICP-MS			Metals-022 ICP-MS	Metals-022 ICP-MS
87792	<0.05	<1	1	22/03/2013	22/03/2013	290	<0.1
87792						290	<0.1
87792	<0.05	130	82	22/03/2013	22/03/2013	<10	8.5

Reference	Cobalt-Total	Chromium-Total	Copper-Total	Iron-Total	Lead-Total	Manganese-Total	Mercury-Total
Units	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L
PQL	1	1	1	10	1	5	0.05
Method	Metals-022 ICP-MS	Metals-022 ICP-MS	Metals-022 ICP-MS	Metals-022 ICP-MS	Metals-022 ICP-MS	Metals-022 ICP-MS	Metals-021 CV-AAS
87792	<1	<1	<1	740	<1	<5	<0.05
87792	<1	<1	<1	760	<1	<5	[NT]
87792	120	1	<1	<10	<1	2300	<0.05

Reference	Nickel-Total	Zinc-Total	Magnesium - Total	Date prepared	Date analysed	Calcium - (0.45µm filtered)	Potassium - Dissolved
Units	µg/L	µg/L	mg/L	-	-	mg/L	mg/L
PQL	1	1	0.5			0.5	0.5
Method	Metals-022 ICP-MS	Metals-022 ICP-MS	Metals-020 ICP-AES			Metals-020 ICP-AES	Metals-020 ICP-AES
87792	<1	2	0.8	22/03/2013	22/03/2013	0.5	<0.5
87792	<1	3					
87792	140	540	110	22/03/2013	22/03/2013	490	8

Reference	Sodium - Dissolved	Magnesium - (0.45µm filtered)	Hydroxide Alkalinity (OH^-)	Bicarbonate Alkalinity as	Carbonate Alkalinity as	Total Alkalinity
			as $CaCO_3$	$CaCO_3$	$CaCO_3$	as $CaCO_3$
Units	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
PQL	0.5	0.5	5	5	5	5
Method	Metals-020 ICP-AES	Metals-020 ICP-AES	Inorg-006	Inorg-006	Inorg-006	Inorg-006
87792	1.7	0.8	<5	17	<5	17
87792						
87792	51	110	<5	45	6	51

Reference	Sulphate, SO4	Chloride, Cl	Ionic Balance	Hardness
Units	mg/L	mg/L	%	$mgCaCO_{3/L}$
PQL	1	1		3
Method	Inorg-081	Inorg-081	Inorg-041	
87792	<1	1	-40	4
87792				
87792	1600	7	2.1	1700

GHD

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Document Status

Rev No.	Author	Reviewer		Approved for Issue		
		Name	Signature	Name	Signature	Date
0	J.Woodworth	G.Metcalfe	*G Metcalfe*	N.Conroy	*N Conroy*	03/06/2013
1	J.Woodworth	G.Metcalfe		G.Metcalfe		01/07/2013

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RP3 Ecotoxicology – December 2013

Vista Gold Australia Pty Ltd

Mt Todd Discharge

Treated Retention Pond 3 Ecotoxicological Report

January 2014

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Appendices

1. Introduction

1.1 Project Background

Vista Gold Australia Pty Ltd (Vista Gold) received a Waste Discharge Licence (WDL 178-3) on 26 November 2013 from the Northern Territory Environment Protection Authority (NT EPA). The WDL outlines environmental requirements for protection of the Edith River from mine wastewater discharges. Previously, Vista Gold derived interim site specific trigger values (ISSTVs) (GHD 2012) to meet the 95% species protection trigger values downstream of the site at SW10 (8.7 km from SW4) to meet the requirements of WDL 178-1. However, the use of dilution factors derived from direct toxicity assessment (DTA) for three water bodies at the Mt Todd mine site have been selected as the most appropriate method for deriving safe concentrations of metals for environmental protection of the Edith River to meet the requirements of WDL 178-3.

The DTA method is recommended by ANZECC & ARMCANZ (2000) where chemicals are present in a complex mixture. DTA allows the determination of direct biological effects on appropriate species to be assessed and also enables metal concentrations to be established that would not cause adverse environmental effects.

This report should be read in conjunction with the Waste Discharge Plan (GHD 2013) (http://mttodd.com.au/content/waste-discharge-licence).

1.2 Objective

WDL 178-3 requires that ecotoxicological assessment be conducted for a wastewater source. This treated retention pond (RP) 3 Ecotox DTA Report has been developed to address the relevant sections of the WDL 178-3 to derive a dilution factor for RP3 prior to discharging treated mine water into the Edith River.

1.3 Scope of Work

This Report addresses the following aspects of the WDL 178-3:

- The methodology for calculating the 80% species protection ANZECC & ARMCANZ (2000) dilution factor for ecosystem protection applied at SW4 for mine discharges from RP3.
- Deriving Monitoring Values for the treated RP3 mine water to be met at SW4 to check that the dilution factor for the treated mine water is being met.

1.4 Limitations

This Vista Gold Australia Retention Pond 3 Ecotoxicological Report ("Report"):

- Has been prepared by GHD Pty Ltd ("GHD") for Vista Gold Australia Pty Ltd (Vista Gold) and the NT EPA.
- May only be used and relied on by Vista Gold and the NT EPA.
- Must not be copied to, used by, or relied on by any person other than Vista Gold without the prior written consent of Vista Gold.
- May only be used for the purpose of addressing WDL 178-3 requirements (and must not be used for any other purpose).

GHD and its servants, employees and officers otherwise expressly disclaim responsibility to any person other than Vista Gold arising from or in connection with this Report.

To the maximum extent permitted by law, all implied warranties and conditions in relation to the services provided by GHD and the Report are excluded unless they are expressly stated to apply in this Report.

The services undertaken by GHD in connection with preparing this Report were limited to those specifically detailed in section 1.3 of this Report.

The opinions, conclusions and any recommendations in this Report are based on assumptions made by GHD when undertaking services and preparing the Report ("Assumptions"), including (but not limited to) those specified in section 1.5 below.

GHD expressly disclaims responsibility for any error in, or omission from, this Report arising from or in connection with any of the Assumptions being incorrect.

Subject to the paragraphs in this section of the Report, the opinions, conclusions and any recommendations in this Report are based on conditions encountered and information reviewed at the time of preparation and may be relied on until 6 months, after which time, GHD expressly disclaims responsibility for any error in, or omission from, this Report arising from or in connection with those opinions, conclusions and any recommendations.

1.5 Assumptions

The assumptions upon which this report is based are:

- The treated mine water tested for DTA was representative of the RP3 water at the time of testing.
- SW2 water used in the DTA was representative of early wet season Edith River water.

2. Direct Toxicity Assessment Methodology

2.1 Direct Toxicity Assessment

Direct Toxicity Assessment (DTA) provides an integrated measure of the toxicity of chemicals within a complex mixture, and accounts for interactions between compounds which may be additive, synergistic or ameliorative. DTA provides a better representation of natural environmental conditions than single chemical testing, and is therefore recommended by ANZECC & ARMCANZ (2000) for assessing the environmental impact from complex effluents.

The Mt Todd DTA program was designed in line with the recommendations contained in ANZECC & ARMCANZ (2000). ANZECC & ARMCANZ (2000) states that a DTA program should address the following design considerations:

- Test species selection
- Receiving water selection
- Nature of contaminant(s)
- Test methods
- Statistical considerations.

2.1.1 Selected Species

ANZECC & ARMCANZ (2000) "Minimum requirements for DTA" (section 8.3.6.8) recommend that toxicity data from between three and five species representing at least four different taxonomic groups (where five species are used) is required for effluent DTA. Further information on species used in the DTAs conducted for Mt Todd discharge to the Edith River is shown in Table 1 below.

Often, due to laboratory issues, not all tests listed in Table 1 are available at the time of testing. DTAs conducted on RP1, RP7 and RP3 Pilot Trial used a maximum of seven species to provide confidence in the species sensitivity distribution calculation.

Tests were selected adhering to the following criteria:

- a minimum of five bioassays covering at least four taxa
- preferably chronic endpoints to avoid the used of application factors
- locally occurring species or species representative of the ecosystem
- dilution water sampled from SW2 (upstream site)
- sufficient test dilutions to obtain a statistically valid EC10 (i.e. tight 95% confidence limits)
- bioassay suite sensitive to a broad range of contaminants

The bioassays for the Mt Todd site are usually conducted by two laboratories: ERISS in Darwin and Ecotox Services Australasia located in Sydney, because not all bioassays are available from one laboratory. The cladoceran (*Moinodaphnia macleayi*) is the most sensitive bioassay species and is only able to be conducted by the ERISS Laboratory in Darwin, however, the laboratory was unable to conduct the test and another cladoceran species, *Ceriodaphnia dubia*, was used in place of the *M. macleayi*. Therefore, all bioassays, shown in Table 1, were conducted by Ecotox Services Australasia.

Table 1 Species used in the Ecotoxicological Assessment of Treated RP3 Discharge

Test Organism	Test Duration	Test Endpoint	Key Reference
Selenastrum caprocornutum (green alga)	72 hour (chronic)	Growth inhibition	USEPA Method 1003.0 (2002)
Lemna aequinoctialis (duckweed)	96 hour (chronic)	Growth (frond number)	OECD Method 221 (2006)
Ceriodaphnia dubia (water flea)	3 brood (chronic)	Reproduction	USEPA Method 1002.0 (2002)
Hydra viridissima Pallas (green hydra)	96 hour (chronic)	Population growth	Riethmuller *et al.* (2003)
Chironomus tepperi (chironomid)	48 hour (acute)	Survival	USEPA (2002), OECD (2011)
Macrobrachium bullatum (shrimp)	96 hour (acute)	Survival	ESA SOP 123 (ESA 2012)
Mealanotaenia splendida (rainbowfish)	10 day (chronic)	Embryonic development and post hatch survival	USEPA (2002)

2.1.2 Concentrations Tested

Edith River water sampled from the upstream site SW2 was used as the diluent to assess the toxicity of the treated RP3 mine water. A laboratory control was run concurrently with all tests to confirm that the natural diluent water was not confounding the bioassays (as can happen in many cases). All samples were serially diluted with the SW2 river water to achieve the required test concentrations. Concentrations differed between bioassays due to methodologies used and sensitivity of the species tested as determined by previous bioassays. Bioassay dilutions for each species are shown in Table 2.

Table 2 Concentrations of Treated RP3 used in Bioassays

Microalga % RP3	Duckweed % RP3	Cladoceran % RP3	Hydra % RP3	Chironomid % RP3	Shrimp % RP3	Fish %RP3
0	0	0	0	0	0	0
0.1	0.2	0.1	6.3	6.3	6.3	3.1
0.2	0.4	0.2	12.5	12.5	12.5	6.3
0.4	0.8	0.4	25	25	25	12.5
0.8	1.5	0.8	50	50	50	25
1.6	3.0	1.5	100	100	100	50
3.1	6.0	3.0	-	-	-	100

2.1.3 Dilution Factors

Dilution factors are derived from results of DTA using bioassays representative of the receiving ecosystem which incorporates all actions of the constituents of the effluent. These results are placed in the BurrliOZ (Campbell *et al.* 2000) statistics program for calculation of a species sensitivity distribution and the concentration of effluent is determined to protect 80% of the populations in the receiving water from a 10% decrease in growth and/or reproduction.

Concentrations of individual chemicals cannot be extrapolated from DTAs for use as trigger values. However, individual chemical concentrations can be used for monitoring purposes to ensure that the dilution factors are met at the appropriate monitoring site. If the dilution factors are not met, the investigation procedure shown in Figure 1 of the Vista Gold Discharge Plan (GHD 2013) will be followed.

2.2 Surface Water Chemistry

Samples from SW2 (diluent) and treated RP3 water were analysed for the suite of analytes shown in Table 3.

Table 3 Analytes for SW2 and Treated RP3

	Analytes
In-Situ	DO, EC, pH
Metals (total and dissolved, i.e. 0.45 µm)	Al, Cd, Co, Cu, Cr, Fe, Pb, Mn, Hg, Ni, Zn
Others	SO_4, Ca, Mg

2.3 Sample Sites

RP3 and SW2 sampling locations are shown in Table 4. The treated RP3 water was sampled at the surface as testing of the top 30 metres has shown it to be homogenous. The proposed pumping depth for discharge will be within the top 30 metres as it provides treated water with the highest pH and lowest metal concentrations.

Table 4 Sampling Sites

Site Name	Site Description	Easting (UTM)	Northing	Latitude (degree, decimal)	Longitude
SW2	Edith River at bridge on Edith Falls Road	0189088	8431347	-14.1718	132.12
RP3	Batman Pit	0187055	8434993	-14.1388	132.1015

3. Results

3.1 Ecotoxicology Testing of RP3 Treated Water (in-situ)

Sites at treated RP3 and SW2 were sampled on the 25 November 2013 and sent directly to the laboratory where testing commenced immediately upon receipt.

The species used were appropriate for the site and the EC10 and LC50 results were of sufficient quality to be used in the BurrliOZ (Campbell *et al.,* 2000) program for species sensitivity distribution (SSD) calculations. Not all species that were used in the ERISS (2005) toxicity testing program were available at the time of testing. The cladoceran (*Moinodaphnia macleayi*) has been replaced with the temperate cladoceran *Ceriodaphnia dubia*.

A copy of the Ecotox Report by Ecotox Services, "*Toxicity Assessment of a Treated Water Sample, December` 2013*" is located in Appendix A. A summary of the results is provided in Appendix B.

The results of the site specific DTA testing performed by Ecotox Services used in this assessment do meet the laboratory NATA requirements for quality assurance/quality control (QA/QC) parameters. It must be noted however, that the majority of the reference toxicant results are in the lower 20% of the Cusum chart limits, indicating that the organisms used were more sensitive to the toxicants than is usual (Appendix A). This sensitivity means that the results presented here from this treated RP3 DTA are very conservative.

Table 5 Summary of Treated RP3 Ecotox Results

Test	EC/IC10 and LC10 (95% confidence limits) % Treated RP3	EC/IC50 and LC50 (95% confidence limits) % Treated RP3
Microalgal 72-hour growth inhibition	0.9 (0.6 – 1.0)	2.1 (1.9 – 2.2)
Duckweed 96-hour growth	0.8 (0.8 – 0.9)	1.1 (1.1 – 1.2)
Cladoceran 6-day reproduction	0.1**	0.2 (0.2 – 0.2)
Hydra 96-hour growth	10.9**	44.6 (36.8 – 57.3)
Chironomid 48-hour survival	100*	>100
Shrimp 96-hour survival	>100	>100
Fish 10 day embryonic development and post hatch survival	60.2**	95.2 (72.9 – 100)

**No observable effect concentration (NOEC) **95% confidence limits not provided*

The results show that the algae, duckweed and cladoceran are the most sensitive species to the treated RP3 water. There was no acute toxicity observed from exposure to the treated RP3 water.

The cladoceran is sensitive to the treated RP3 water, which may be a reflection of the toxicity of the treated RP3 water to their food source (i.e. the unicellular alga).

The chironomid tests showed an interrupted dose response, therefore, the NOEC value has been used as it is not significantly different from the control results.

The chemistry results shown in Table 8 indicate that the toxicity observed in the algal, duckweed and cladoceran bioassays can be attributed to the presence of zinc and possibly cadmium and nickel, as other toxic metals were below detection limits and below the default 95% species protection trigger values.

3.2 Acute to Chronic Ratios (ACR)

Two of the bioassays used in this study were acute bioassays where survival was the end point. The chironomid and shrimp are acute bioassays and the LC50 results need to be adjusted so that they can be incorporated into the statistics to calculate the SSD. ANZECC & ARMCANZ (2000) recommend an acute to chronic factor of 10 to go from an acute LC50 to a chronic NOEC or EC10. This acute to chronic ratio (ACR) was applied to the chironomid and shrimp LC50 data to obtain a conservative chronic EC10.

3.3 Concentrations used to calculate the SSD

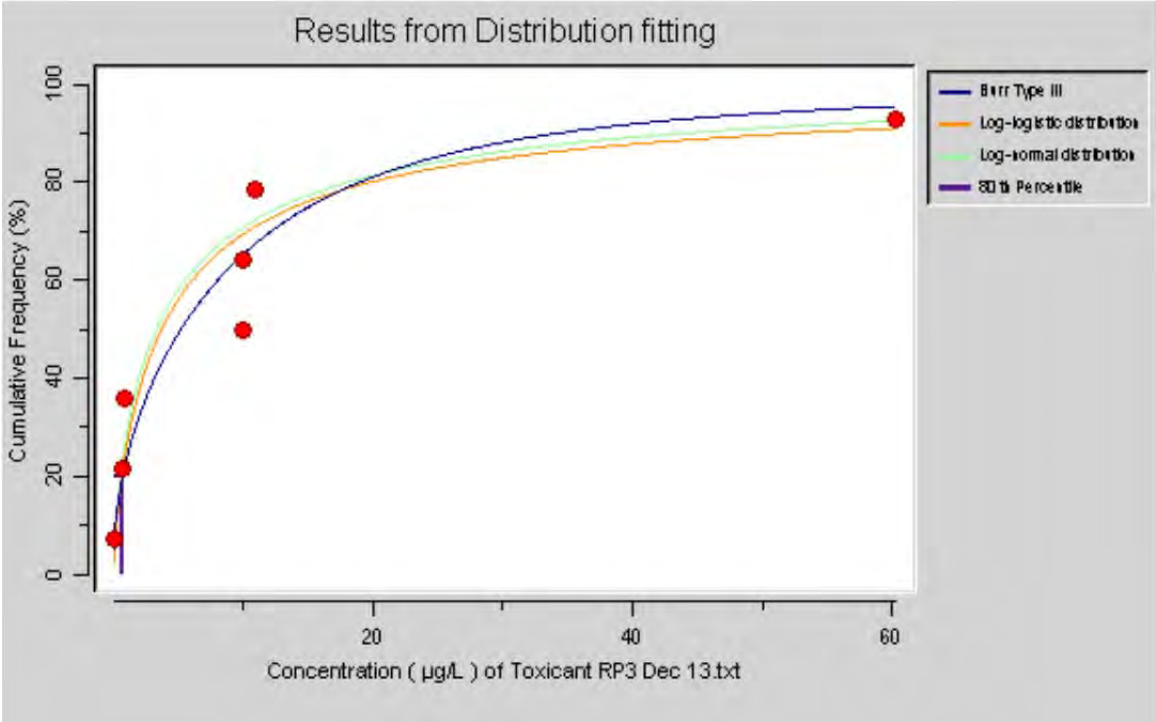
The acute bioassays required an ACR adjustment, applied to the LC50 values for the chironomid (LC50/10) and shrimp (LC50/10). The values used to calculate the SSD and dilution factors are shown in Table 6.

Table 6 Values used to Calculate SSD

Bioassay	EC10 % RP3
Microalgal 72-hour growth inhibition	0.9
Duckweed 96-hour growth inhibition	0.8
Cladoceran 6-day reproduction	0.1
Hydra 96-hour growth	10.9
Chironomid 48-hour survival	10
Shrimp 96-hour survival	10
Fish 10-day Embryo hatching	60.2

3.4 Species Protection Values

The EC10 and LC50/10 values from Table 6 were input into the BurrliOZ (Campbell *et al*., 2000) statistics program for calculation of the 80% species protection value using the SSD. The calculated value and the dilution factor required to reach the concentration are provided in Table 7. The 80% SSD graph is shown in Figure 1.



PC80 50% = 0.76 (501 Bootstrap samples)

Burr Type III distribution fitted to 7 observations

Figure 1 Species Sensitivity Distribution Graph

Table 7 Species Protection Values

Species Protection Level	Concentration of Treated RP3 (%)	Dilution Factor
80%	0.76	132

3.5 Dilution Factor

The 80% species protection was selected as appropriate to provide a level of protection for the Edith River from the discharge of treated RP3 water over the dewatering period as required by the WDL 178-3. A dilution factor of 1:132 for the RP3 treated water (as at time of testing, 25/11/13) discharged into the Edith River will provide 80% of the species with protection from a 10% decrease in reproduction or growth (Table 7). The concentrations of chemicals at this dilution are shown in Table 8. This dilution factor will provide a conservative result as the water quality within RP3 is expected to improve during the wet season.

3.6 80% Species Protection Monitoring Values

The chemistry of the RP3 treated water at the 1:132 dilution is shown in Table 8. The complete chemistry results are located in Appendix B.

The levels of the 80% species protection dilution concentrations for cadmium (0.4 µg/L) and zinc (33.3 µg/L) have been selected as monitoring values to be met at SW4. These monitoring values will ensure that the dilution of the treated RP3 water is at 1:132 to meet the requirements of WDL 178-3. It must be noted that the concentrations of other metals and metalloids in the discharge will be below the 95% species protection trigger values at SW4 and, in most cases, also below the laboratory detection limits.

The concentrations of cadmium at 0.4 µg/L and zinc at 33.3 µg/L will be met at SW4 to ensure environmental protection of the Edith River. If this monitoring value is not met then action is triggered as shown in Figure 1 of the Discharge Plan (GHD 2013).

Table 8 Dilution Rates of RP3 Treated Water (in-situ) to Meet 80 % SSD Dilution (1:132)

Analytes (metals 0.45 µm)	SW2 25/11/13	RP3 Treated Water 25/11/13	Conc. in RP3 treated water at 1:132 to be met at SW4	ANZECC 80% species protection TVs/ISSTV	ANZECC 95% species protection TVs/ISSTV
pH	6.7	7.6	-	6-8	6-8
DO (%)	6.2	8.5	-	85-120	85-120
Conductivity (µS/cm)	19	2,700	-	20-250	20-250
Mg (mg/L)	<0.5	210	1.6	-	2.5*
Ca (mg/L)	<0.5	480	3.6	-	-
SO_4 (mg/L)	<1.0	1,900	14.4	129	129
Al (µg/L)	<10	<10	<10	150	149
Cd (µg/L)	<0.1	51	0.4	0.8	0.2
Co (µg/L)	<1.0	510	3.9	90	90
Cr (µg/L)	<1.0	<1.0	<1.0	40	1.0
Cu (µg/L)	<1.0	<1.0	<1.0	2.5	1.4
Fe (µg/L)	110	<10	<10	300	300
Mn (µg/L)	<5.0	6,000	45.5	3,600	1,700
Ni (µg/L)	<1.0	500	3.8	17	11
Pb (µg/L)	<1.0	<1.0	<1.0	9.4	3.4
Hg (µg/L)	<0.05	<0.05	<0.05	5.4	0.6
Zn (µg/L)	1.0	4,400	33.3	31	8.0

*Note: Cells in green are below the ISSTVs and ANZECC & ARMCANZ (2000) default 95% trigger values and below the laboratory detection limits *van Dam et al (2010)*

The water quality sampled in November 2013 showed elevated cadmium, nickel and zinc when compared to the March 2013 sample used for the previous DTA. The elevated metals are related to the drop in pH from 8.8 in March 2013 to pH 7.6 in November 2013. This drop in pH is most likely due to mixing within the top 30 metres and turnover of the pit as the concentration of zinc below 30m has decreased since the March 2013 sampling.

Table 9 shows the monitoring values for each discharge point as of 24 December 2013.

Table 9 Monitoring Values for SW4 Wet Season 2013/2014

Discharge	Chemical	Concentration (µg/L) 0.45 µm filtered
RP3 Treated Water	Cd	0.4
	Zn	33.3
RP1 Untreated Water	Cu	No discharge
RP7 Untreated Water	Cu	No discharge

RP3 treated water will be assessed for toxicity prior to the 2014/2015 wet season and a revised dilution factor and revised monitoring values will be calculated.

Vista Gold are not proposing to discharge from RP1 or RP7 during the 2013/2014 wet season at this date. RP1 and RP7 mine waters will be assessed for toxicity prior to any discharge and revised dilution factors and monitoring values will be calculated.

4. Conclusions and Recommendations

4.1 Conclusions

Ongoing treatment of water in RP3 has resulted in substantial improvements in water quality since treatment began in November 2012 (Table 10).

The results of this direct toxicity assessment on treated water from RP3 show that a conservative dilution factor of 1:132 will provide an 80% species protection level at the downstream monitoring point SW4 on the Edith River. That is, one unit of RP3 water is to be diluted with 132 units flowing down the Edith River to mitigate any adverse impact on organisms within the receiving ecosystem. All metal concentrations will be below the 95% species protection default trigger values at SW4 at this dilution with the exception of cadmium and zinc.

Table 10 RP3 Chemistry (Top 30 metres)

Analyte Dissolved µg/L	20 Oct 2012	23 Nov 2012	20 Dec 2012	17 Jan 2013	14 Feb 2013	21 Mar 2013	25 Nov 2013
Aluminium	57,000	31,000	1,700	270	<10	<10	<10
Chromium	2	2	1	<1	<1	1	<1
Copper	10,000	11,000	9,900	5,400	140	<1	<1
Lead	210	190	160	86	<1	<1	<1
Cadmium	140	140	140	130	45	5.5	51
Zinc	36,000	34,000	38,000	39,000	8,400	82	4,400

Treatment of RP3 is changing the water quality throughout the pit. Even though treatment has ceased, the water quality below 30m is still improving with elevated pH and reduced metal concentrations (Table 10). The dilution factor of 1: 132 calculated in this report will be applied for discharging in the 2013/2014 wet season.

4.2 Recommendations

To meet the 80% species protection level required by WDL 178-3, based on ecotoxicological testing, it is recommended that a dilution factor of 1:132 be applied to the discharge of RP3 treated mine water, with the following monitoring values applied at SW4:

- Cd 0.4 µg/L
- Zn 33.3 µg/L

These monitoring values will apply until a further round of ecotoxicological testing is conducted to derive a new dilution factor and monitoring values prior to discharging in the 2014/2015 wet season.

5. References

ANZECC & ARMCANZ (2000) Australian and New Zealand Environment and Conservation Council and Agriculture and Resource Management Council of Australia and New Zealand. National water quality management strategy, Australian and New Zealand guidelines for fresh and marine water quality. ANZECC and ARMCANZ, Canberra, Australia.

Campbell E., Palmer M.J., Shao Q., Warne M.StJ. and Wilson D. (2000) BurrliOZ: A computer program for calculating toxicant trigger values for the ANZECC and ARMCANZ water quality guidelines. Perth, Western Australia.

ERISS. 2005. Ecotoxicological assessment of retention pond water from Mount Todd gold mine. Internal Report 499. R. van Dam, A. Hogan, S. Nou and S. Markich. March 2005. Darwin, NT.

GHD (2013). Vista Gold Australia Pty. Ltd. Discharge Plan. Revision 1. February 2013.

OECD (2006) Lemna sp. Growth inhibition test. Method 221. OECD guideline for the testing of chemicals. Organisation for Economic Cooperation and Development. Paris.

OECD (2011) OECD guideline for the testing of chemicals. Test Guideline 235: Chironomus sp. Acute immobilisation test. Organisation for Economic Cooperation and Development. Paris.

Riethmuller N., Camilleri C., Franklin N., Hogan A., King A., Markich S.J., Turley C. and van Dam R. (2003) Green hydra population growth test. In: Ecotoxicological Testing Protocols for Australian Tropical Freshwater Ecosystems. Supervising Scientist Report 173. ERISS, Darwin, NT.

USEPA (2002) Short-term methods for measuring the chronic toxicity of effluents and receiving waters to freshwater organisms. 4th Edition. United States Environmental Protection Agency, Office of Water, Washington DC.

van Dam R.A., Hogan A.C., McCullough C.D., Houston M.A., Humphrey C.L. and Harford A.J. 2010. Aquatic toxicity of magnesium sulfate and the influence of calcium in very low ionic concentration water. Environmental Toxicology and Chemistry. 29(2):410-421.

Appendices

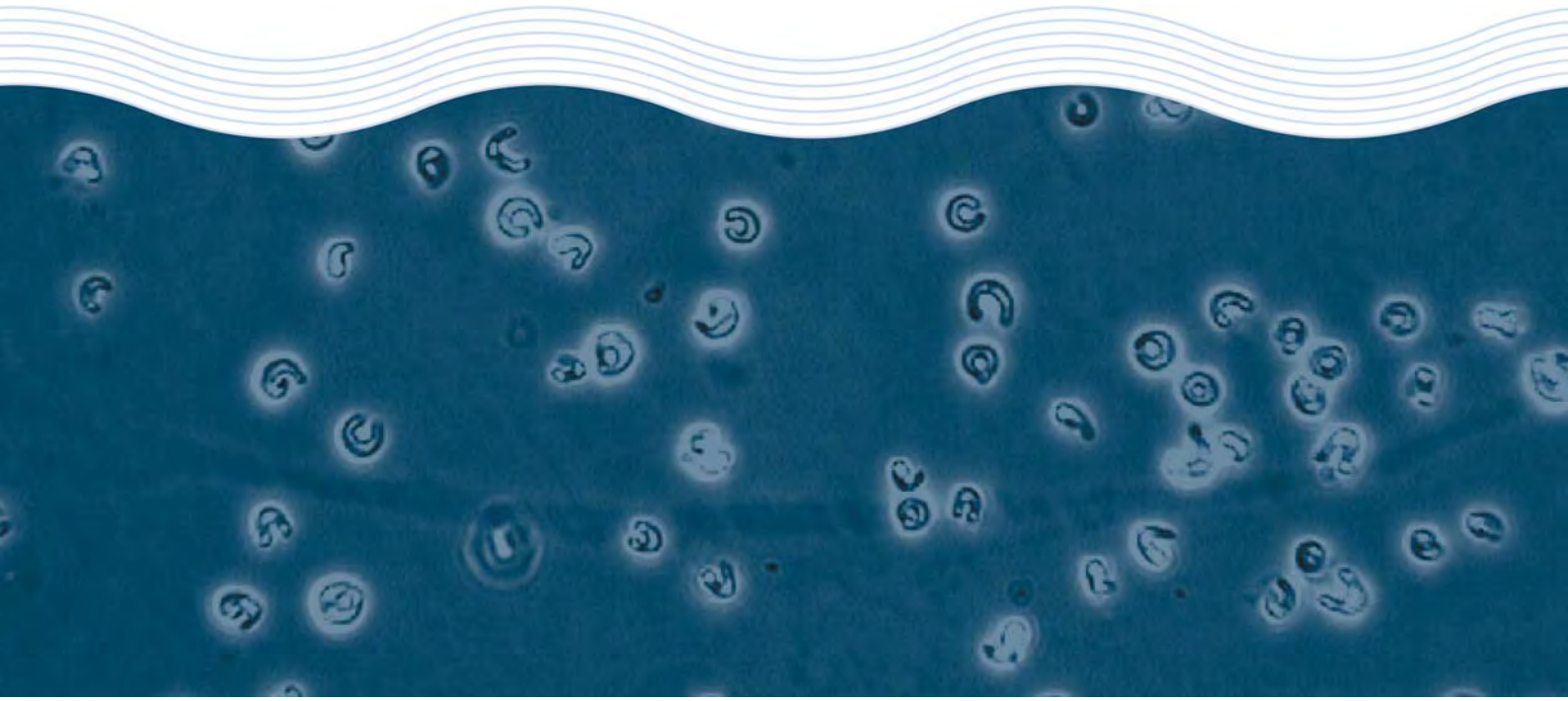
ecotox
SERVICES AUSTRALASIA

Toxicity Assessment of a Treated Water Sample from Mt Todd

Vista Gold Australia Pty Ltd

Test Report

December 2013



Toxicity Assessment of a Treated Water Sample from Mt Todd

Vista Gold Australia Pty Ltd

Test Report

December 2013

ECOTOX Services Australasia Pty Ltd ABN>45 094 714 904
unit 27/2 chaplin drive lane cove nsw 2066 T>61 2 9420 9481 F>61 2 9420 9484 W>www.ecotox.com.au

Toxicity Test Report: TR1108/1

This document is issued in accordance with NATA's accreditation requirements

Client:	Vista Gold Pty Ltd Level 3, 43 Cavenagh Street Darwin NT 0801	**ESA Job #:** **Date Sampled:** **Date Received:**	PR1108 25 November 2013 26 November 2013
Attention:	Austin Brandis	**Sampled By:**	Client
Client Ref:	Not applicable	**ESA Quote #:**	PL1108_q03

Lab ID No.:	Sample Name:	Sample Description:
6368	SW2	Aqueous sample, pH 7.5, conductivity 21.7µS/cm. Sample received at room temperature in apparent good condition
6369	RP3	Aqueous sample, pH 7.7, conductivity 2900µS/cm. Sample received at room temperature in apparent good condition

Test Performed:	72-hr microalgal growth inhibition test using the green alga *Selenastrum capricornutum*
Test Protocol:	ESA SOP 103 (ESA 2013), based on USEPA (2002)
Test Temperature:	The test was performed at 25±1°C.
Deviations from Protocol:	Nil
Comments on Solution Preparation:	The samples were filtered to 0.45µm prior to testing. The highest concentration was prepared by diluting sample 6369 'RP3' with sample 6368 'SW2'. Sample 6369 'RP3' was then serially diluted with sample 6368 'SW2' to achieve the test concentrations. A USEPA control and a diluent control (SW2) were tested concurrently with the sample.
Source of Test Organisms:	ESA Laboratory culture, originally sourced from CSIRO Microalgal Supply Service, TAS
Test Initiated:	13 December 2013 at 1330h

Sample 6369: *RP3*		*Vacant*	*Vacant*
Concentration (%)	**Cell Yield x10^4 cells/mL (Mean ± SD)**		
USEPA Control	18.1 ± 3.0		
Diluent Control	20.7 ± 1.0		
0.1	19.8 ± 0.6		
0.2	19.9 ± 1.1		
0.4	20.4 ± 0.6		
0.8	19.4 ± 0.6		
1.6	13.2 ± 0.6 *		
3.1	4.4 ± 1.0 *		
72-hr IC10 = 0.9 (0.6-1.0)% **72-hr IC50 = 2.1 (2.0-2.2)%** **NOEC = 0.8%** **LOEC = 1.6%**			

*Significantly lower cell yield compared with the Diluent Control (Dunnett's Test, 1-tailed, P=0.05)

Toxicity Test Report: TR1108/1

QA/QC Parameter	Criterion	This Test	Criterion met?
Control mean cell density	≥16.0x10^4 cells/mL	19.1x10^4 cells/mL	Yes
Control coefficient of variation	<20%	16.3%	Yes
Reference Toxicant within cusum chart limits	1.3-5.3g KCl/L	2.6g KCl/L	Yes

Test Report Authorised by: Dr Rick Krassoi, Director on 23 December 2013

Results are based on the samples in the condition as received by ESA.

NATA Accredited Laboratory Number: 14709

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Citations:

ESA (2013) *ESA SOP 103 – Green Alga, Selenastrum capricornutum, Growth Test*. Issue No 10. Ecotox Services Australasia, Sydney, NSW.

USEPA (2002) *Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms*. Fourth Edition. EPA-821-R-02-013. United States Environmental Protection Agency, Office of Research and Development, Washington DC, USA,

Toxicity Test Report: TR1108/2

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Client:	Vista Gold Pty Ltd Level 3, 43 Cavenagh Street Darwin NT 0801	**ESA Job #:** **Date Sampled:** **Date Received:**	PR1108 25 November 2013 26 November 2013
Attention:	Austin Brandis	**Sampled By:**	Client
Client Ref:	Not applicable	**ESA Quote #:**	PL1108_q03

Lab ID No.:	Sample Name:	Sample Description:
6368	SW2	Aqueous sample, pH 7.5, conductivity 21.7µS/cm. Sample received at room temperature in apparent good condition
6369	RP3	Aqueous sample, pH 7.7, conductivity 2900µS/cm. Sample received at room temperature in apparent good condition

Test Performed:	96-hr Growth inhibition of the freshwater aquatic duckweed *Lemna aequinoctialis*
Test Protocol:	ESA SOP 112 (ESA 2012), based on OECD method 221 (2006)
Test Temperature:	The test was performed at 29±2°C.
Deviations from Protocol:	Nil
Comments on Solution Preparation:	The highest concentration was prepared by diluting sample 6369 'RP3' with sample 6368 'SW2'. Sample 6369 'RP3' was then serially diluted with sample 6368 'SW2' to achieve the test concentrations. A CAAC control and a diluent control (SW2) were tested concurrently with the sample.
Source of Test Organisms:	ESA Laboratory culture
Test Initiated:	11 December 2013 at 1500h

Sample 6369: *RP3*		*Vacant*	*Vacant*
Concentration (%)	**Specific Growth Rate (Mean ± SD)**		
CAAC Control	0.32 ± 0.02		
Diluent Control	0.31 ± 0.01		
0.2	0.33 ± 0.03		
0.4	0.33 ± 0.02		
0.8	0.31 ± 0.01		
1.5	0.01 ± 0.02 *		
3.0	0.00 ± 0.00		
6.0	0.00 ± 0.00		
96-hr IC10 = 0.8 (0.8-0.9)% **96-hr IC50 = 1.1 (1.1-1.2)%** **NOEC = 0.8%** **LOEC = 1.5%**			

*Significantly lower specific growth rate compared with the Diluent Control (Steel's Many-One Rank Test, 1-tailed, P=0.05)

Toxicity Test Report: TR1108/2

QA/QC Parameter	Criterion	This Test	Criterion met?
Control frond doubling time	<3.0 days	2.2days	Yes
Reference Toxicant within cusum chart limits	7.4-59.4mg Mg/L	13.2mg Mg/L	Yes

Test Report Authorised by: Dr Rick Krassoi, Director on 23 December 2013

Results are based on the samples in the condition as received by ESA.

NATA Accredited Laboratory Number: 14709

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Citations:

ESA (2012) *SOP 112 – Duckweed Growth Inhibition Test.* Issue No. 5. Ecotox Services Australasia, Sydney NSW

OECD (2006) *Lemna sp*. Growth Inhibition Test. Method 221. OECD Guideline for the Testing of Chemicals. Organisation for Economic Cooperation and Development, Paris

Toxicity Test Report: TR1108/3

This document is issued in accordance with NATA's accreditation requirements

Client:	Vista Gold Pty Ltd Level 3, 43 Cavenagh Street Darwin NT 0801	**ESA Job #:** **Date Sampled:** **Date Received:**	PR1108 25 November 2013 26 November 2013
Attention:	Austin Brandis	**Sampled By:**	Client
Client Ref:	Not applicable	**ESA Quote #:**	PL1108_q03

Lab ID No.:	Sample Name:	Sample Description:
6368	SW2	Aqueous sample, pH 7.5, conductivity 21.7µS/cm. Sample received at room temperature in apparent good condition
6369	RP3	Aqueous sample, pH 7.7, conductivity 2900µS/cm. Sample received at room temperature in apparent good condition

Test Performed:	48-hr acute (survival) toxicity test using the freshwater chironomid *Chironomus tepperi*
Test Protocol:	ESA SOP 121 (ESA 2012), based on OECD (2011) USEPA (2002) and Bailey *et al*. (2000)
Test Temperature:	The test was performed at 25±1°C.
Deviations from Protocol:	Nil
Comments on Solution Preparation:	Sample 6369 'RP3' was serially diluted with sample 6368 'SW2' to achieve the test concentrations. A DMW control and a diluent control (SW2) were tested concurrently with the sample.
Source of Test Organisms:	ESA Laboratory culture
Age of Test Organisms:	8-9 days old
Test Initiated:	29 November 2013 at 1530h

Sample 6369: *RP3*		*Vacant*	*Vacant*
Concentration (%)	**% Survival (Mean ± SD)**		
DMW Control	100 ± 0.0		
Diluent Control	100 ± 0.0		
6.3	100 ± 0.0		
12.5	85.0 ± 19.2		
25	80.0 ± 16.3		
50	90.0 ± 20.0		
100	75.0 ± 30.0		
48-hr EC10 = 15.6%*			
48-hr EC50 = >100%			
NOEC = 100%			
LOEC = >100%			

*95% confidence limits are not available

Toxicity Test Report: TR1108/3

QA/QC Parameter	Criterion	This Test	Criterion met?
Control mean % survival	≥85.0%	100%	Yes
Reference Toxicant within cusum chart limits	25.3-1355.2µg Cu/L	215.2µg Cu/L	Yes

Test Report Authorised by: Dr Rick Krassoi, Director on 23 December 2013

Results are based on the samples in the condition as received by ESA.

NATA Accredited Laboratory Number: 14709

This document is issued in accordance with NATA's accreditation requirements. Accredited for compliance with ISO/IEC 17025. NATA is a signatory to the APLAC mutual recognition arrangement for the mutual recognition of the equivalence of testing, calibration and inspection reports. This document shall not be reproduced except in full.

Citations:

Bailey, H.C., Krassoi, R., Elphick, J.R., Mulhall, A., Hunt, 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* 19:88-93.

ESA (2012) *SOP 121 – Acute toxicity test using Chironomus tepperi*. Issue No. 1. Ecotox Services Australasia, Sydney, New South Wales.

OECD (2011) OECD Guideline for the Testing of Chemicals. Test Guideline 235: *Chironomus sp*, Acute Immobilisation Test.

USEPA (2002) *Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms. 4th Ed.* United States Environmental Protection Agency, Office of Water, Washington DC.

Toxicity Test Report: TR1108/4

This document is issued in accordance with NATA's accreditation requirements

Client:	Vista Gold Pty Ltd Level 3, 43 Cavenagh Street Darwin NT 0801	**ESA Job #:** **Date Sampled:** **Date Received:**	PR1108 25 November 2013 26 November 2013
Attention:	Austin Brandis	**Sampled By:**	Client
Client Ref:	Not applicable	**ESA Quote #:**	PL1108_q03

Lab ID No.:	Sample Name:	Sample Description:
6368	SW2	Aqueous sample, pH 7.5, conductivity 21.7µS/cm. Sample received at room temperature in apparent good condition
6369	RP3	Aqueous sample, pH 7.7, conductivity 2900µS/cm. Sample received at room temperature in apparent good condition

*Ammonia analysis is not covered by Ecotox Services Australasia's scope of accreditation

Test Performed:	96-hr acute toxicity test using the freshwater hydra *hydra viridissima*
Test Protocol:	ESA SOP 125 (2012), based on Riethmuller et al. (2003)
Test Temperature:	The test was performed at 27±1°C.
Deviations from Protocol:	Nil
Comments on Solution Preparation:	Sample 6369 'RP3' was serially diluted with sample 6368 'SW2' to achieve the test concentrations. A Laboratory Water control and a diluent control (SW2) were tested concurrently with the sample.
Source of Test Organisms:	ESA Laboratory culture
Test Initiated:	27 November 2013 at 1500h

Sample 6369: *RP3*		*Vacant*	*Vacant*
Concentration (%)	**Population Growth Rate (Mean ± SD)**		
Lab Control	0.36 ± 0.01		
Diluent Control	0.40 ± 0.01		
6.3	0.37 ± 0.02		
12.5	0.35 ± 0.02 *		
25	0.28 ± 0.02 *		
50	0.18 ± 0.04 *		
100	0.00 ± 0.00		
96-hr IC10 = 10.9%**			
96-hr IC50 = 44.6 (36.8-57.3)%			
NOEC = 6.3%			
LOEC = 12.5%			

*Significantly lower population growth rate compared with the Diluent Control (Dunnett's Test, 1-tailed, P=0.05)
**95% confidence limits are not available

Toxicity Test Report: TR1108/4

QA/QC Parameter	Criterion	This Test	Criterion met?
Control mean population growth rate	≥0.25	0.36	Yes
Reference Toxicant within cusum chart limits	1.0-17.0µg Cu/L	2.1µg Cu/L	Yes

Test Report Authorised by: Dr Rick Krassoi, Director on 23 December 2013

Results are based on the samples in the condition as received by ESA.

NATA Accredited Laboratory Number: 14709

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Citations:

ESA (2012) SOP 125 –*Hydra Population Growth Test.* Issue No 1. Ecotox Services Australasia, Sydney, NSW

Riethmuller N, Camilleri C, Franklin N, Hogan A, King A, Koch A, Markich SJ, Turley C and van Dam R (2003). Green Hydra Population Growth Test. In: *Ecotoxicological testing protocols for Australian tropical freshwater ecosystems*. Supervising Scientist Report 173, Supervising Scientist, Darwin NT.

Toxicity Test Report: TR1108/5

This document is issued in accordance with NATA's accreditation requirements

Client:	Vista Gold Pty Ltd Level 3, 43 Cavenagh Street Darwin NT 0801	**ESA Job #:** **Date Sampled:** **Date Received:**	PR1108 25 November 2013 26 November 2013
Attention:	Austin Brandis	**Sampled By:**	Client
Client Ref:	Not applicable	**ESA Quote #:**	PL1108_q03

Lab ID No.:	Sample Name:	Sample Description:
6368	SW2	Aqueous sample, pH 7.5, conductivity 21.7µS/cm. Sample received at room temperature in apparent good condition
6369	RP3	Aqueous sample, pH 7.7, conductivity 2900µS/cm. Sample received at room temperature in apparent good condition

Test Performed:	Rainbowfish embryo hatching test using *Melanotaenia splendida splendida*
Test Protocol:	ESA SOP 126 (2013), based on USEPA (2002), but adapted for use with native rainbowfish
Test Temperature:	The test was performed at 25±1°C.
Deviations from Protocol:	Solution were renewed every 48 hours instead of every 24 hours
Comments on Solution Preparation:	Sample 6369 'RP3' was serially diluted with sample 6368 'SW2' to achieve the test concentrations. A DMW control and a diluent control (SW2) were tested concurrently with the sample.
Source of Test Organisms:	ESA Laboratory culture
Test Initiated:	27 November 2013 at 1600h

Sample 6369: *RP3*		*Vacant*	*Vacant*
Concentration (%)	**% Survival (Mean ± SD)**		
DMW Control	85.0 ± 19.2		
Diluent Control	80.0 ± 16.3		
3.1	65.0 ± 19.2		
6.3	90.0 ± 11.6		
12.5	95.0 ± 10.0		
25	100 ± 0.0		
50	90.0 ± 11.6		
100	40.0 ± 40.0		
11-d IC10 = 60.2%*			
11-d EC50 = 95.2 (72.9-100)%			
NOEC = 100%			
LOEC = >100%			

*95% confidence limits are not available

QA/QC Parameter	Criterion	This Test	Criterion met?
Control mean % survival	≥80.0%	85.0%	Yes
Reference Toxicant within cusum chart limit	11.5-459.5µg Cu/L	44.2µg Cu/L	Yes

Test Report Authorised by: Dr Rick Krassoi, Director on 23 December 2013

Results are based on the samples in the condition as received by ESA.

NATA Accredited Laboratory Number: 14709

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Citations:

ESA (2013) *SOP 126- Rainbowfish Embryo Hatching Test*. Issue N°3. Ecotox Services Australasia, Sydney NSW

USEPA (2002) *Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms.4th Ed.* United States Environmental Protection Agency, Office of Water, Washington DC.

ecotox SERVICES AUSTRALASIA

Toxicity Test Report: TR1108/6

Client:	Vista Gold Pty Ltd Level 3, 43 Cavenagh Street Darwin NT 0801	**ESA Job #:** **Date Sampled:** **Date Received:**	PR1108 25 November 2013 26 November 2013
Attention:	Austin Brandis	**Sampled By:**	Client
Client Ref:	Not applicable	**ESA Quote #:**	PL1108_q03

Lab ID No.:	Sample Name:	Sample Description:
6368	SW2	Aqueous sample, pH 7.5, conductivity 21.7µS/cm. Sample received at room temperature in apparent good condition
6369	RP3	Aqueous sample, pH 7.7, conductivity 2900µS/cm. Sample received at room temperature in apparent good condition

Test Performed:	96-hr acute survival test using the freshwater shrimp *Macrobrachium bullatum*
Test Protocol:	ESA SOP 123 (ESA 2012), based on USEPA (1996)
Test Temperature:	The test was performed at 25±1°C.
Deviations from Protocol:	Nil
Comments on Solution Preparation:	Sample 6369 'RP3' was serially diluted with sample 6368 'SW2' to achieve the test concentrations. A DMW control and a diluent control (SW2) were tested concurrently with the sample.
Source of Test Organisms:	Hatchery reared, NT
Test Initiated:	3 December 2013 at 1600h

Sample 6369: *RP3*		*Vacant*	*Vacant*
Concentration (%)	**% Unaffected (Mean ± SD)**		
DMW Control	95.0 ± 10.0		
Diluent Control	95.0 ± 10.0		
6.3	100 ± 0.0		
12.5	100 ± 0.0		
25	95.0 ± 10.0		
50	95.0 ± 10.0		
100	100 ± 0.0		
96-hr EC10 = >100%			
96-hr EC50 = >100%			
NOEC = 100%			
LOEC = >100%			

QA/QC Parameter	Criterion	This Test	Criterion met?
Control mean % unaffected	≥90.0%	95.0%	Yes
Reference Toxicant within cusum chart limits	27.3-306.9µg Cu/L	112.0µg Cu/L	Yes

Toxicity Test Report: TR1108/6

Test Report Authorised by: Dr Rick Krassoi, Director on 23 December 2013

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

Citations:

ESA (2012) SOP 123 –*Acute Toxicity Test Using Freshwater Shrimp.* Issue No 1. Ecotox Services Australasia, Sydney, NSW

USEPA (1996) Ecological Effects Test Guidelines: OPPTS 850.1035 Mysid Acute Toxicity Test. Public Draft. United States Environmental Protection Agency, Washington DC, USA.

Toxicity Test Report: TR1108/7

This document is issued in accordance with NATA's accreditation requirements

Client:	Vista Gold Pty Ltd Level 3, 43 Cavenagh Street Darwin NT 0801	**ESA Job #:** **Date Sampled:** **Date Received:**	PR1108 25 November 2013 26 November 2013
Attention:	Austin Brandis	**Sampled By:**	Client
Client Ref:	Not applicable	**ESA Quote #:**	PL1108_q03

Lab ID No.:	Sample Name:	Sample Description:
6368	SW2	Aqueous sample, pH 7.5, conductivity 21.7µS/cm. Sample received at room temperature in apparent good condition
6369	RP3	Aqueous sample, pH 7.7, conductivity 2900µS/cm. Sample received at room temperature in apparent good condition

Test Performed:	Partial life-cycle toxicity test using the freshwater cladoceran *Ceriodaphnia cf dubia*
Test Protocol:	ESA SOP 102 (ESA 2011), based on USEPA (2002) and Bailey *et al*. (2000)
Test Temperature:	The test was performed at 25±1°C.
Deviations from Protocol:	Nil
Comments on Solution Preparation:	Sample 6369 'RP3' was serially diluted with sample 6368 'SW2' to achieve the test concentrations. A DMW control and a diluent control (SW2) were tested concurrently with the sample.
Source of Test Organisms:	ESA Laboratory culture
Test Initiated:	16 December 2013 at 1330h

Sample 6369: *RP3*

Concentration (%)	% Survival at 7 days (Mean ± SD)
DMW Control	100 ± 0.0
Diluent Control	100 ± 0.0
0.1	100 ± 0.0
0.2	100 ± 0.0
0.4	100 ± 0.0
0.8	100 ± 0.0
1.5	50.0 ± 52.7 *
3.0	50.0 ± 52.7 *

7 day EC10 (survival) = 0.9 (0.2-1.3)%
7 day EC50 (survival) = 2.4%***
NOEC = 0.8%
LOEC = 1.5%

Sample 6369: *RP3*

Concentration (%)	Number of Young (Mean ± SD)
DMW Control	15.6 ± 3.2
Diluent Control	14.5 ± 3.5
0.1	14.7 ± 3.5
0.2	6.7 ± 2.5 **
0.4	2.9 ± 1.2 **
0.8	1.8 ± 1.6 **
1.5	1.7 ± 1.1 **
3.0	1.6 ± 1.2 **

7 day IC10 (reproduction) = 0.1%***
7 day IC50 (reproduction) = 0.2 (0.2-0.2)%
NOEC = 0.1%
LOEC = 0.2%

*Significantly lower survival compared with the Diluent Control (Fisher's Exact Test, 1-tailed, P=0.05)
** Significantly lower number of young compared with the Diluent Control (Wilcoxon Rank Sum Test, 1-tailed, P=0.05)
***95% confidence limits not reliable

QA/QC Parameter	Criterion	This Test	Criterion met?
Control mean % survival	≥80.0%	100%	Yes
Control mean number of young	≥15.0	15.6	Yes
Reference Toxicant within cusum chart limits	161.0-342.3mgKCl/L	205.1mgKCl/L	Yes

Toxicity Test Report: TR1108/7

Test Report Authorised by: Dr Rick Krassoi, Director on 23 December 2013

Results are based on the samples in the condition as received by ESA.

NATA Accredited Laboratory Number: 14709

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Citations:

Bailey, H.C., Krassoi, R., Elphick, J.R., Mulhall, A., Hunt, 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* 19:88-93.

ESA (2011) ESA SOP 102 – *Acute Toxicity Test Using Ceriodaphnia dubia.* Issue No 8. Ecotox Services Australasia, Sydney, NSW.

USEPA (2002) *Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms.4th Ed.* United States Environmental Protection Agency, Office of Water, Washington DC.

Chain-of-Custody Documentation

Datasheet ID: 601.2
Last Revised: 30 April 2009

Sample Receipt Notification

Attention : Austin Brandis

Client : Vista Gold Pty Ltd
Level 3, 43 Cavenagh St
Darwin NT 0801

Email :
Telephone :
Facsimile :

Date : 20/12/2013

Re : Receipt of Samples **Pages :** **2**

ESA Project : PR1108 ☑ For Review ☐ Additional Documentation Required - Please Respond

Sample Delivery Details

Completed Chain of Custody accompanied samples: **YES**
Samples received in apparent good condition and correctly bottled: **YES**
Security seals on sample bottles and esky intact: **YES**

Date samples received : 26/11/2013
Time samples received : 10:00
No. of samples received : 2
Sample matrix : Aqueous
Sample temperature : room temperature

Comments : Includes 5x20L SW2 (ESA ID#6368) aqnd 2x20L RP3 (ESA ID# 6369)

Contact Details

Customer Services Officer : Tina Micevska
Telephone : 61 2 9420 9481
Facsimile : 61 2 9420 9484
Email : tmicevska@ecotox.com.au

Please contact customer services officer for all queries or issues regarding samples

Note that the chain-of-custody provides definitive information on the tests to be performed

Ecotox Services Australia
ABN 45 094 714 904
Unit 27, 2 Chaplin Drive
Lane Cove NSW 2066 Australia

Phone : 61 2 9420 9481
Fax : 61 2 9420 9484
Email : info@ecotox.com.au

Chain-of-Custody / Service Request Form

Datasheet ID: 601.1
Last Revised: 22 January 2013

ecotox SERVICES AUSTRALASIA

Customer: Vista Gold Australia Pty Ltd
Ship To: Ecotox Services Australasia
Contact Name: Austin Brandis
Attention: Tina Micevska
Phone: 0889017730 Email: abrandis@envirotechmonitoring.com.au (please provide an email address for sample receipt notification)
Sampled by: Vista Gold Staff

	Sample Date (day/month /year)	Sample Time	Sample Name (exactly as written on the sample vessel)	Sample Method (eg. Grab, composite etc.)	Number and Volume of Containers (eg 2 x 1L)	Tests Requested (See reverse for guidance): See page 3 for ecotox tests and concentrations	Subcontracted Water Chemistry as per Page 4					Comments / Instructions: **Note that testing will be delayed if an incomplete chain of custody is received** • Additional treatment of samples (i.e. spiking) • Sub-contracted services (i.e. chemical analyses) • Dilutions required (if different than 100% down to 6.25%) • Sample holding time restriction (if applicable) • Sample used for litigation (if applicable) *Note: An MSDS must be attached if Available* **ESA Project Number: PR** 1108
6367	25/11/2013	11 :00	RP3	Grab	2 x 20L	x	x					Test Water – Mine Water. Contains some heavy metals. pH neutral
6368	25/11/2013	11 :00	SW2	Grab	5 x 20L		x					Diluent – Natural River Water

1) Released By:	Date:	2) Received By:	Date:	3) Released By:	Date:	4) Received By:	Date:
Austin Brandis	25/11/2013	Tina	26/11/13				
Of:	Time:	Of:	Time:	Of:	Time:	Of:	Time:
		ESA	10:00				

Note that the chain-of-custody documentation will provide definitive information on the tests to be performed.

Ecotox Services Australasia . Unit 27, 2 Chaplin Drive, Lane Cove NSW 2066 AUSTRALIA
Phone: 61 2 9420-9481 Fax 61 2 9420-9484 info@ecotox.com.au

ecotox
SERVICES AUSTRALASIA

Statistical Printouts for the *Selenastrum* Growth Inhibition Tests

Microalgal Growth inhibition Test-Growth-Cell Yield

Start Date:	13/12/2013 13:30	Test ID:	PR1108/11	Sample ID:	RP3
End Date:	16/12/2013 12:00	Lab ID:	6369	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 103	Test Species:	SC-Selenastrum capricornutum
Comments:					

Conc-%	1	2	3	4	5	6	7	8
USEPA Control	216300	160300	238300	180300	165300	163300	162300	165300
Diluent Control	198300	210300	219300	201300				
0.1	190300	200300	204300	197300				
0.2	190300	205300	211300	188300				
0.4	205300	196300	210300	203300				
0.8	192300	187300	202300	193300				
1.6	140300	130300	132300	125300				
3.1	36300	44300	36300	57300				

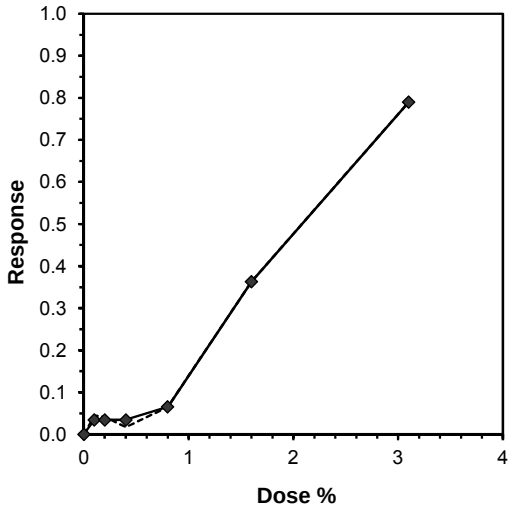
			Transform: Untransformed						1-Tailed		Isotonic	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
USEPA Control	181425	0.8752	181425	160300	238300	16.288	8					
Diluent Control	207300	1.0000	207300	198300	219300	4.576	4	*			207300	1.0000
0.1	198050	0.9554	198050	190300	204300	2.984	4	1.611	2.451	14078.57	200216.7	0.9658
0.2	198800	0.9590	198800	188300	211300	5.669	4	1.480	2.451	14078.57	200216.7	0.9658
0.4	203800	0.9831	203800	196300	210300	2.847	4	0.609	2.451	14078.57	200216.7	0.9658
0.8	193800	0.9349	193800	187300	202300	3.222	4	2.351	2.451	14078.57	193800	0.9349
*1.6	132050	0.6370	132050	125300	140300	4.724	4	13.103	2.451	14078.57	132050	0.6370
*3.1	43550	0.2101	43550	36300	57300	22.760	4	28.513	2.451	14078.57	43550	0.2101

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.05)	0.933804	0.924	0.361873	-0.9502
Bartlett's Test indicates equal variances (p = 0.85)	2.656183	16.81189		
The control means are not significantly different (p = 0.13)	1.672463	2.228139		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	0.8	1.6	1.131371	125	14078.57	0.067914	1.47E+10	65964286	6.6E-18	6, 21
Treatments vs Diluent Control										

Linear Interpolation (200 Resamples)

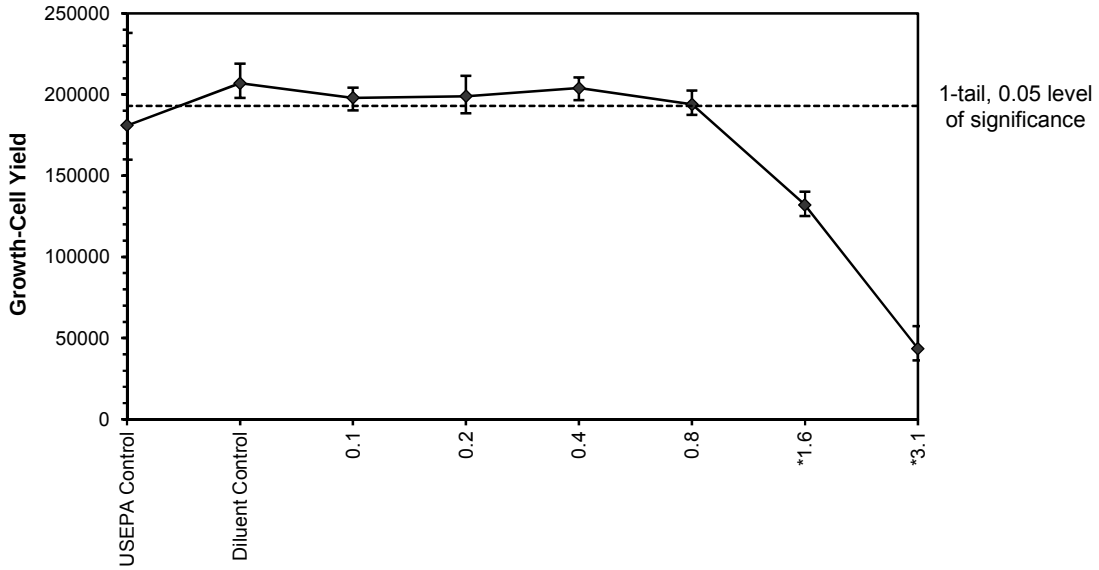
Point	%	SD	95% CL(Exp)		Skew
IC05	0.6046	0.2547	0.0000	0.9784	-0.7624
IC10	0.8937	0.0685	0.6115	1.0216	-1.6731
IC15	1.0280	0.0496	0.8620	1.1500	-0.3851
IC20	1.1622	0.0462	1.0053	1.2810	-0.3239
IC25	1.2965	0.0443	1.1494	1.4107	-0.2037
IC40	1.7300	0.0553	1.5741	1.8761	-0.0399
IC50	2.0814	0.0516	1.9537	2.2309	0.2147



Microalgal Growth inhibition Test-Growth-Cell Yield

Start Date:	13/12/2013 13:30	Test ID:	PR1108/11	Sample ID:	RP3
End Date:	16/12/2013 12:00	Lab ID:	6369	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 103	Test Species:	SC-Selenastrum capricornutum
Comments:					

Dose-Response Plot



Microalgal Growth inhibition Test-Growth-Cell Yield

Start Date:	13/12/2013 13:30	Test ID:	PR1108/11	Sample ID:	RP3
End Date:	16/12/2013 12:00	Lab ID:	6369	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 103	Test Species:	SC-Selenastrum capricornutum
Comments:					

Auxiliary Data Summary

Conc-%	Parameter	Mean	Min	Max	SD	CV%	N
USEPA Control	Cell Yield	18.14	16.03	23.83	2.96	9.48	8
Diluent Control		20.73	19.83	21.93	0.95	4.70	4
0.1		19.81	19.03	20.43	0.59	3.88	4
0.2		19.88	18.83	21.13	1.13	5.34	4
0.4		20.38	19.63	21.03	0.58	3.74	4
0.8		19.38	18.73	20.23	0.62	4.08	4
1.6		13.21	12.53	14.03	0.62	5.98	4
3.1		4.36	3.63	5.73	0.99	22.86	4
USEPA Control	pH	7.50	7.50	7.50	0.00	0.00	1
Diluent Control		7.20	7.20	7.20	0.00	0.00	1
0.1		7.20	7.20	7.20	0.00	0.00	1
0.2		7.20	7.20	7.20	0.00	0.00	1
0.4		7.20	7.20	7.20	0.00	0.00	1
0.8		7.10	7.10	7.10	0.00	0.00	1
1.6		7.10	7.10	7.10	0.00	0.00	1
3.1		7.10	7.10	7.10	0.00	0.00	1
USEPA Control	Conductivity uS/cm	96.40	96.40	96.40	0.00	0.00	1
Diluent Control		111.80	111.80	111.80	0.00	0.00	1
0.1		117.20	117.20	117.20	0.00	0.00	1
0.2		122.40	122.40	122.40	0.00	0.00	1
0.4		134.90	134.90	134.90	0.00	0.00	1
0.8		146.90	146.90	146.90	0.00	0.00	1
1.6		186.10	186.10	186.10	0.00	0.00	1
3.1		235.00	235.00	235.00	0.00	0.00	1

Statistical Printouts for the Duckweed Growth Inhibition Tests

Duckweed Growth Inhibtion Test-Specific Growth Rate

Start Date:	11/12/2013 15:00	Test ID:	PR1108/02	Sample ID:	RP3
End Date:	15/12/2013 14:15	Lab ID:	6369	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 112	Test Species:	LA-Lemna aequinoctialis
Comments:					

Conc-%	1	2	3	4
CAAC Control	0.2974	0.3375	0.3280	0.2974
Diluent Control	0.3080	0.3280	0.3080	0.3080
0.2	0.3280	0.3720	0.3182	0.3182
0.4	0.3280	0.3553	0.3182	0.3080
0.8	0.2974	0.3182	0.3080	0.3080
1.5	0.0334	0.0000	0.0000	0.0000
3	0.0000	0.0000	0.0000	0.0000
6	0.0000	0.0000	0.0000	0.0000

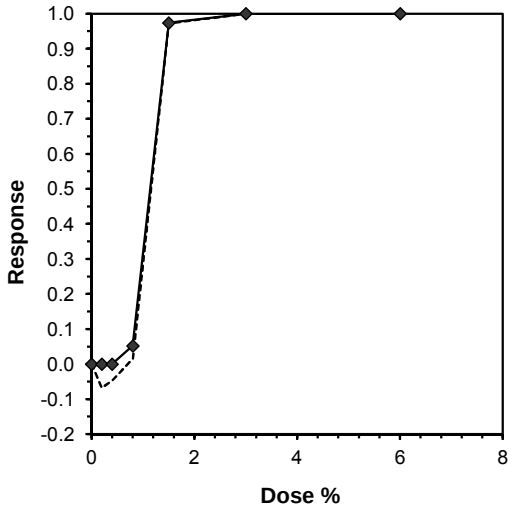
			Transform: Untransformed					Rank	1-Tailed	Isotonic	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Mean	N-Mean
CAAC Control	0.3151	1.0065	0.3151	0.2974	0.3375	6.595	4				
Diluent Control	0.3130	1.0000	0.3130	0.3080	0.3280	3.196	4	*		0.3249	1.0000
0.2	0.3341	1.0674	0.3341	0.3182	0.3720	7.684	4	23.50	10.00	0.3249	1.0000
0.4	0.3274	1.0459	0.3274	0.3080	0.3553	6.210	4	22.00	10.00	0.3249	1.0000
0.8	0.3079	0.9837	0.3079	0.2974	0.3182	2.764	4	16.00	10.00	0.3079	0.9479
*1.5	0.0083	0.0267	0.0083	0.0000	0.0334	200.000	4	10.00	10.00	0.0083	0.0257
3	0.0000	0.0000	0.0000	0.0000	0.0000	0.000	4			0.0000	0.0000
6	0.0000	0.0000	0.0000	0.0000	0.0000	0.000	4			0.0000	0.0000

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.05)	0.868032	0.905	1.202266	0.739986
Bartlett's Test indicates equal variances (p = 0.39)	4.137926	13.2767		
The control means are not significantly different (p = 0.87)	0.177034	2.446912		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	0.8	1.5	1.095445	125
Treatments vs Diluent Control				

Linear Interpolation (200 Resamples)

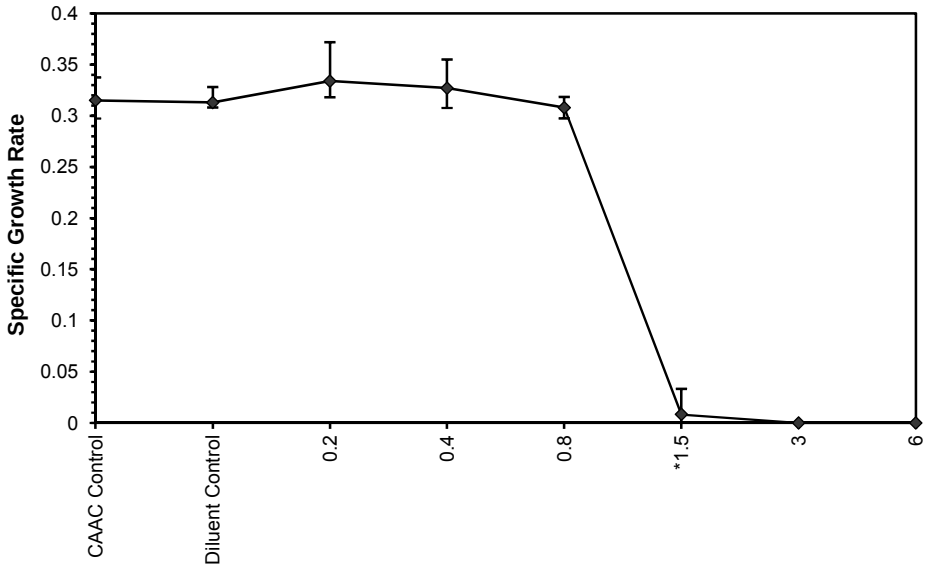
Point	%	SD	95% CL(Exp)		Skew
IC05	0.7836	0.1010	0.2524	0.8467	-1.4236
IC10	0.8363	0.0142	0.7915	0.8721	-0.0384
IC15	0.8743	0.0134	0.8330	0.9095	0.0166
IC20	0.9122	0.0128	0.8734	0.9473	0.0319
IC25	0.9502	0.0123	0.9138	0.9851	0.0640
IC40	1.0641	0.0115	1.0298	1.0992	0.2860
IC50	1.1400	0.0117	1.1080	1.1781	0.4961



Duckweed Growth Inhibtion Test-Specific Growth Rate

Start Date:	11/12/2013 15:00	Test ID:	PR1108/02	Sample ID:	RP3
End Date:	15/12/2013 14:15	Lab ID:	6369	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 112	Test Species:	LA-Lemna aequinoctialis
Comments:					

Dose-Response Plot



Duckweed Growth Inhibtion Test-Specific Growth Rate

Start Date:	11/12/2013 15:00	Test ID:	PR1108/02	Sample ID:	RP3
End Date:	15/12/2013 14:15	Lab ID:	6369	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 112	Test Species:	LA-Lemna aequinoctialis
Comments:					

		Auxiliary Data Summary					
Conc-%	**Parameter**	**Mean**	**Min**	**Max**	**SD**	**CV%**	**N**
CAAC Control	Specific Growth Rate	0.32	0.30	0.34	0.02	45.75	4
Diluent Control		0.31	0.31	0.33	0.01	31.95	4
0.2		0.33	0.32	0.37	0.03	47.95	4
0.4		0.33	0.31	0.36	0.02	43.55	4
0.8		0.31	0.30	0.32	0.01	29.96	4
1.5		0.01	0.00	0.03	0.02	1548.04	4
3		0.00	0.00	0.00	0.00		4
6		0.00	0.00	0.00	0.00		4
CAAC Control	pH	6.10	6.10	6.10	0.00	0.00	1
Diluent Control		6.40	6.40	6.40	0.00	0.00	1
0.2		6.40	6.40	6.40	0.00	0.00	1
0.4		6.40	6.40	6.40	0.00	0.00	1
0.8		6.40	6.40	6.40	0.00	0.00	1
1.5		6.40	6.40	6.40	0.00	0.00	1
3		6.40	6.40	6.40	0.00	0.00	1
6		6.30	6.30	6.30	0.00	0.00	1
CAAC Control	Cond uS/cm	37.70	37.70	37.70	0.00	0.00	1
Diluent Control		49.90	49.90	49.90	0.00	0.00	1
0.2		59.00	59.00	59.00	0.00	0.00	1
0.4		68.30	68.30	68.30	0.00	0.00	1
0.8		86.90	86.90	86.90	0.00	0.00	1
1.5		123.50	123.50	123.50	0.00	0.00	1
3		185.60	185.60	185.60	0.00	0.00	1
6		316.00	316.00	316.00	0.00	0.00	1

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Statistical Printouts for the Acute Test with *Chironomus tepperi*

Chironomid Acute Toxicity Test-48hr Survival

Start Date:	29/11/2013 15:30	Test ID:	PR1108/06	Sample ID:	RP3
End Date:	1/12/2013 13:00	Lab ID:	6369	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 121	Test Species:	CT-Chironomus tepperi
Comments:					

Conc-%	1	2	3	4
DMW Control	1.0000	1.0000	1.0000	1.0000
Diluent Control	1.0000	1.0000	1.0000	1.0000
6.3	1.0000	1.0000	1.0000	1.0000
12.5	1.0000	1.0000	0.8000	0.6000
25	0.6000	0.8000	1.0000	0.8000
50	1.0000	0.6000	1.0000	1.0000
100	1.0000	0.6000	0.4000	1.0000

			Transform: Arcsin Square Root					Rank	1-Tailed		Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical		Resp	Number
DMW Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4					
Diluent Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	*			0	20
6.3	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00		0	20
12.5	0.8500	0.8500	1.1709	0.8861	1.3453	18.840	4	14.00	10.00		3	20
25	0.8000	0.8000	1.1114	0.8861	1.3453	16.874	4	12.00	10.00		4	20
50	0.9000	0.9000	1.2305	0.8861	1.3453	18.660	4	16.00	10.00		2	20
100	0.7500	0.7500	1.0653	0.6847	1.3453	31.308	4	14.00	10.00		5	20

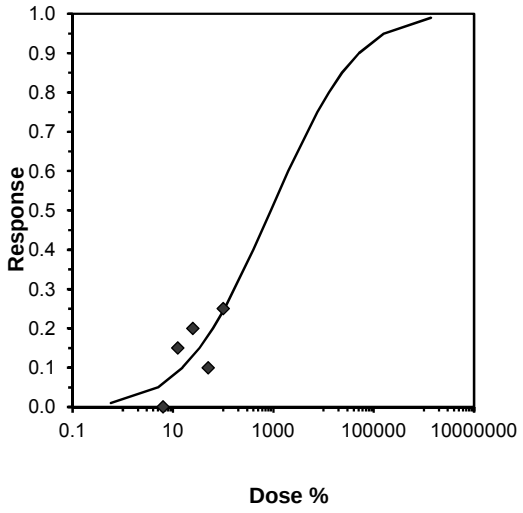
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.05)	0.917818	0.916	-0.52866	-0.03472
Equality of variance cannot be confirmed				
The control means are not significantly different (p = 1.00)	0	2.446912		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	100	>100		1
Treatments vs Diluent Control				

Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	0.72947	0.389976	-0.03488	1.493822	0	3.978342	7.814728	0.26	2.950671	1.370859	4
Intercept	2.847575	0.609858	1.652254	4.042896							
TSCR											

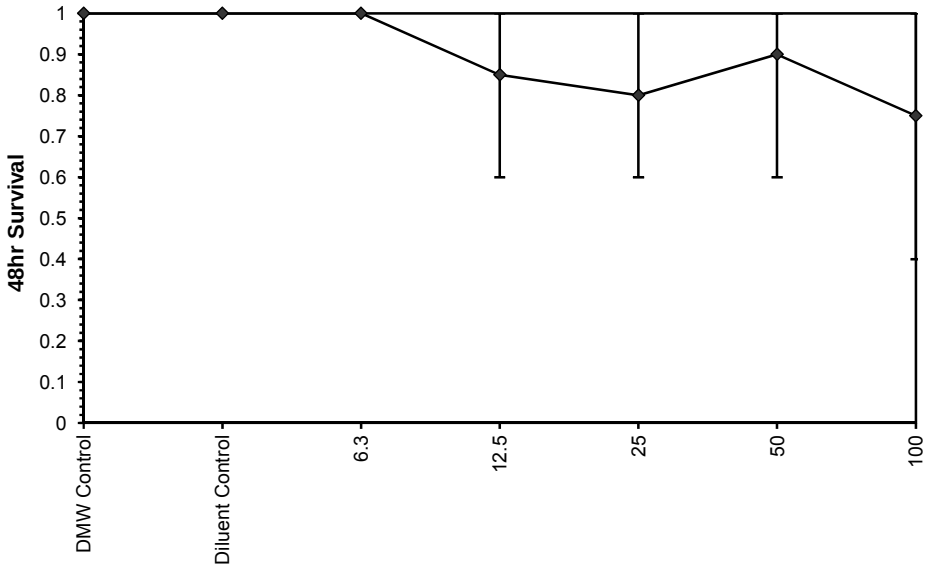
Point	Probits	%	95% Fiducial Limits
EC01	2.674	0.577532	
EC05	3.355	4.963735	
EC10	3.718	15.62588	
EC15	3.964	33.87404	
EC20	4.158	62.65086	
EC25	4.326	106.1795	
EC40	4.747	401.2065	
EC50	5.000	892.6292	
EC60	5.253	1985.978	
EC75	5.674	7504.149	
EC80	5.842	12717.89	
EC85	6.036	23522.06	
EC90	6.282	50991.46	
EC95	6.645	160521.6	
EC99	7.326	1379643	



Chironomid Acute Toxicity Test-48hr Survival

Start Date:	29/11/2013 15:30	Test ID:	PR1108/06	Sample ID:	RP3
End Date:	1/12/2013 13:00	Lab ID:	6369	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 121	Test Species:	CT-Chironomus tepperi
Comments:					

Dose-Response Plot



Chironomid Acute Toxicity Test-48hr Survival

Start Date:	29/11/2013 15:30	Test ID:	PR1108/06	Sample ID:	RP3
End Date:	1/12/2013 13:00	Lab ID:	6369	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 121	Test Species:	CT-Chironomus tepperi
Comments:					

		Auxiliary Data Summary					
Conc-%	**Parameter**	**Mean**	**Min**	**Max**	**SD**	**CV%**	**N**
DMW Control	% Survival	100.00	100.00	100.00	0.00	0.00	4
Diluent Control		100.00	100.00	100.00	0.00	0.00	4
6.3		100.00	100.00	100.00	0.00	0.00	4
12.5		85.00	60.00	100.00	19.15	5.15	4
25		80.00	60.00	100.00	16.33	5.05	4
50		90.00	60.00	100.00	20.00	4.97	4
100		75.00	40.00	100.00	30.00	7.30	4
DMW Control	pH	8.20	8.20	8.20	0.00	0.00	1
Diluent Control		6.70	6.70	6.70	0.00	0.00	1
6.3		6.70	6.70	6.70	0.00	0.00	1
12.5		6.90	6.90	6.90	0.00	0.00	1
25		7.10	7.10	7.10	0.00	0.00	1
50		7.40	7.40	7.40	0.00	0.00	1
100		7.80	7.80	7.80	0.00	0.00	1
DMW Control	DO (%)	98.90	98.90	98.90	0.00	0.00	1
Diluent Control		96.60	96.60	96.60	0.00	0.00	1
6.3		96.80	96.80	96.80	0.00	0.00	1
12.5		97.60	97.60	97.60	0.00	0.00	1
25		98.60	98.60	98.60	0.00	0.00	1
50		103.80	103.80	103.80	0.00	0.00	1
100		109.20	109.20	109.20	0.00	0.00	1
DMW Control	Conductivity (uS/cm)	172.90	172.90	172.90	0.00	0.00	1
Diluent Control		21.60	21.60	21.60	0.00	0.00	1
6.3		300.00	300.00	300.00	0.00	0.00	1
12.5		531.00	531.00	531.00	0.00	0.00	1
25		947.00	947.00	947.00	0.00	0.00	1
50		1667.00	1667.00	1667.00	0.00	0.00	1
100		2910.00	2910.00	2910.00	0.00	0.00	1

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Statistical Printouts for *Hydra* Population Growth Tests

Hydra Population Growth Test-Growth Rate

Start Date:	27/11/2013 15:00	Test ID:	PR1108/03	Sample ID:	RP3
End Date:	1/12/2013 17:45	Lab ID:	6369	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 125	Test Species:	HV-Hydra viridissima
Comments:					

Conc-%	1	2	3	4
Lab Control	0.3631	0.3408	0.3735	0.3577
Diluent Control	0.4113	0.3835	0.3931	0.3978
6.3	0.3785	0.3631	0.3522	0.3931
12.5	0.3408	0.3785	0.3522	0.3349
25	0.2747	0.2508	0.2964	0.2821
50	0.2052	0.1495	0.2151	0.1366
100	0.0000	0.0000	0.0000	0.0000

			Transform: Untransformed						1-Tailed		Isotonic	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
Lab Control	0.3588	0.9050	0.3588	0.3408	0.3735	3.801	4					
Diluent Control	0.3964	1.0000	0.3964	0.3835	0.4113	2.918	4	*			0.3964	1.0000
6.3	0.3717	0.9377	0.3717	0.3522	0.3931	4.812	4	1.493	2.360	0.0390	0.3717	0.9377
*12.5	0.3516	0.8870	0.3516	0.3349	0.3785	5.493	4	2.709	2.360	0.0390	0.3516	0.8870
*25	0.2760	0.6962	0.2760	0.2508	0.2964	6.904	4	7.282	2.360	0.0390	0.2760	0.6962
*50	0.1766	0.4455	0.1766	0.1366	0.2151	22.252	4	13.294	2.360	0.0390	0.1766	0.4455
100	0.0000	0.0000	0.0000	0.0000	0.0000	0.000	4				0.0000	0.0000

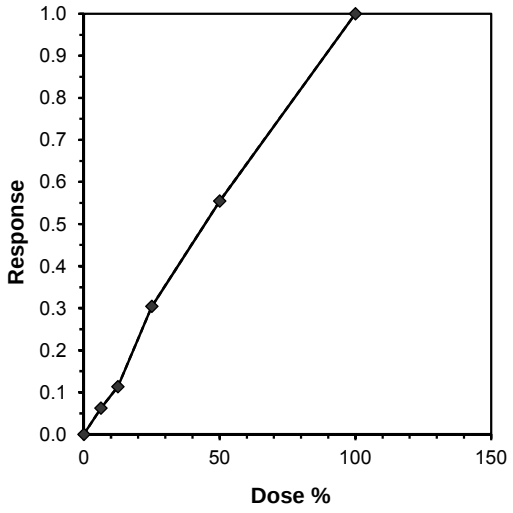
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.05)	0.986267	0.905	0.039134	-0.5998
Bartlett's Test indicates equal variances (p = 0.34)	4.493741	13.2767		
The control means are significantly different (p = 5.62E-03)	4.210484	2.446912		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	6.3	12.5	8.87412	15.87302	0.039023	0.098441	0.031861	0.000547	5.8E-09	4, 15
Treatments vs Diluent Control										

Linear Interpolation (200 Resamples)

Point	%	SD	95% CL(Exp)		Skew
IC05*	5.058	1.778	1.741	11.554	1.0502
IC10	10.912	2.081	3.003	16.251	-0.4746
IC15	14.926	1.215	11.420	18.657	-0.0332
IC20	18.202	1.094	15.134	21.302	-0.0506
IC25	21.478	1.188	18.378	25.348	0.0922
IC40	34.596	2.070	29.625	41.557	0.3929
IC50	44.566	3.337	36.762	57.349	0.5809

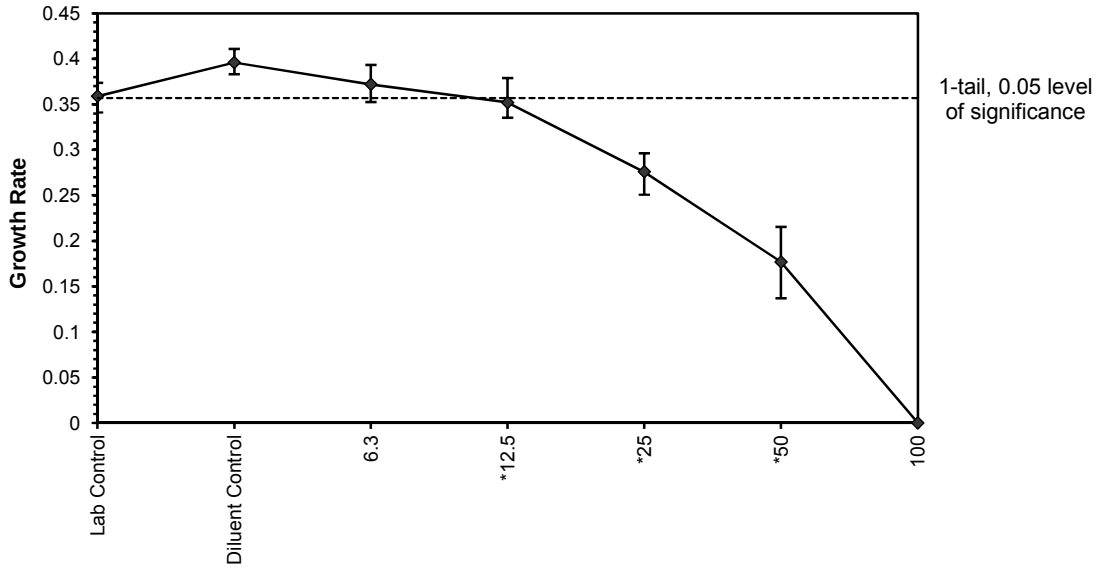
* indicates IC estimate less than the lowest concentration



Hydra Population Growth Test-Growth Rate

Start Date:	27/11/2013 15:00	Test ID:	PR1108/03	Sample ID:	RP3
End Date:	1/12/2013 17:45	Lab ID:	6369	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 125	Test Species:	HV-Hydra viridissima
Comments:					

Dose-Response Plot



Hydra Population Growth Test-Growth Rate

Start Date:	27/11/2013 15:00	Test ID:	PR1108/03	Sample ID:	RP3
End Date:	1/12/2013 17:45	Lab ID:	6369	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 125	Test Species:	HV-Hydra viridissima
Comments:					

		Auxiliary Data Summary					
Conc-%	**Parameter**	**Mean**	**Min**	**Max**	**SD**	**CV%**	**N**
Lab Control	Growth Rate	0.36	0.34	0.37	0.01	32.55	4
Diluent Control		0.40	0.38	0.41	0.01	27.13	4
6.3		0.37	0.35	0.39	0.02	35.98	4
12.5		0.35	0.33	0.38	0.02	39.52	4
25		0.28	0.25	0.30	0.02	50.02	4
50		0.18	0.14	0.22	0.04	112.25	4
100		0.00	0.00	0.00	0.00		4
Lab Control	Conductivity	29.70	29.70	29.70	0.00	0.00	1
Diluent Control		19.10	19.10	19.10	0.00	0.00	1
6.3		303.00	303.00	303.00	0.00	0.00	1
12.5		537.00	537.00	537.00	0.00	0.00	1
25		945.00	945.00	945.00	0.00	0.00	1
50		1648.00	1648.00	1648.00	0.00	0.00	1
100		2890.00	2890.00	2890.00	0.00	0.00	1
Lab Control	pH	6.90	6.90	6.90	0.00	0.00	1
Diluent Control		6.60	6.60	6.60	0.00	0.00	1
6.3		6.80	6.80	6.80	0.00	0.00	1
12.5		6.90	6.90	6.90	0.00	0.00	1
25		7.10	7.10	7.10	0.00	0.00	1
50		7.50	7.50	7.50	0.00	0.00	1
100		7.80	7.80	7.80	0.00	0.00	1
Lab Control	DO, % sat	99.10	99.10	99.10	0.00	0.00	1
Diluent Control		95.30	95.30	95.30	0.00	0.00	1
6.3		98.90	98.90	98.90	0.00	0.00	1
12.5		98.90	98.90	98.90	0.00	0.00	1
25		99.60	99.60	99.60	0.00	0.00	1
50		100.60	100.60	100.60	0.00	0.00	1
100		103.50	103.50	103.50	0.00	0.00	1

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Statistical Printouts for the Fish Embryonic Post Hatch Survival Tests

Fish Embryonic Development and Post-Hatch Survival Test-Survival

Start Date:	27/11/2013 16:00	Test ID:	PR1108/07	Sample ID:	RP3
End Date:	8/12/2013 12:15	Lab ID:	6369	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 126	Test Species:	MS-Melanotaenia splendida
Comments:					

Conc-%	1	2	3	4
DMW Control	1.0000	0.6000	1.0000	0.8000
Diluent Control	0.6000	1.0000	0.8000	0.8000
3.1	0.6000	0.4000	0.8000	0.8000
6.3	1.0000	1.0000	0.8000	0.8000
12.5	1.0000	0.8000	1.0000	1.0000
25	1.0000	1.0000	1.0000	1.0000
50	0.8000	1.0000	1.0000	0.8000
100	1.0000	0.2000	0.2000	0.2000

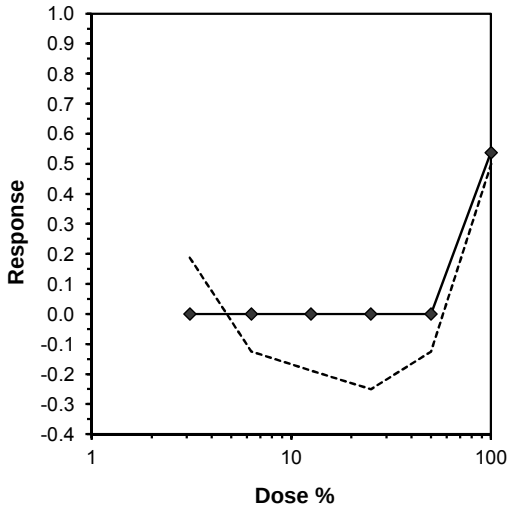
			Transform: Arcsin Square Root					Rank	1-Tailed	Isotonic	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Mean	N-Mean
DMW Control	0.8500	1.0625	1.1709	0.8861	1.3453	18.840	4				
Diluent Control	0.8000	1.0000	1.1114	0.8861	1.3453	16.874	4	*		0.8667	1.0000
3.1	0.6500	0.8125	0.9463	0.6847	1.1071	21.467	4	14.50	10.00	0.8667	1.0000
6.3	0.9000	1.1250	1.2262	1.1071	1.3453	11.212	4	21.00	10.00	0.8667	1.0000
12.5	0.9500	1.1875	1.2857	1.1071	1.3453	9.261	4	22.50	10.00	0.8667	1.0000
25	1.0000	1.2500	1.3453	1.3453	1.3453	0.000	4	24.00	10.00	0.8667	1.0000
50	0.9000	1.1250	1.2262	1.1071	1.3453	11.212	4	21.00	10.00	0.8667	1.0000
100	0.4000	0.5000	0.6841	0.4636	1.3453	64.442	4	13.50	10.00	0.4000	0.4615

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.05)	0.882017	0.924	1.430131	4.281494
Equality of variance cannot be confirmed				
The control means are not significantly different (p = 0.70)	0.411217	2.446912		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	100	>100		1
Treatments vs Diluent Control				

Log-Logit Interpolation (200 Resamples)

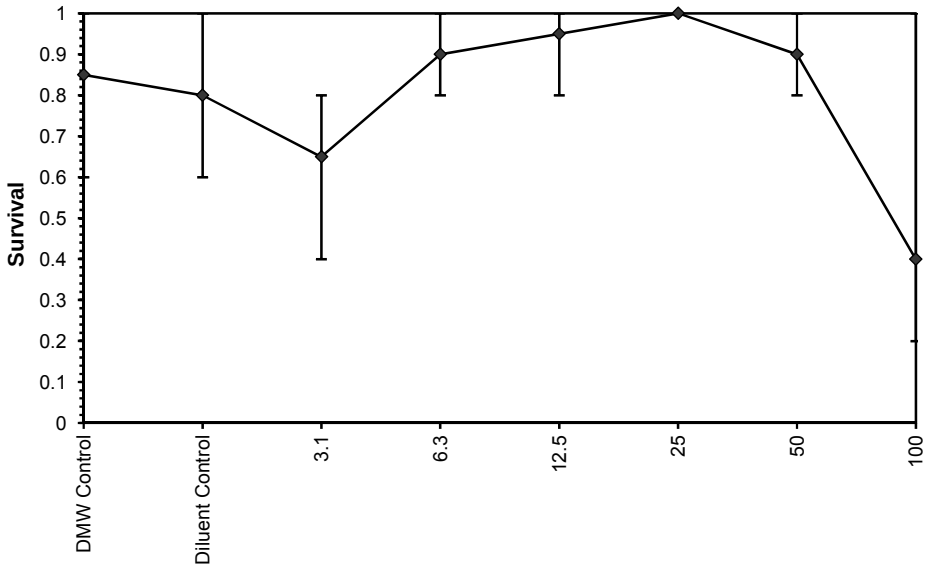
Point	%	SD	95% CL(Exp)	Skew
IC05	55.354			
IC10	60.172			
IC15	64.680			
IC20	69.014			
IC25	73.271			
IC40	86.308			
IC50	95.927			



Fish Embryonic Development and Post-Hatch Survival Test-Survival

Start Date:	27/11/2013 16:00	Test ID:	PR1108/07	Sample ID:	RP3
End Date:	8/12/2013 12:15	Lab ID:	6369	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 126	Test Species:	MS-Melanotaenia splendida
Comments:					

Dose-Response Plot



Fish Embryonic Development and Post-Hatch Survival Test-Survival

Start Date:	27/11/2013 16:00	Test ID:	PR1108/07	Sample ID:	RP3
End Date:	8/12/2013 12:15	Lab ID:	6369	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 126	Test Species:	MS-Melanotaenia splendida
Comments:					

		Auxiliary Data Summary					
Conc-%	**Parameter**	**Mean**	**Min**	**Max**	**SD**	**CV%**	**N**
DMW Control	% Survival	85.00	60.00	100.00	19.15	5.15	4
Diluent Control		80.00	60.00	100.00	16.33	5.05	4
3.1		65.00	40.00	80.00	19.15	6.73	4
6.3		90.00	80.00	100.00	11.55	3.78	4
12.5		95.00	80.00	100.00	10.00	3.33	4
25		100.00	100.00	100.00	0.00	0.00	4
50		90.00	80.00	100.00	11.55	3.78	4
100		40.00	20.00	100.00	40.00	15.81	4
DMW Control	pH	8.00	8.00	8.00	0.00	0.00	1
Diluent Control		7.50	7.50	7.50	0.00	0.00	1
3.1		7.00	7.00	7.00	0.00	0.00	1
6.3		6.90	6.90	6.90	0.00	0.00	1
12.5		6.80	6.80	6.80	0.00	0.00	1
25		6.80	6.80	6.80	0.00	0.00	1
50		7.00	7.00	7.00	0.00	0.00	1
100		7.70	7.70	7.70	0.00	0.00	1
DMW Control	DO (%)	99.70	99.70	99.70	0.00	0.00	1
Diluent Control		89.20	89.20	89.20	0.00	0.00	1
3.1		93.00	93.00	93.00	0.00	0.00	1
6.3		94.10	94.10	94.10	0.00	0.00	1
12.5		94.60	94.60	94.60	0.00	0.00	1
25		96.20	96.20	96.20	0.00	0.00	1
50		100.70	100.70	100.70	0.00	0.00	1
100		103.00	103.00	103.00	0.00	0.00	1
DMW Control	Conductivity (uS/cm)	173.30	173.30	173.30	0.00	0.00	1
Diluent Control		21.70	21.70	21.70	0.00	0.00	1
3.1		161.30	161.30	161.30	0.00	0.00	1
6.3		285.00	285.00	285.00	0.00	0.00	1
12.5		523.00	523.00	523.00	0.00	0.00	1
25		920.00	920.00	920.00	0.00	0.00	1
50		1662.00	1662.00	1662.00	0.00	0.00	1
100		2900.00	2900.00	2900.00	0.00	0.00	1

Fish Embryonic Development and Post-Hatch Survival Test-Survival

Start Date:	27/11/2013 16:00	Test ID:	PR1108/07	Sample ID:	RP3
End Date:	8/12/2013 12:15	Lab ID:	6369	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 126	Test Species:	MS-Melanotaenia splendida
Comments:					

Conc-%	1	2	3	4
DMW Control	1.0000	0.6000	1.0000	0.8000
Diluent Control	0.6000	1.0000	0.8000	0.8000
3.1	0.6000	0.4000	0.8000	0.8000
6.3	1.0000	1.0000	0.8000	0.8000
12.5	1.0000	0.8000	1.0000	1.0000
25	1.0000	1.0000	1.0000	1.0000
50	0.8000	1.0000	1.0000	0.8000
100	1.0000	0.2000	0.2000	0.2000

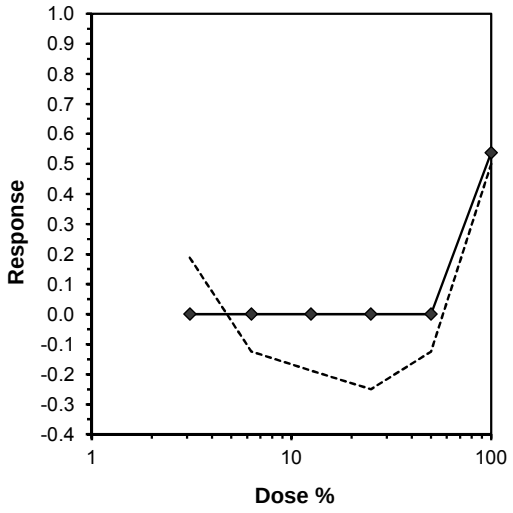
			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
DMW Control	0.8500	1.0625	1.1709	0.8861	1.3453	18.840	4				
Diluent Control	0.8000	1.0000	1.1114	0.8861	1.3453	16.874	4	*		4	20
3.1	0.6500	0.8125	0.9463	0.6847	1.1071	21.467	4	14.50	10.00	7	20
6.3	0.9000	1.1250	1.2262	1.1071	1.3453	11.212	4	21.00	10.00	2	20
12.5	0.9500	1.1875	1.2857	1.1071	1.3453	9.261	4	22.50	10.00	1	20
25	1.0000	1.2500	1.3453	1.3453	1.3453	0.000	4	24.00	10.00	0	20
50	0.9000	1.1250	1.2262	1.1071	1.3453	11.212	4	21.00	10.00	2	20
100	0.4000	0.5000	0.6841	0.4636	1.3453	64.442	4	13.50	10.00	12	20

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.05)	0.882017	0.924	1.430131	4.281494
Equality of variance cannot be confirmed				
The control means are not significantly different (p = 0.70)	0.411217	2.446912		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	100	>100		1
Treatments vs Diluent Control				

Trimmed Spearman-Karber

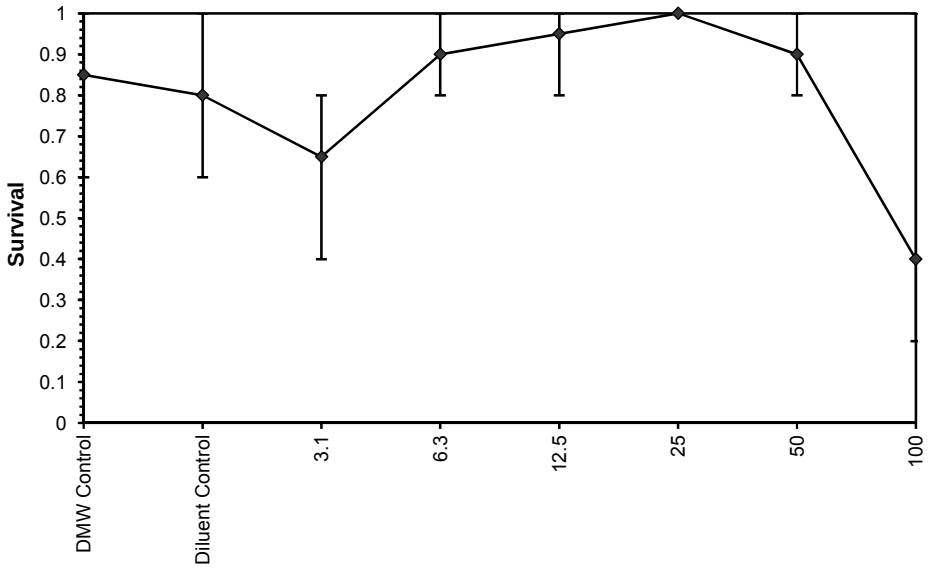
Trim Level	EC50	95% CL	
0.0%			
5.0%			
10.0%			
20.0%			
Auto-46.2%	95.170	72.906	124.232



Fish Embryonic Development and Post-Hatch Survival Test-Survival

Start Date:	27/11/2013 16:00	Test ID:	PR1108/07	Sample ID:	RP3
End Date:	8/12/2013 12:15	Lab ID:	6369	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 126	Test Species:	MS-Melanotaenia splendida
Comments:					

Dose-Response Plot



Fish Embryonic Development and Post-Hatch Survival Test-Survival

Start Date:	27/11/2013 16:00	Test ID:	PR1108/07	Sample ID:	RP3
End Date:	8/12/2013 12:15	Lab ID:	6369	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 126	Test Species:	MS-Melanotaenia splendida
Comments:					

		Auxiliary Data Summary					
Conc-%	**Parameter**	**Mean**	**Min**	**Max**	**SD**	**CV%**	**N**
DMW Control	% Survival	85.00	60.00	100.00	19.15	5.15	4
Diluent Control		80.00	60.00	100.00	16.33	5.05	4
3.1		65.00	40.00	80.00	19.15	6.73	4
6.3		90.00	80.00	100.00	11.55	3.78	4
12.5		95.00	80.00	100.00	10.00	3.33	4
25		100.00	100.00	100.00	0.00	0.00	4
50		90.00	80.00	100.00	11.55	3.78	4
100		40.00	20.00	100.00	40.00	15.81	4
DMW Control	pH	8.00	8.00	8.00	0.00	0.00	1
Diluent Control		7.50	7.50	7.50	0.00	0.00	1
3.1		7.00	7.00	7.00	0.00	0.00	1
6.3		6.90	6.90	6.90	0.00	0.00	1
12.5		6.80	6.80	6.80	0.00	0.00	1
25		6.80	6.80	6.80	0.00	0.00	1
50		7.00	7.00	7.00	0.00	0.00	1
100		7.70	7.70	7.70	0.00	0.00	1
DMW Control	DO (%)	99.70	99.70	99.70	0.00	0.00	1
Diluent Control		89.20	89.20	89.20	0.00	0.00	1
3.1		93.00	93.00	93.00	0.00	0.00	1
6.3		94.10	94.10	94.10	0.00	0.00	1
12.5		94.60	94.60	94.60	0.00	0.00	1
25		96.20	96.20	96.20	0.00	0.00	1
50		100.70	100.70	100.70	0.00	0.00	1
100		103.00	103.00	103.00	0.00	0.00	1
DMW Control	Conductivity (uS/cm)	173.30	173.30	173.30	0.00	0.00	1
Diluent Control		21.70	21.70	21.70	0.00	0.00	1
3.1		161.30	161.30	161.30	0.00	0.00	1
6.3		285.00	285.00	285.00	0.00	0.00	1
12.5		523.00	523.00	523.00	0.00	0.00	1
25		920.00	920.00	920.00	0.00	0.00	1
50		1662.00	1662.00	1662.00	0.00	0.00	1
100		2900.00	2900.00	2900.00	0.00	0.00	1

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Statistical Printouts for the Freshwater Shrimp Tests

Freshwater Shrimp Acute Toxicity Test-96 hr Survival

Start Date:	3/12/2013 16:00	Test ID:	PR1108/08	Sample ID:	RP3
End Date:	7/12/2013 16:30	Lab ID:	6369	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 123	Test Species:	MB-Macrobrachium bullatum
Comments:					

Conc-%	1	2	3	4
DMW Control	1.0000	1.0000	1.0000	0.8000
Diluent Control	1.0000	1.0000	0.8000	1.0000
6.3	1.0000	1.0000	1.0000	1.0000
12.5	1.0000	1.0000	1.0000	1.0000
25	1.0000	1.0000	0.8000	1.0000
50	1.0000	1.0000	0.8000	1.0000
100	1.0000	1.0000	1.0000	1.0000

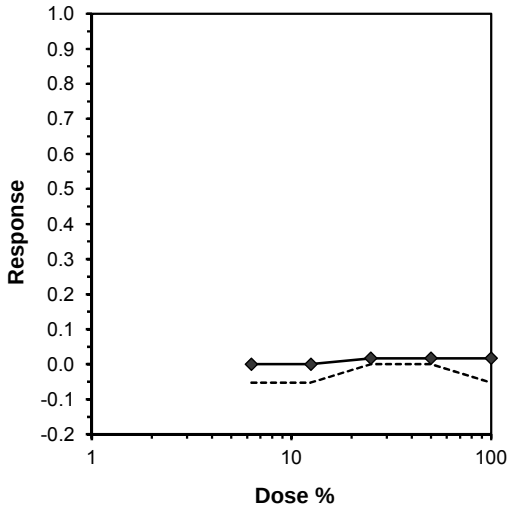
			Transform: Arcsin Square Root					Rank	1-Tailed	Isotonic	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Mean	N-Mean
DMW Control	0.9500	1.0000	1.2857	1.1071	1.3453	9.261	4				
Diluent Control	0.9500	1.0000	1.2857	1.1071	1.3453	9.261	4	*		0.9833	1.0000
6.3	1.0000	1.0526	1.3453	1.3453	1.3453	0.000	4	20.00	10.00	0.9833	1.0000
12.5	1.0000	1.0526	1.3453	1.3453	1.3453	0.000	4	20.00	10.00	0.9833	1.0000
25	0.9500	1.0000	1.2857	1.1071	1.3453	9.261	4	18.00	10.00	0.9667	0.9831
50	0.9500	1.0000	1.2857	1.1071	1.3453	9.261	4	18.00	10.00	0.9667	0.9831
100	1.0000	1.0526	1.3453	1.3453	1.3453	0.000	4	20.00	10.00	0.9667	0.9831

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.05)	0.668306	0.916	-1.74394	2.373016
Equality of variance cannot be confirmed				
The control means are not significantly different (p = 1.00)	0	2.446912		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	100	>100		1
Treatments vs Diluent Control				

Log-Logit Interpolation (200 Resamples)

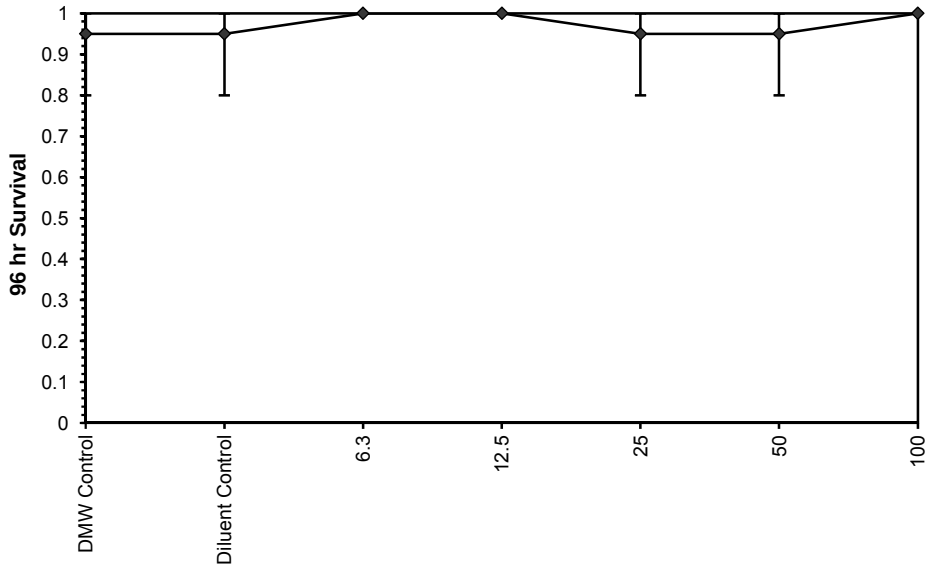
Point	%	SD	95% CL(Exp)	Skew
IC05	>100			
IC10	>100			
IC15	>100			
IC20	>100			
IC25	>100			
IC40	>100			
IC50	>100			



Freshwater Shrimp Acute Toxicity Test-96 hr Survival

Start Date:	3/12/2013 16:00	Test ID:	PR1108/08	Sample ID:	RP3
End Date:	7/12/2013 16:30	Lab ID:	6369	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 123	Test Species:	MB-Macrobrachium bullatum
Comments:					

Dose-Response Plot



 ToxCalc v5.0.23 Reviewed by:_____

Freshwater Shrimp Acute Toxicity Test-96 hr Survival

Start Date:	3/12/2013 16:00	Test ID:	PR1108/08	Sample ID:	RP3
End Date:	7/12/2013 16:30	Lab ID:	6369	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 123	Test Species:	MB-Macrobrachium bullatum
Comments:					

		Auxiliary Data Summary					
Conc-%	**Parameter**	**Mean**	**Min**	**Max**	**SD**	**CV%**	**N**
DMW Control	% Survival	95.00	80.00	100.00	10.00	3.33	4
Diluent Control		95.00	80.00	100.00	10.00	3.33	4
6.3		100.00	100.00	100.00	0.00	0.00	4
12.5		100.00	100.00	100.00	0.00	0.00	4
25		95.00	80.00	100.00	10.00	3.33	4
50		95.00	80.00	100.00	10.00	3.33	4
100		100.00	100.00	100.00	0.00	0.00	4
DMW Control	pH	8.10	8.10	8.10	0.00	0.00	1
Diluent Control		7.50	7.50	7.50	0.00	0.00	1
6.3		7.50	7.50	7.50	0.00	0.00	1
12.5		7.00	7.00	7.00	0.00	0.00	1
25		7.10	7.10	7.10	0.00	0.00	1
50		7.30	7.30	7.30	0.00	0.00	1
100		7.70	7.70	7.70	0.00	0.00	1
DMW Control	DO (%)	100.70	100.70	100.70	0.00	0.00	1
Diluent Control		97.10	97.10	97.10	0.00	0.00	1
6.3		97.50	97.50	97.50	0.00	0.00	1
12.5		97.70	97.70	97.70	0.00	0.00	1
25		97.40	97.40	97.40	0.00	0.00	1
50		96.90	96.90	96.90	0.00	0.00	1
100		97.10	97.10	97.10	0.00	0.00	1
DMW Control	Conductivity (uS/cm)	173.60	173.60	173.60	0.00	0.00	1
Diluent Control		18.60	18.60	18.60	0.00	0.00	1
6.3		291.00	291.00	291.00	0.00	0.00	1
12.5		528.00	528.00	528.00	0.00	0.00	1
25		942.00	942.00	942.00	0.00	0.00	1
50		1661.00	1661.00	1661.00	0.00	0.00	1
100		2910.00	2910.00	2910.00	0.00	0.00	1

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Statistical Printouts for the 7-d Chronic Test with *Ceriodaphnia dubia*

Ceriodaphnia Partial Life-Cycle Test-Reproduction

Start Date:	16/12/2013 13:30	Test ID:	PR1108/02	Sample ID:	RP3
End Date:	23/12/2013 10:30	Lab ID:	6369	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 102	Test Species:	CD-Ceriodaphnia dubia
Comments:					

Conc-%	1	2	3	4	5	6	7	8	9	10
DMW Control	20.000	19.000	16.000	13.000	18.000	16.000	16.000	9.000	14.000	15.000
Diluent Control	14.000	15.000	17.000	11.000	7.000	13.000	17.000	17.000	15.000	19.000
0.1	16.000	17.000	14.000	17.000	8.000	13.000	17.000	10.000	16.000	19.000
0.2	7.000	6.000	5.000	11.000	8.000	7.000	8.000	7.000	7.000	1.000
0.4	1.000	5.000	3.000	4.000	4.000	3.000	2.000	3.000	2.000	2.000
0.8	1.000	3.000	5.000	1.000	2.000	2.000	0.000	2.000	0.000	
1.5	2.000	3.000	2.000	0.000	2.000	1.000	3.000	2.000	2.000	0.000
3	2.000	2.000	2.000	2.000	0.000	1.000	2.000	0.000	1.000	4.000

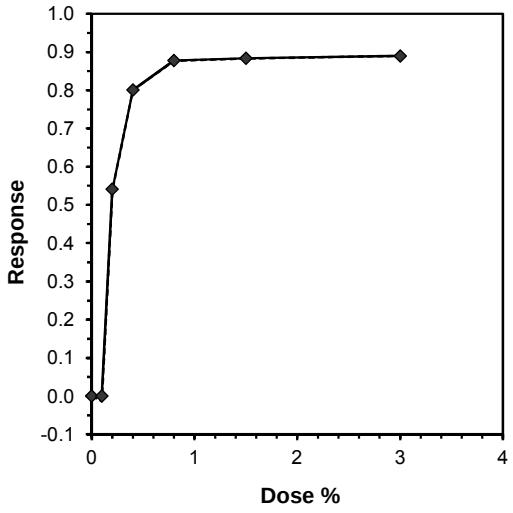
			Transform: Untransformed					Rank	1-Tailed	Isotonic	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Mean	N-Mean
DMW Control	15.600	1.0759	15.6000	9.0000	20.0000	20.316	10				
Diluent Control	14.500	1.0000	14.5000	7.0000	19.0000	24.165	10	*		14.600	1.0000
0.1	14.700	1.0138	14.7000	8.0000	19.0000	23.576	10	107.00	73.00	14.600	1.0000
*0.2	6.700	0.4621	6.7000	1.0000	11.0000	37.922	10	60.50	73.00	6.700	0.4589
*0.4	2.900	0.2000	2.9000	1.0000	5.0000	41.283	10	55.00	73.00	2.900	0.1986
*0.8	1.778	0.1226	1.7778	0.0000	5.0000	87.945	9	45.00	60.00	1.778	0.1218
*1.5	1.700	0.1172	1.7000	0.0000	3.0000	62.315	10	55.00	73.00	1.700	0.1164
*3	1.600	0.1103	1.6000	0.0000	4.0000	73.362	10	55.00	73.00	1.600	0.1096

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates non-normal distribution (p <= 0.05)	1.186323	0.895	-0.98421	2.561473
Bartlett's Test indicates unequal variances (p = 1.59E-04)	26.77824	16.81189		
The control means are not significantly different (p = 0.47)	0.736248	2.100922		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Wilcoxon Rank Sum Test	0.1	0.2	0.141421	1000
Treatments vs Diluent Control				

Linear Interpolation (200 Resamples)

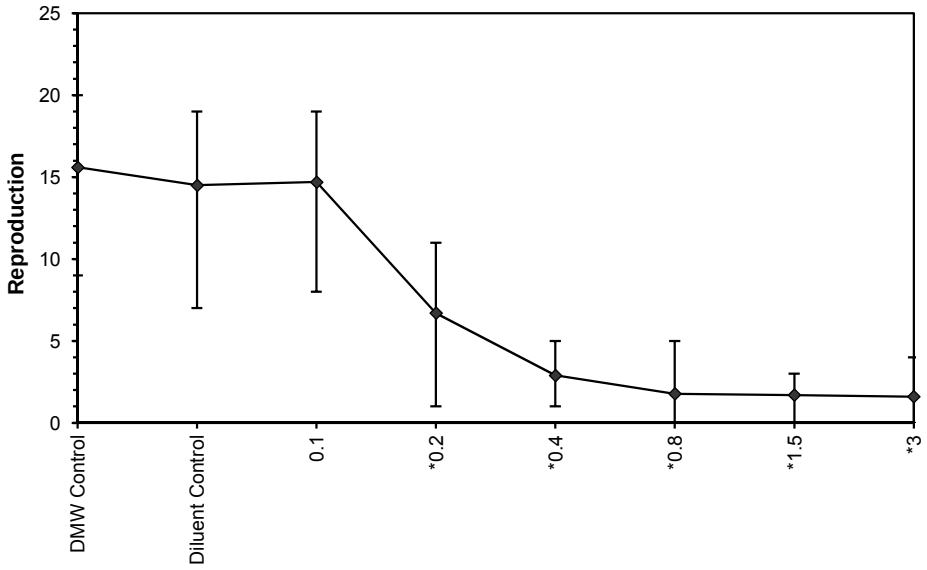
Point	%	SD	95% CL		Skew
IC05	0.1092	0.0252	0.0285	0.1113	-1.7822
IC10	0.1185	0.0166	0.0570	0.1227	-2.6684
IC15	0.1277	0.0123	0.0855	0.1340	-2.7940
IC20	0.1370	0.0105	0.1068	0.1454	-2.2541
IC25	0.1462	0.0096	0.1193	0.1567	-1.5647
IC40	0.1739	0.0093	0.1506	0.1908	0.0900
IC50	0.1924	0.0153	0.1738	0.2353	1.5507



Ceriodaphnia Partial Life-Cycle Test-Reproduction

Start Date:	16/12/2013 13:30	Test ID:	PR1108/02	Sample ID:	RP3
End Date:	23/12/2013 10:30	Lab ID:	6369	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 102	Test Species:	CD-Ceriodaphnia dubia
Comments:					

Dose-Response Plot



Ceriodaphnia Partial Life-Cycle Test-Reproduction

Start Date:	16/12/2013 13:30	Test ID:	PR1108/02	Sample ID:	RP3
End Date:	23/12/2013 10:30	Lab ID:	6369	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 102	Test Species:	CD-Ceriodaphnia dubia
Comments:					

Auxiliary Data Summary

Conc-%	Parameter	Mean	Min	Max	SD	CV%	N
DMW Control	No of Young	15.60	9.00	20.00	3.17	11.41	10
Diluent Control		14.50	7.00	19.00	3.50	12.91	10
0.1		14.70	8.00	19.00	3.47	12.66	10
0.2		6.70	1.00	11.00	2.54	23.79	10
0.4		2.90	1.00	5.00	1.20	37.73	10
0.8		1.78	0.00	5.00	1.56	70.33	9
1.5		1.70	0.00	3.00	1.06	60.54	10
3		1.60	0.00	4.00	1.17	67.71	10
DMW Control	% survival	100.00	100.00	100.00	0.00	0.00	10
Diluent Control		100.00	100.00	100.00	0.00	0.00	10
0.1		100.00	100.00	100.00	0.00	0.00	10
0.2		100.00	100.00	100.00	0.00	0.00	10
0.4		100.00	100.00	100.00	0.00	0.00	10
0.8		100.00	100.00	100.00	0.00	0.00	9
1.5		50.00	0.00	100.00	52.70	14.52	10
3		50.00	0.00	100.00	52.70	14.52	10
DMW Control	pH	8.10	8.10	8.10	0.00	0.00	1
Diluent Control		7.30	7.30	7.30	0.00	0.00	1
0.1		7.20	7.20	7.20	0.00	0.00	1
0.2		7.10	7.10	7.10	0.00	0.00	1
0.4		7.10	7.10	7.10	0.00	0.00	1
0.8		6.90	6.90	6.90	0.00	0.00	1
1.5		6.90	6.90	6.90	0.00	0.00	1
3		6.90	6.90	6.90	0.00	0.00	1
DMW Control	DO %	99.00	99.00	99.00	0.00	0.00	1
Diluent Control		100.90	100.90	100.90	0.00	0.00	1
0.1		100.50	100.50	100.50	0.00	0.00	1
0.2		99.90	99.90	99.90	0.00	0.00	1
0.4		99.70	99.70	99.70	0.00	0.00	1
0.8		99.80	99.80	99.80	0.00	0.00	1
1.5		99.80	99.80	99.80	0.00	0.00	1
3		100.40	100.40	100.40	0.00	0.00	1
DMW Control	Cond uS/cm	179.80	179.80	179.80	0.00	0.00	1
Diluent Control		179.80	179.80	179.80	0.00	0.00	1
0.1		32.90	32.90	32.90	0.00	0.00	1
0.2		37.20	37.20	37.20	0.00	0.00	1
0.4		46.40	46.40	46.40	0.00	0.00	1
0.8		64.40	64.40	64.40	0.00	0.00	1
1.5		100.10	100.10	100.10	0.00	0.00	1
3		170.20	170.20	170.20	0.00	0.00	1

Ceriodaphnia Partial Life-Cycle Test-7 Day Survival

Start Date:	16/12/2013 13:30	Test ID:	PR1108/02	Sample ID:	RP3
End Date:	23/12/2013 10:30	Lab ID:	6369	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 102	Test Species:	CD-Ceriodaphnia dubia
Comments:					

Conc-%	1	2	3	4	5	6	7	8	9	10
DMW Control	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
Diluent Control	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
0.1	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
0.2	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
0.4	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
0.8	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	
1.5	0.0000	1.0000	1.0000	0.0000	0.0000	1.0000	0.0000	1.0000	0.0000	1.0000
3	0.0000	1.0000	0.0000	1.0000	1.0000	0.0000	1.0000	1.0000	0.0000	0.0000

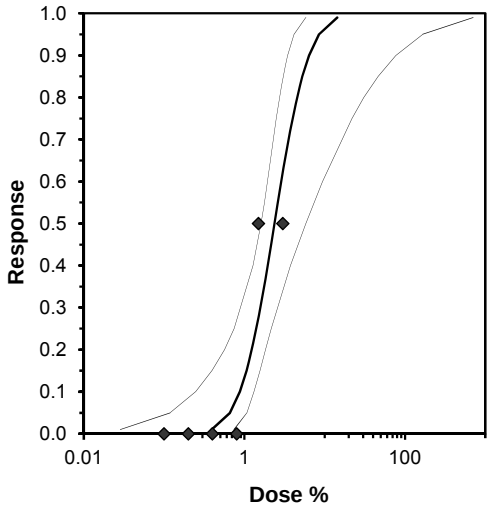
Conc-%	Mean	N-Mean	Resp	Not Resp	Total	N	Fisher's Exact P	1-Tailed Critical	Number Resp	Total Number
DMW Control	1.0000	1.0000	0	10	10	10	0.6238			
Diluent Control	1.0000	1.0000	0	10	10	10	*		0	10
0.1	1.0000	1.0000	0	10	10	10	1.0000	0.0500	0	10
0.2	1.0000	1.0000	0	10	10	10	1.0000	0.0500	0	10
0.4	1.0000	1.0000	0	10	10	10	1.0000	0.0500	0	10
0.8	1.0000	1.0000	0	9	9	9	1.0000	0.0500	0	9
*1.5	0.5000	0.5000	5	5	10	10	0.0163	0.0500	5	10
*3	0.5000	0.5000	5	5	10	10	0.0163	0.0500	5	10

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Fisher's Exact Test	0.8	1.5	1.095445	125
Treatments vs Diluent Control				

Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	2.976816	0.971097	1.073466	4.880166	0	3.97566	9.487729	0.41	0.374219	0.335929	7
Intercept	3.88602	0.329684	3.239839	4.532201							
TSCR											

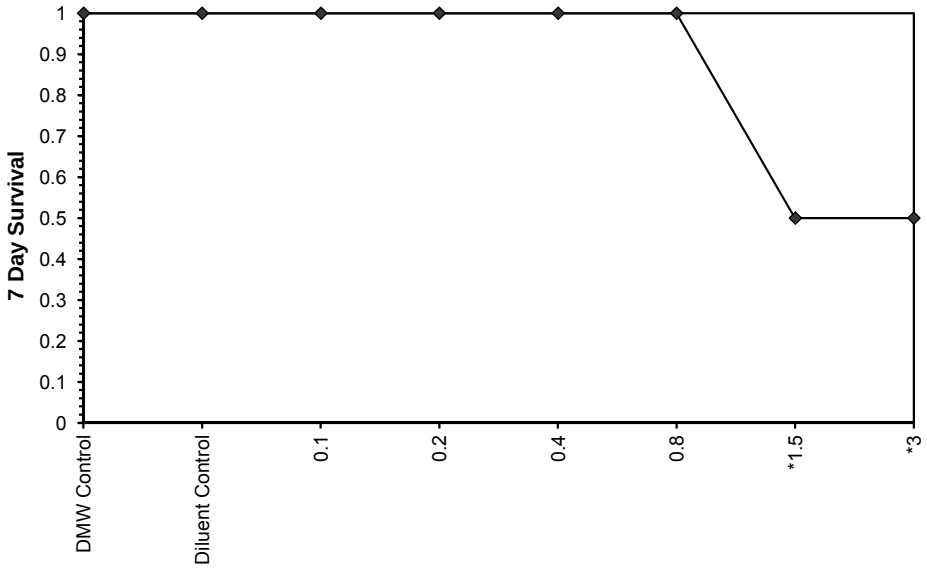
Point	Probits	%	95% Fiducial Limits	
EC01	2.674	0.391498	0.028529	0.74125
EC05	3.355	0.66323	0.117986	1.066382
EC10	3.718	0.878431	0.245824	1.324347
EC15	3.964	1.061818	0.394916	1.565639
EC20	4.158	1.234506	0.562604	1.829756
EC25	4.326	1.404875	0.742772	2.146252
EC40	4.747	1.945861	1.280952	3.746453
EC50	5.000	2.367111	1.606831	5.795692
EC60	5.253	2.879555	1.92853	9.370682
EC75	5.674	3.988408	2.491194	21.83772
EC80	5.842	4.53883	2.734199	30.81237
EC85	6.036	5.277	3.036866	46.18792
EC90	6.282	6.37866	3.452778	77.15344
EC95	6.645	8.448377	4.155259	165.8857
EC99	7.326	14.31223	5.827223	703.794



Ceriodaphnia Partial Life-Cycle Test-7 Day Survival

Start Date:	16/12/2013 13:30	Test ID:	PR1108/02	Sample ID:	RP3
End Date:	23/12/2013 10:30	Lab ID:	6369	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 102	Test Species:	CD-Ceriodaphnia dubia
Comments:					

Dose-Response Plot



 ToxCalc v5.0.23 Reviewed by:_____

Ceriodaphnia Partial Life-Cycle Test-7 Day Survival

Start Date:	16/12/2013 13:30	Test ID:	PR1108/02	Sample ID:	RP3
End Date:	23/12/2013 10:30	Lab ID:	6369	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 102	Test Species:	CD-Ceriodaphnia dubia
Comments:					

		Auxiliary Data Summary					
Conc-%	**Parameter**	**Mean**	**Min**	**Max**	**SD**	**CV%**	**N**
DMW Control	No of Young	15.60	9.00	20.00	3.17	11.41	10
Diluent Control		14.50	7.00	19.00	3.50	12.91	10
0.1		14.70	8.00	19.00	3.47	12.66	10
0.2		6.70	1.00	11.00	2.54	23.79	10
0.4		2.90	1.00	5.00	1.20	37.73	10
0.8		1.78	0.00	5.00	1.56	70.33	9
1.5		1.70	0.00	3.00	1.06	60.54	10
3		1.60	0.00	4.00	1.17	67.71	10
DMW Control	% survival	100.00	100.00	100.00	0.00	0.00	10
Diluent Control		100.00	100.00	100.00	0.00	0.00	10
0.1		100.00	100.00	100.00	0.00	0.00	10
0.2		100.00	100.00	100.00	0.00	0.00	10
0.4		100.00	100.00	100.00	0.00	0.00	10
0.8		100.00	100.00	100.00	0.00	0.00	9
1.5		50.00	0.00	100.00	52.70	14.52	10
3		50.00	0.00	100.00	52.70	14.52	10
DMW Control	pH	8.10	8.10	8.10	0.00	0.00	1
Diluent Control		7.30	7.30	7.30	0.00	0.00	1
0.1		7.20	7.20	7.20	0.00	0.00	1
0.2		7.10	7.10	7.10	0.00	0.00	1
0.4		7.10	7.10	7.10	0.00	0.00	1
0.8		6.90	6.90	6.90	0.00	0.00	1
1.5		6.90	6.90	6.90	0.00	0.00	1
3		6.90	6.90	6.90	0.00	0.00	1
DMW Control	DO %	99.00	99.00	99.00	0.00	0.00	1
Diluent Control		100.90	100.90	100.90	0.00	0.00	1
0.1		100.50	100.50	100.50	0.00	0.00	1
0.2		99.90	99.90	99.90	0.00	0.00	1
0.4		99.70	99.70	99.70	0.00	0.00	1
0.8		99.80	99.80	99.80	0.00	0.00	1
1.5		99.80	99.80	99.80	0.00	0.00	1
3		100.40	100.40	100.40	0.00	0.00	1
DMW Control	Cond uS/cm	179.80	179.80	179.80	0.00	0.00	1
Diluent Control		179.80	179.80	179.80	0.00	0.00	1
0.1		32.90	32.90	32.90	0.00	0.00	1
0.2		37.20	37.20	37.20	0.00	0.00	1
0.4		46.40	46.40	46.40	0.00	0.00	1
0.8		64.40	64.40	64.40	0.00	0.00	1
1.5		100.10	100.10	100.10	0.00	0.00	1
3		170.20	170.20	170.20	0.00	0.00	1

Appendix B – Chemistry Reports

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12 Ashley St Chatswood NSW 2067
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enquiries@envirolabservices.com.au
www.envirolabservices.com.au

CERTIFICATE OF ANALYSIS 101665

Client:
Vista Gold Australia Pty Ltd
Mt Todd Mine Site
Edith Falls Rd
NT 2067

Attention: Austin Brandis

Sample log in details:

Your Reference:	**PR1108**		
No. of samples:	2 Waters		
Date samples received / completed instructions received	2/12/2013	/	2/12/2013

Analysis Details:
Please refer to the following pages for results, methodology summary and quality control data.
Samples were analysed as received from the client. Results relate specifically to the samples as received.
Results are reported on a dry weight basis for solids and on an as received basis for other matrices.
Please refer to the last page of this report for any comments relating to the results.

Report Details:

Date results requested by: / Issue Date:	9/12/13	/	9/12/13
Date of Preliminary Report:	None Issued		

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Accredited for compliance with ISO/IEC 17025. **Tests not covered by NATA are denoted with *.**

Results Approved By:

Jacinta Hurst
Laboratory Manager

HM in water - dissolved Our Reference: Your Reference Date Sampled Type of sample	UNITS ---------- ----------	101665-1 SW2 25/11/2013 Water	101665-2 RP3 25/11/2013 Water
Date prepared	-	03/12/2013	03/12/2013
Date analysed	-	03/12/2013	03/12/2013
Aluminium-Dissolved	µg/L	<10	<10
Cadmium-Dissolved	µg/L	<0.1	51
Cobalt-Dissolved	µg/L	<1	510
Chromium-Dissolved	µg/L	<1	<1
Copper-Dissolved	µg/L	<1	<1
Iron-Dissolved	µg/L	110	<10
Manganese-Dissolved	µg/L	<5	6,000
Nickel-Dissolved	µg/L	<1	500
Lead-Dissolved	µg/L	<1	<1
Mercury-Dissolved	µg/L	<0.05	<0.05
Zinc-Dissolved	µg/L	1	4,400

Miscellaneous Inorganics			
Our Reference:	UNITS	101665-1	101665-2
Your Reference	----------	SW2	RP3
Date Sampled	----------	25/11/2013	25/11/2013
Type of sample		Water	Water
Date prepared	-	03/12/2013	03/12/2013
Date analysed	-	03/12/2013	03/12/2013
pH	pH Units	6.7	7.6
Dissolved Oxygen*	mg/L	6.2	8.5
Electrical Conductivity	µS/cm	19	2,700
Sulphate, SO4	mg/L	<1	1,900

Cations in water Dissolved			
Our Reference:	UNITS	101665-1	101665-2
Your Reference	----------	SW2	RP3
Date Sampled	----------	25/11/2013	25/11/2013
Type of sample		Water	Water
Date digested	-	03/12/2013	03/12/2013
Date analysed	-	03/12/2013	03/12/2013
Calcium - Dissolved	mg/L	<0.5	480
Magnesium - Dissolved	mg/L	<0.5	210

Method ID	Methodology Summary
Metals-022 ICP-MS	Determination of various metals by ICP-MS.
Metals-021 CV-AAS	Determination of Mercury by Cold Vapour AAS.
Inorg-001	pH - Measured using pH meter and electrode in accordance with APHA 22nd ED, 4500-H+.
Inorg-112	Dissolved Oxygen using membrane electrode. The method is based upon APHA 4500-O G. Note this analysis should ideally be carried out immediately after sampling.
Inorg-002	Conductivity and Salinity - measured using a conductivity cell and dedicated meter, in accordance with APHA 22nd ED 2510 and Rayment & Lyons.
Inorg-081	Anions - a range of Anions are determined by Ion Chromatography, in accordance with APHA 22nd ED, 4110 -B.
Metals-020 ICP-AES	Determination of various metals by ICP-AES.

Client Reference: PR1108

QUALITY CONTROL HM in water - dissolved	UNITS	PQL	METHOD	Blank	Duplicate Sm#	Duplicate results Base II Duplicate II %RPD	Spike Sm#	Spike % Recovery
Date prepared	-			03/12/2013	101665-1	03/12/2013 || 03/12/2013	LCS-W1	03/12/2013
Date analysed	-			03/12/2013	101665-1	03/12/2013 || 03/12/2013	LCS-W1	03/12/2013
Aluminium-Dissolved	µg/L	10	Metals-022 ICP-MS	<10	101665-1	<10 || [N/T]	LCS-W1	104%
Cadmium-Dissolved	µg/L	0.1	Metals-022 ICP-MS	<0.1	101665-1	<0.1 || [N/T]	LCS-W1	100%
Cobalt-Dissolved	µg/L	1	Metals-022 ICP-MS	<1	101665-1	<1 || [N/T]	LCS-W1	94%
Chromium-Dissolved	µg/L	1	Metals-022 ICP-MS	<1	101665-1	<1 || [N/T]	LCS-W1	93%
Copper-Dissolved	µg/L	1	Metals-022 ICP-MS	<1	101665-1	<1 || [N/T]	LCS-W1	97%
Iron-Dissolved	µg/L	10	Metals-022 ICP-MS	<10	101665-1	110 || [N/T]	LCS-W1	100%
Manganese-Dissolved	µg/L	5	Metals-022 ICP-MS	<5	101665-1	<5 || [N/T]	LCS-W1	98%
Nickel-Dissolved	µg/L	1	Metals-022 ICP-MS	<1	101665-1	<1 || [N/T]	LCS-W1	95%
Lead-Dissolved	µg/L	1	Metals-022 ICP-MS	<1	101665-1	<1 || [N/T]	LCS-W1	101%
Mercury-Dissolved	µg/L	0.05	Metals-021 CV-AAS	<0.05	101665-1	<0.05 || <0.05	LCS-W1	92%
Zinc-Dissolved	µg/L	1	Metals-022 ICP-MS	<1	101665-1	1 || [N/T]	LCS-W1	99%

QUALITY CONTROL Miscellaneous Inorganics	UNITS	PQL	METHOD	Blank	Duplicate Sm#	Duplicate results Base II Duplicate II %RPD	Spike Sm#	Spike % Recovery
Date prepared	-			03/12/2013	[NT]	[NT]	LCS-W1	03/12/2013
Date analysed	-			03/12/2013	[NT]	[NT]	LCS-W1	03/12/2013
pH	pH Units		Inorg-001	[NT]	[NT]	[NT]	LCS-W1	100%
Dissolved Oxygen*	mg/L	0.1	Inorg-112	<0.1	[NT]	[NT]	LCS-W1	105%
Electrical Conductivity	µS/cm	1	Inorg-002	<1	[NT]	[NT]	[NR]	[NR]
Sulphate, SO4	mg/L	1	Inorg-081	<1	[NT]	[NT]	LCS-W1	117%

QUALITY CONTROL Cations in water Dissolved	UNITS	PQL	METHOD	Blank	Duplicate Sm#	Duplicate results Base II Duplicate II %RPD	Spike Sm#	Spike % Recovery
Date digested	-			03/12/2013	[NT]	[NT]	LCS-W1	03/12/2013
Date analysed	-			03/12/2013	[NT]	[NT]	LCS-W1	03/12/2013
Calcium - Dissolved	mg/L	0.5	Metals-020 ICP-AES	<0.5	[NT]	[NT]	LCS-W1	104%
Magnesium - Dissolved	mg/L	0.5	Metals-020 ICP-AES	<0.5	[NT]	[NT]	LCS-W1	105%

QUALITY CONTROL HM in water - dissolved	UNITS	Dup. Sm#	Duplicate Base + Duplicate + %RPD	Spike Sm#	Spike % Recovery
Date prepared	-	[NT]	[NT]	101665-2	03/12/2013
Date analysed	-	[NT]	[NT]	101665-2	03/12/2013
Aluminium-Dissolved	µg/L	[NT]	[NT]	[NR]	[NR]
Cadmium-Dissolved	µg/L	[NT]	[NT]	[NR]	[NR]
Cobalt-Dissolved	µg/L	[NT]	[NT]	[NR]	[NR]
Chromium-Dissolved	µg/L	[NT]	[NT]	[NR]	[NR]
Copper-Dissolved	µg/L	[NT]	[NT]	[NR]	[NR]
Iron-Dissolved	µg/L	[NT]	[NT]	[NR]	[NR]
Manganese-Dissolved	µg/L	[NT]	[NT]	[NR]	[NR]
Nickel-Dissolved	µg/L	[NT]	[NT]	[NR]	[NR]
Lead-Dissolved	µg/L	[NT]	[NT]	[NR]	[NR]
Mercury-Dissolved	µg/L	[NT]	[NT]	101665-2	96%
Zinc-Dissolved	µg/L	[NT]	[NT]	[NR]	[NR]

Report Comments:

Asbestos ID was analysed by Approved Identifier:	Not applicable for this job
Asbestos ID was authorised by Approved Signatory:	Not applicable for this job

INS: Insufficient sample for this test	PQL: Practical Quantitation Limit	NT: Not tested
NA: Test not required	RPD: Relative Percent Difference	NA: Test not required
<: Less than	>: Greater than	LCS: Laboratory Control Sample

Quality Control Definitions

Blank: This is the component of the analytical signal which is not derived from the sample but from reagents, glassware etc, can be determined by processing solvents and reagents in exactly the same manner as for samples.

Duplicate: This is the complete duplicate analysis of a sample from the process batch. If possible, the sample selected should be one where the analyte concentration is easily measurable.

Matrix Spike : A portion of the sample is spiked with a known concentration of target analyte. The purpose of the matrix spike is to monitor the performance of the analytical method used and to determine whether matrix interferences exist.

LCS (Laboratory Control Sample) : This comprises either a standard reference material or a control matrix (such as a blank sand or water) fortified with analytes representative of the analyte class. It is simply a check sample.

Surrogate Spike: Surrogates are known additions to each sample, blank, matrix spike and LCS in a batch, of compounds which are similar to the analyte of interest, however are not expected to be found in real samples.

Laboratory Acceptance Criteria

Duplicate sample and matrix spike recoveries may not be reported on smaller jobs, however, were analysed at a frequency to meet or exceed NEPM requirements. All samples are tested in batches of 20. The duplicate sample RPD and matrix spike recoveries for the batch were within the laboratory acceptance criteria.
Filters, swabs, wipes, tubes and badges will not have duplicate data as the whole sample is generally extracted during sample extraction.
Spikes for Physical and Aggregate Tests are not applicable.
For VOCs in water samples, three vials are required for duplicate or spike analysis.

Duplicates: <5xPQL - any RPD is acceptable; >5xPQL - 0-50% RPD is acceptable.
Matrix Spikes, LCS and Surrogate recoveries: Generally 70-130% for inorganics/metals; 60-140% for organics and 10-140% for SVOC and speciated phenols is acceptable.

In circumstances where no duplicate and/or sample spike has been reported at 1 in 10 and/or 1 in 20 samples respectively, the sample volume submitted was insufficient in order to satisfy laboratory QA/QC protocols.

When samples are received where certain analytes are outside of recommended technical holding times (THTs), the analysis has proceeded. Where analytes are on the verge of breaching THTs, every effort will be made to analyse within the THT or as soon as practicable.

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Document Status

Rev No.	Author	Reviewer		Approved for Issue		
		Name	Signature	Name	Signature	Date
0	J.Woodworth	G.Metcalfe	*G Metcalfe**	G.Metcalfe	*G Metcalfe**	24/12/2013
1	J.Woodworth	G.Metcalfe	*G Metcalfe**	G.Metcalfe	*G Metcalfe**	06/01/2014

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Document Status

Rev No.	Author	Reviewer		Approved for Issue		
		Name	Signature	Name	Signature	Date
0	J. Woodworth A. Koscielski	J. Woodworth	*Jill Woodworth*	N. Conroy	*Nikki Conroy*	10/10/14

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