

Toxicity Identification Evaluation (TIE) of County of San Diego and Copermittees Agua Hedionda Creek Sample

Prepared for:

County of San Diego and Copermittees

Prepared by:

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Carlsbad, California 92010

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1.0 INTRODUCTION

The San Diego municipal stormwater monitoring permit (National Pollutant Discharge Elimination System [NPDES] Order 2007-0001) for the San Diego, California region requires monitoring of runoff at six mass loading stations (MLS) and nine temporary watershed assessment stations (TWAS) within San Diego's watersheds. The six MLS evaluated in the 2007 to 2008 monitoring period included San Luis Rey River, Agua Hedionda Creek, Escondido Creek, San Dieguito River, Los Penasquitos Creek, and Chollas Creek. The nine TWAS evaluated in the 2007 to 2008 monitoring period included Buena Vista Creek, Loma Alta Creek, Escondido Creek, San Luis Rey River, Agua Hedionda Creek, Los Penasquitos Creek (2), and San Dieguito Creek (2). Samples were collected from two wet weather and two dry weather events and analyzed for toxicity using *Ceriodaphnia dubia*, *Hyaella azteca*, and *Selenastrum capricornutum*. The initial toxicity test from the November 30, 2007 storm event demonstrated that Agua Hedionda Creek MLS stormwater caused significant toxicity to *H. azteca*. Survival in the 6.25, 12.5, 25, 50, and 100 percent sample concentrations was 92.5, 100, 82.5, 65.0, and 37.5%, respectively. The median lethal concentration (LC50) at 96 hours was estimated to be 77.3%, while the no observed effect concentration (NOEC) was 12.5%. As a result, a Phase I toxicity identification evaluation (TIE) was conducted to establish the potential cause or causes of toxicity. Weston Solutions Inc. (Weston) initiated the Phase I TIE on December 11, 2007.

2.0 BIOASSAY TEST METHODS

Stormwater toxicity tests and TIEs using the freshwater amphipod *H. azteca* were performed according to a modified version of the United States Environmental Protection Agency (USEPA) protocol for testing sediment-associated contaminants with freshwater invertebrates (EPA/600/R-99/064) (USEPA, 2000). TIEs were conducted according to guidelines for characterizing chronically toxic effluents (USEPA, 1991, 1992, 1993a, and 1993b). Phase I TIE testing involved manipulating the sample at the initial pH using the manipulations presented in Table 1. A detailed description of each manipulation including set-up procedures are presented in Appendix I of the 2006-2007 San Diego County Municipal Copermittees Urban Runoff Monitoring report (Weston, 2008).

Table 1. Phase I TIE Sample Manipulations

Physical and Chemical Manipulations (Tests) on Stormwater Samples	Purpose of Test
Filtration	Detects filterable compounds (e.g., TSS related)
Aeration	Detects volatile, oxidizable, sublutable, or spargeable compounds
Ethylenediaminetetraacetic Acid (EDTA) Addition	Detects cationic metals (e.g., cadmium)
Sodium Thiosulfate (STS) Addition	Detects oxidative compounds (e.g., chlorine)
Solid Phase Extraction (SPE) over C ₁₈ Column, followed by Methanol Elution	Detects non-polar organics and some surfactants
Piperonyl Butoxide (PBO) Addition	Detects organophosphate pesticides and pyrethroids
Carboxyl esterase Addition	Detects pyrethroids
Bovine serum albumin (BSA) Addition	The protein BSA is used as a control for the Carboxyl esterase

3.0 BIOASSAY TEST RESULTS

Water quality parameters were within the appropriate limits (Table 2). Mean percent control survival of *H. azteca* was 96.0%, which met the minimum acceptable control survival criterion ($\geq 90\%$). The Baseline test confirmed the presence of toxicity in the stormwater sample, however toxicity was reduced relative to the initial toxicity test with this species; Survival in the 100% concentration of the Baseline test was 56% while survival in the 100% concentration of the initial test was 37.5%. The Baseline test acts as a benchmark for comparison to toxicity in other TIE treatments. The only treatments which significantly altered toxicity of the sample were Filtration, C₁₈ SPE, and PBO. The Filtration treatment significantly reduced toxicity in the 100% concentration (100% survival) relative to the Baseline test, indicating that the causative agent may be associated with particulates in the stormwater sample. The C₁₈ SPE treatment demonstrated a similar reduction in toxicity (100% survival). Since this sample was pre-filtered prior to the C₁₈ manipulation, the reduction in toxicity was most likely due to pre-filtration of the sample prior to C₁₈ elution. The PBO treatment significantly increased toxicity (4% survival) relative to the Baseline test. This suggests that pyrethroids may be a causative agent in this stormwater sample (Budavari, 1989). Treatments that showed no significant effect on toxicity included Carboxyl esterase, BSA, Aeration, STS, and EDTA. The Carboxyl esterase treatment (68% survival) slightly reduced toxicity relative to the Baseline test; however, this reduction was not significant. Toxicity in the BSA treatment (52% survival) was similar to the Baseline treatment. Although toxicity was slightly reduced in the Aeration (76% survival) and STS treatments (72% survival) relative to the Baseline test, it was not significant. Toxicity in the EDTA treatment (44% survival) was similar to the Baseline test.

The copper sulfate reference toxicant test was conducted at concentrations of 0, 62.5, 125, 250, 500 and 1000 $\mu\text{g Cu}^{2+}/\text{L}$. The LC₅₀ was 164 $\mu\text{g Cu}^{2+}/\text{L}$, which was inside two standard deviations ($\pm 203 \mu\text{g Cu}^{2+}/\text{L}$) of the Weston laboratory mean of 236 $\mu\text{g Cu}^{2+}/\text{L}$. This indicates that the sensitivity of *H. azteca* used in the assessment fell within the normal range.

Complete survival results for the TIE test using *H. azteca* are presented in Table 3.

Table 2. Test Conditions for the 96 Hour TIE Using *H. azteca*

Test Conditions: <i>Hyalella azteca</i> TIE Test		
Sample Identification	Agua Hedionda Creek	
Dates sampled	November 30, 2007	
Date received at Weston Carlsbad Laboratory	December 1, 2007	
Approximate volume received	20 L	
Sample storage conditions	4°C, dark	
Test Species	<i>H. azteca</i>	
Supplier	Aquatic Indicators	
Date acquired	December 11, 2007	
Acclimation/holding time	0 days	
Age	9 days	
Test Procedures	USEPA (1991, 1992, 1993a, 1993b, 2000)	
Test location	Weston Solutions Carlsbad Laboratory, Room 5	
Test type/duration	Static / 96 hours	
Test dates	December 11 – 15, 2007 ¹	
Control water	Evian™ mineral water diluted with deionized water to achieve a moderate hardness (80-100 mg/L as CaCO ₃)	
Test temperature	Target: 23 ± 1°C	Actual: 22 - 24°C
Test conductivity	No recommended concentration	Actual: 0.17 – 0.89 mS/cm
Test dissolved oxygen	Target: 4.0 mg/L	Actual: 5.2 – 10.0 mg/L
Test pH	Target: Watch for pH drift	Actual: 7.4 – 8.6
Test photoperiod	16 hours light: 8 hours dark	
Test chamber	125 mL glass jar	
Concentrations	Baseline: 0% (Control), 50%, and 100% TIE Treatments: 0% (Blank) and 100%, with the exception of the Methanol Elution treatment. This treatment was spiked to a concentration of 2.0 times the concentration of the potential contaminants in the original stormwater sample.	
Replicates	5	
Organisms/replicate	5	
Exposure volume	50 mL	
Feeding	Day 0 and 2	
Water renewal	None	
Deviations from Test Protocol	Six amphipods were counted in replicate 5 of the PBO treatment blank. This indicates that an extra animal was added to this chamber at test initiation. This replicate was eliminated from statistical analysis.	

¹Test dates for the Methanol Elution treatment was December 12 - 16, 2007.

Table 3. Results of the 96 Hour TIE Using *H. azteca*

TIE Treatment	% Survival			NOEC	LC ₅₀
	Control (Blank) – Dilution Water	50% Stormwater	100% Stormwater (2X ¹)		
Baseline	96	84	56	50	>100
Aeration	100		76		
Filtration	100		100		
Solid Phase Extraction (C ₁₈)	100		100		
Methanol Elution	96		96		
STS – 10 mg/L	100		72		
EDTA – 3.0 mg/L	100		44		
PBO – 0.025 mg/L	100 ²		4		
Carboxyl esterase	100		68		
BSA (esterase control)	52 ³		52		
Copper Sulfate Reference Toxicant	Concentration (µg/L)	% Survival		LC ₅₀ (µg/L)	
	Control	100		164	
	62.5	90.0			
	125	77.5			
	250	12.5			
	500	0.00			
1000	0.00				

¹ 2X concentration is associated with Methanol Elution treatment only.

² An extra animal was added to test chamber at test initiation. This replicate was dropped from statistical analysis.

³ Low survival in BSA blank possibly due to contamination. Fuzzy material observed in test chambers on Day 3.

4.0 DISCUSSION

The results from the Phase I TIE performed on the Agua Hedionda Creek stormwater sample collected in November indicated that pyrethroids may be the cause of toxicity observed in the initial toxicity test. The lack of toxicity reduction in the EDTA, STS, and aeration treatments indicates that the causative agent was likely not a metal, an oxidative chemical, or a volatile chemical or surfactant, respectively. However, the reduction in toxicity of *H. azteca* following filtration of the stormwater sample indicates that the causative agent was highly bound to particulates in the sample. Pyrethroids have physicochemical properties that match the results of the present TIE; pyrethroids are insoluble in water but soluble in solvents (have high K_{ow} s), have low vapor pressures (indicating low volatility), and have high adsorption coefficients, indicating their tendency to adsorb to particulates (Kidd and James, 1991). In addition, toxicity was increased in the diluted PBO-treated stormwater samples, as compared to toxicity in the baseline test (untreated diluted stormwater). These results also indicate pyrethroids as the causative agents because pyrethroid toxicity is potentiated by PBO (Budavari, 1989). The carboxyl esterase treatment caused a reduction in toxicity in the undiluted stormwater sample; however, it was not significant, possibly due to the reduced toxicity demonstrated in the Baseline test (56% survival) compared to the initial toxicity test (37.5% survival). Toxicity was not removed in the BSA treatment, used as a control for the carboxyl esterase. If toxicity was reduced in both the BSA treatment and the carboxyl esterase treatment, this would indicate that both BSA and esterase are adsorbing the chemicals in the water samples. In contrast, if toxicity was only reduced in the carboxyl esterase treatment, this would indicate that the chemicals in the water sample have been enzymatically altered by the carboxyl esterase, and that these chemicals may be pyrethroids due to the affinity of carboxyl esterase for these compounds.

In addition to TIE tests, a review of chemical analyses of Agua Hedionda Creek MLS stormwater indicates that pyrethroids are likely the primary causative agents of toxicity. The concentration of bifenthrin (0.0816 $\mu\text{g/L}$) was measured at a level that exceeded aqueous 96-hour LC_{50} values for *H. azteca* (0.0093 $\mu\text{g/L}$) (Anderson et al., 2006). A number of other pyrethroids were detected in stormwater samples including cyfluthrin, esfenvalerate, fenvalerate, and L-cyhalothrin¹. The aqueous 96 hour LC_{50} values for *H. azteca* exposed to these pyrethroids are currently unknown.

Chemicals other than pyrethroids were detected in Agua Hedionda Creek MLS stormwater; however, there is little evidence that any of these chemicals were a major cause of toxicity. All organophosphate pesticides were below their detection limits, with the exception of chlorpyrifos, diazinon, and malathion. The concentration of chlorpyrifos (0.094 $\mu\text{g/L}$) may have contributed to toxicity observed in the November storm event because the concentration detected exceeded the water quality objective (0.020 $\mu\text{g/L}$). In addition, the 10-day LC_{50} for this species has been reported to be 0.09 $\mu\text{g/L}$ (Phipps et al., 1995). However, because the addition of PBO increased toxicity (consistent with its synergistic effects on pyrethroids), rather than decreased toxicity (consistent with its antagonistic effects on organophosphate pesticides), it is unlikely that chlorpyrifos was the primary cause of toxicity in the sample. Diazinon (0.035 $\mu\text{g/L}$) and malathion (0.398 $\mu\text{g/L}$) were both detected at concentrations below their water quality objectives and were measured at concentrations below those which have been shown to cause toxicity to this species in 10-day solid phase tests (6.51 $\mu\text{g/L}$ [Ankley et al., 1995] and 80.3 $\mu\text{g/L}$ [Weston

¹ Concentration of L-cyhalothrin was estimated.

Bioassay Lab data], respectively). Thus, it unlikely that diazinon and malathion contributed to toxicity of this sample. Concentrations of total copper and lead were above their respective water quality objectives; however, concentrations of dissolved copper and lead were below their water quality objectives. Because dissolved forms of metals are more bioavailable to organisms, it is unlikely that copper and lead were responsible for the observed toxicity.

5.0 REFERENCES

- Anderson, B.S., Phillips, B.M., Hunt, J.W., Connor, V., Richard, N., and R.S. Tjeerdema. 2006. Identifying primary stressors impacting macroinvertebrates in the Salinas River (California, USA): relative effects of pesticides and suspended particles. *Environmental Pollution*, 141:402-408.
- Ankley, G.T., and S.A. Collyard. 1995. Influence of piperonyl butoxide on the toxicity of organophosphate insecticides to three species of freshwater benthic invertebrates. *Comparative Biochemistry and Physiology C*, 110:149-155.
- Budavari, S., (ed.). 1989. *The Merck Index*. Merck & Co. Rahway, NJ, USA.
- Kidd, H. and D.R. James, (Eds.). 1991. *The Agrochemicals Handbook*, Third Edition. Royal Society of Chemistry Information Services, Cambridge, UK, pp. 2-13.
- Phipps, G.L., V.R. Mattson, and G.T. Ankley. 1995. Relative sensitivity of three freshwater benthic macroinvertebrates to ten contaminants. *Archives of Environmental Contamination and Toxicology*, 28:281-286.
- United States Environmental Protection Agency (USEPA). 1991. *Methods for Aquatic Toxicity Identification Evaluations. Phase I Toxicity Characterization Procedures*. EPA/600/6-91/003. EPA Office of Research and Development. Second Edition. February .
- United States Environmental Protection Agency (USEPA). 1992. *Toxicity Identification Evaluation. Characterization of Chronically Toxic Effluents, Phase I*. EPA/600/6-91/005F. EPA Office of Research and Development. May.
- United States Environmental Protection Agency (USEPA). 1993a. *Methods for Aquatic Toxicity Identification Evaluations. Phase II Toxicity Characterization Procedures for Samples Exhibiting Acute and Chronic Toxicity* EPA/600/R-92/080. EPA Office of Research and Development. September.
- United States Environmental Protection Agency (USEPA). 1993b. *Methods for Aquatic Toxicity Identification Evaluations. Phase III Toxicity Characterization Procedures for Samples Exhibiting Acute and Chronic Toxicity* EPA/600/R-92/081. EPA Office of Research and Development. September.
- United States Environmental Protection Agency (USEPA). 2000. *Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates*. EPA/600/R-99/064. EPA Office of Water. March.
- Weston Solutions, Inc. 2008. *San Diego County Municipal Copermittees 2007-2008 Urban Runoff Monitoring. Final Report*. January.

Client:	County of SDT Copermitted
Project:	T/E
Client Sample ID:	AHC-mis baseline
WESTON Test ID:	CO71201.1678
Species:	Hyalella azteca

Date Received:	12/11/07
Date Test Started:	12/11/07
Date Test Ended:	12/15/07
Study Director:	A. Margolis
# Organisms/Chamber:	5

	Conc.	meter #	D.O. (mg/L)	meter #	Temp (°C)	meter #	Cond. (mS/cm)	meter #	pH	Hardness (mg/L CaCO ₃)	Alkalinity (mg/L CaCO ₃)	Chlorine (mg/L)
Day 0 (0 Hours) Date: 12/11/07 Sample ID: CO71201.16a Dilutions (Tech): 0.1M WQ* Time: 1500 Technician: NS	Control	6	8.6	8a	21.5	6	0.20	13	8.6	84	70	0.00
	50		9.5		22.5		0.39		8.3			
	100		9.0		22.0		0.60		8.0	176	52	0.00
24 Hours Date: 12/12/07 WQ Time: 15:208 Replicate: WQ SURR Technician: AEL	Control	1	9.3	4A	22.8	6	0.27	13	8.0			
	50		7.8		22.7		0.47		7.9			
	100		7.7		22.7		0.71		7.8			
48 Hours Date: 12/13/07 WQ Time: 1020 Replicate: SURR Technician: NS	Control	6	8.2	11	24.3	5	0.26	10	8.4			
	50		7.6		23.0		0.48		8.1			
	100		7.6		23.0		0.69		7.9			
72 Hours Date: 12/14/07 WQ Time: 1129 Replicate: SURR Technician: AEL	Control	1	8.7	8A	22.5	6	0.27	13	8.1			
	50		8.4		22.0		0.35		8.0			
	100		8.4		21.7		0.55		8.0			
96 Hours Date: 12/15/07 WQ Time: 1510 Replicate: WQ SURR Technician: E/B	Control	6	8.2	4	22.0	5	0.28	10	8.3			
	50		7.8		23.1		0.50		8.2			
	100		7.9		21.6		0.79		8.1			

*water quality performed on stock dilutions on Day 0.

Start Time:	1700 AL
End Time:	1525 AL
Supplier:	Aquatic Indicators
Organism Batch:	AI 9211 Age: 9 days

Dilution Water Batch:	DMW 362
Hobo Temp. No.:	119280
Test Location:	rm 5
Test Acceptability:	X ≥ 90% Survival in Control

① IE 12/11/07 NS (METER 8a used)

Weston Test ID: C071201.1678	Client: County of SD + Copermittes	Client Sample ID: AHC mls baseline
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Survival Data									
Conc.	Rep	24 Hours		48 Hours		72 Hours		96 Hours	
		Date: 12/12/07		Date: 12/13/07		Date: 12/14/07		Date: 12/15/07	
		Time: 0935		Time: 1320		Time: 1025		End Time: 1525	
		Tech: am		Tech: am		Tech: am		Tech: AL	
		# Alive	# Dead	# Alive	# Dead	# Alive	# Dead	# Alive	# Dead
Control	1	5	0	5	0	5	0	5	0
	2	5	0	5	0	5	0	5	0
	3	5	0	5	0	5	0	5	0
	4/5	4/5	1/0	4/5	0/0	4/5	0/0	4/5	0/0
50	1	5	0	4	1	4	0	3	1
	2	5	0	5	0	5	0	5	5
	3	5	0	5	0	5	0	5	5
	4/5	5/5	0/0	5/5	0/0	5/4	0/1	4/4	1/0
100	1	5	0	5	0	5	0	3	2
	2	4	0 NB	5	0 FB	5	0	4	1
	3	5	0	3	2	1	2	1	0
	4/5	5/5	0/0	5/5	0/0	5/4	0/1	3/3	2/1
	1								
	2								
	3								
	4								
	1								
	2								
	3								
	4								

FEEDING INFORMATION					
Day 0 (0 Hours)			48 Hours		
Date: 12/11/07	Time: 1800	Initials: EB	Date: 12/13/07	Time: 1515	Initials: NS

Hyalella Acute Test-96 Hr Survival

Start Date: 12/11/2007 17:00	Test ID: AHC MLS TIE	Sample ID: Baseline
End Date: 12/15/2007 15:25	Lab ID: CCA-Weston, Carlsbad	Sample Type: SRW1-Municipal stormwater
Sample Date: 11/30/2007	Protocol: EPAA 91-EPA Acute	Test Species: HA-Hyalella azteca

Conc-%	1	2	3	4	5
Control	1.0000	1.0000	1.0000	0.8000	1.0000
50	0.6000	1.0000	1.0000	0.8000	0.8000
100	0.6000	0.8000	0.2000	0.6000	0.6000

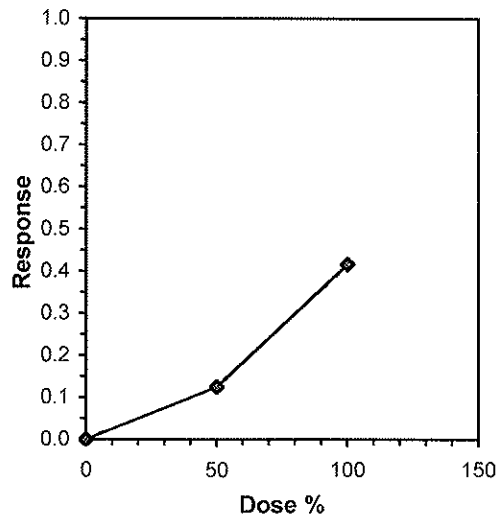
Conc-%	Mean	N-Mean	Transform: Untransformed					N	t-Stat	1-Tailed Critical	MSD	Isotonic	
			Mean	Min	Max	CV%	Mean					N-Mean	
Control	0.9600	1.0000	0.9600	0.8000	1.0000	9.317	5				0.9600	1.0000	
50	0.8400	0.8750	0.8400	0.6000	1.0000	19.920	5	1.134	2.110	0.2233	0.8400	0.8750	
*100	0.5600	0.5833	0.5600	0.2000	0.8000	39.123	5	3.780	2.110	0.2233	0.5600	0.5833	

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.89027	0.835	-0.9363	1.08059
Bartlett's Test indicates equal variances (p = 0.28)	2.56956	9.21034		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	50	100	70.7107	2	0.2233	0.23261	0.21067	0.028	0.00763	2, 12

Linear Interpolation (200 Resamples)					
Point	%	SD	95% CL(Exp)		Skew
IC05*	20.000	15.739	4.633	78.700	0.7376
IC10*	40.000	14.433	9.267	80.025	-0.1237
IC15	54.286	12.562	17.329	86.109	-0.3720
IC20	62.857				
IC25	71.429				
IC40	97.143				
IC50	>100				

* indicates IC estimate less than the lowest concentration



Test: HA-Hyalella Acute Test

Species: HA-Hyalella azteca

Sample ID: Baseline

Start Date: 12/11/2007 17:00

Test ID: AHC TIE

Protocol: EPAA 91-EPA Acute

Sample Type: SRW1-Municipal stormwater

End Date: 12/15/2007 15:25 Lab ID: CCA-Weston, Carlsbad

Pos	ID	Rep	Group	Start	24 Hr	48 Hr	72 Hr	96 Hr	Notes
	1	1	Control	5				5	
	2	2	Control	5				5	
	3	3	Control	5				5	
	4	4	Control	5				4	
	5	5	Control	5				5	
	6	1	50,000	5				3	
	7	2	50,000	5				5	
	8	3	50,000	5				5	
	9	4	50,000	5				4	
	10	5	50,000	5				4	
	11	1	100,000	5				3	
	12	2	100,000	5				4	
	13	3	100,000	5				1	
	14	4	100,000	5				3	
	15	5	100,000	5				3	

Comments:



Hyalella 96-Hour Acute Toxicity Test

BIO078

Client:	County of SD+ Copermitttees
Project:	TIE
Client Sample ID:	AHC-MLS P130-0.025mg/L
WESTON Test ID:	C071201.1678
Species:	Hyalella azteca

Date Received:	12/11/07
Date Test Started:	12/11/07
Date Test Ended:	12/15/07
Study Director:	A. Margolis
# Organisms/Chamber:	5

	Conc.	meter #	D.O. (mg/L)	meter #	Temp (°C)	meter #	Cond. (mS/cm)	meter #	pH	Hardness (mg/L CaCO ₃)	Alkalinity (mg/L CaCO ₃)	Chlorine (mg/L)
Day 0 (0 Hours)	Control	6		8a		6		13				
Date: 12/11/07	Blank		8.3		22.5		0.18		8.4			
Sample ID: C071201.16a	100		8.0		23.0		0.60		7.7			
Dilutions (Tech): AM												
WQ* Time: 15:15												
Technician: NS												
24 Hours	Control	1		9A		6		13				
Date: 12/12/07	Blank		9.2		23.1		0.20		8.0			
WQ Time: 13:10	100		8.8		23.2		0.71		7.8			
Replicate: WQ SURV												
Technician: AEL												
48 Hours	Control	6		11		5		10				
Date: 12/13/07	Blank		8.7		24.4		0.20		8.4			
WQ Time: 10:15	100		8.4		24.3		0.69		8.1			
Replicate: SURV												
Technician: NS												
72 Hours	Control	6		8A		6		13				
Date: 12/14/07	Blank		8.2		22.1		0.20		8.1			
WQ Time: 11:16	100		8.3		22.1		0.75		7.9			
Replicate: SURV												
Technician: AEL												
96 Hours	Control	6		6		5		10				
Date: 12/15/07	Blank		8.0		22.5		0.23		8.4			
WQ Time: 15:40	100		8.1		21.5		0.76		8.1			
Replicate: WQ SURV												
Technician: AEL												

*water quality performed on stock dilutions on Day 0.

Start Time:	1723 EB
End Time:	1701 EB
Supplier:	Aquatic Indicators
Organism Batch:	AI 9211 Age: 9 days

Dilution Water Batch:	DMW 362
Hobo Temp. No.:	119280
Test Location:	rm 5
Test Acceptability: \leq \geq 90% Survival in Control	

- ① WT 12/11/07 NS
- ② changed meter AEL 12/14/07
- ③ WC 12/14/07 AEL

Weston Test ID: C071201.1678	Client: County of SBT Coopermitters	Client Sample ID: AHC-MLS P30-0025 mg/l
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Survival Data									
Conc.	Rep	24 Hours		48 Hours		72 Hours		96 Hours	
		Date: 12/12/07		Date: 12/13/07		Date: 12/14/07		Date: 12/15/07	
		Time: 1100		Time: 1400		Time: 1140		End Time: 1701	
		Tech: AM		Tech: AM		Tech: AM		Tech: EB	
		# Alive	# Dead	# Alive	# Dead	# Alive	# Dead	# Alive	# Dead
Control	1								
	2								
	3								
	4								
blank	1	5	0	5	0	5	0	5	0
	2	5	0	5	0	5	0	5	0
	3	5	0	5	0	5	0	5	0
	4	5	0	5	0	5	0	5	0
100	1	5	0	4	1	2	2	0	2
	2	5	0	4	1	3	1	0	3
	3	5	0	4	1	2	2	0	2
	4	5	0	4	1	2	2	0	2
	1	5	0	4	1	2	2	0	2
	2								
	3								
	4								
	1								
	2								
	3								
	4								
	1								
	2								
	3								
	4								

FEEDING INFORMATION					
Day 0 (0 Hours)			48 Hours		
Date: 12/11/07	Time: 1460	Initials: EB	Date: 12/13/07	Time: 1515	Initials: EB

① extra body found 12/13/07 am
 ② IE 12/14/07 am



Hyalella 96-Hour Acute Toxicity Test

BIO078

Client:	County of SD + Copermittacs
Project:	TIE
Client Sample ID:	AHC-MLS C-18
WESTON Test ID:	C071201.1678
Species:	Hyalella azteca

Date Received:	12/11/07
Date Test Started:	12/11/07
Date Test Ended:	12/15/07
Study Director:	A. Margolis
# Organisms/Chamber:	5

	Conc.	meter #	D.O. (mg/L)	meter #	Temp (°C)	meter #	Cond. (mS/cm)	meter #	pH	Hardness (mg/L CaCO ₃)	Alkalinity (mg/L CaCO ₃)	Chlorine (mg/L)
Day 0 (0 Hours)	-Control	6		8a		6		13				
Date: 12/11/07	Blank		8.7		22.5		0.18		8.3			
Sample ID: C071201.16a	100		8.2		22.5		0.59		8.1			
Dilutions (Tech): Ann												
WQ* Time: 1520												
Technician: NS												
24 Hours	Control	8	-	9A	-	6	-	13	-			
Date: 12/12/07	Blank		10.0		22.5		0.21		8.1			
WQ Time: 1255	100		9.1		23.0		0.68		7.9			
Replicate: WQ SURR												
Technician: AEL												
48 Hours	Control	6		11		5		10				
Date: 12/13/07	Blank		8.1		23.5		0.23		8.3			
WQ Time: 1150	100		6.2		23.2		0.71		7.8			
Replicate: SURR												
Technician: NS												
72 Hours	Control	1		8A		6		13				
Date: 12/14/07	Blank		7.7		21.7		0.24		8.0			
WQ Time: 1200	100		8.4		21.9		0.76		7.9			
Replicate: SURR												
Technician: AEL												
96 Hours	Control	6		6		5		10				
Date: 12/15/07	Blank		7.9		22.0		0.26		8.3			
WQ Time: 1532	100		8.0		21.7		0.79		8.2			
Replicate: WQ SURR												
Technician: AB												

*water quality performed on stock dilutions on Day 0.

Start Time:	1720 AL
End Time:	1745 AB
Supplier:	Aquatic Indicators
Organism Batch:	AI9211
Age:	9 days

Dilution Water Batch:	DMW 362
Hobo Temp. No.:	119280
Test Location:	rm 5
Test Acceptability: <input checked="" type="checkbox"/> ≥ 90% Survival in Control	



Hyalella 96-Hour Acute Toxicity Test

BIO078

Weston Test ID: C071201.1678	Client: County of SD+ Coopermittees	Client Sample ID: AHC-MLS C-18
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Survival Data											
Conc.	Rep	24 Hours		48 Hours		72 Hours		96 Hours			
		Date: 12/12/07	Date: 12/13/07	Date: 12/14/07	Date: 12/15/07	Time: 1005	Time: 1345	Time: 1105	End Time: 1745	Tech: arm	Tech: arm
		# Alive	# Dead	# Alive	# Dead	# Alive	# Dead	# Alive	# Dead		
Control	1										
	2										
	3										
	4										
blank	1	5	0	5	0	5	0	5	0		
	2	5	0	5	0	5	0	5	0		
	3	5	0	5	0	5	0	5	0		
	4	5	0	5	0	5	0	5	0		
100	1	5	0	5	0	5	0	5	0		
	2	5	0	5	0	5	0	5	0		
	3	5	0	5	0	5	0	5	0		
	4	5	0	5	0	5	0	5	0		
	1										
	2										
	3										
	4										
	1										
	2										
	3										
	4										
	1										
	2										
	3										
	4										

FEEDING INFORMATION					
Day 0 (0 Hours)			48 Hours		
Date: 12/11/07	Time: 800	Initials: NS	Date: 12/13/07	Time: 1515	Initials: NS



Hyaella 96-Hour Acute Toxicity Test

BIO078

Client:	County of SD + Coopermittees
Project:	TIE
Client Sample ID:	AHC-MLS filtration
WESTON Test ID:	071201.1678
Species:	Hyaella azteca

Date Received:	12/11/07
Date Test Started:	12/11/07
Date Test Ended:	12/15/07
Study Director:	R. Margolis
# Organisms/Chamber:	5

	Conc.	meter #	D.O. (mg/L)	meter #	Temp (°C)	meter #	Cond. (mS/cm)	meter #	pH	Hardness (mg/L CaCO ₃)	Alkalinity (mg/L CaCO ₃)	Chlorine (mg/L)
Day 0 (0 Hours)	Control	6		8a		6		13				
Date: 12/11/07	blank		8.5		22.0		0.17		8.5			
Sample ID: 071201.16a	100		8.6		22.0		0.59		8.1			
Dilutions (Tech):												
WQ* Time: 1505												
Technician: NS												
24 Hours	Control	1		9A		6		13				
Date: 12/12/07	blank		9.7		23.0		0.23		8.0			
WQ Time: 1238	100		8.8		23.3		0.69		7.7			
Replicate: WQ SURR												
Technician: AEC												
48 Hours	Control	6		6/6 ⁵		5		10				
Date: 12/13/07	Blank		9.1		24.0		0.25		8.4			
WQ Time: 1005	100		8.6		24.0		0.70		8.1			
Replicate: SURR												
Technician: NS												
72 Hours	Control	6		8A		6		13				
Date: 12/14/07	Blank		8.4		22.1		0.22		8.0			
WQ Time: 1113	100		8.3		22.0		0.72		7.9			
Replicate: SURR												
Technician: AEL												
96 Hours	Control	6		6		5		10				
Date: 12/15/07	Blank		8.5		21.5		0.29		8.3			
WQ Time: 1525	100		8.4		21.9		0.78		8.3			
Replicate: WQ SURR												
Technician: WB												

*water quality performed on stock dilutions on Day 0.

Start Time:	1715 AL
End Time:	1735 WB
Supplier:	Aquatic Indicators
Organism Batch:	AI 9211 Age: 9 days

Dilution Water Batch:	DMW 362
Hobo Temp. No.:	119280
Test Location:	rm 5
Test Acceptability: X ≥ 90% Survival in Control	

① TEMP METER - H used 12/13/07 NS
 ② changed meter 12/14/07 AEL



Hyalella 96-Hour Acute Toxicity Test

BIO078

Weston Test ID: C071201.1678	Client: County of SD + Copermittes	Client Sample ID: AHCMLS filtration
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Survival Data									
Conc.	Rep	24 Hours		48 Hours		72 Hours		96 Hours	
		Date: 12/12/07		Date: 12/13/07		Date: 12/14/07		Date: 12/15/07	
		Time: 0955		Time: 1330		Time: 1055		End Time: 1735	
		Tech: AM		Tech: EB		Tech: AM		Tech: EB	
		# Alive	# Dead	# Alive	# Dead	# Alive	# Dead	# Alive	# Dead
Control	1								
	2								
	3								
	4	5							
blank	1	5	0	5	0	5	0	5	0
	2	5	0	5	0	5	0	5	0
	3	5	0	5	0	5	0	5	0
	4	5	0	5	0	5	0	5	0
100	1	5	0	5	0	5	0	5	0
	2	5	0	5	0	5	0	5	0
	3	5	0	5	0	5	0	5	0
	4	5	0	5	0	5	0	5	0
	1								
	2								
	3								
	4								
	1								
	2								
	3								
	4								

FEEDING INFORMATION					
Day 0 (0 Hours)			48 Hours		
Date: 12/11/07	Time: 1900	Initials: EB	Date: 12/13/07	Time: 1515	Initials: NS



Hyalella 96-Hour Acute Toxicity Test

BIO078

Client:	County of SD + Cooperatives
Project:	TIE
Client Sample ID:	AHC-MLS EDTA 3.0mg/L
WESTON Test ID:	CO712011678
Species:	Hyalella azteca

Date Received:	12/11/07
Date Test Started:	12/11/07
Date Test Ended:	12/15/07
Study Director:	A. Margolis
# Organisms/Chamber:	5

	Conc.	meter #	D.O. (mg/L)	meter #	Temp (°C)	meter #	Cond. (mS/cm)	meter #	pH	Hardness (mg/L CaCO ₃)	Alkalinity (mg/L CaCO ₃)	Chlorine (mg/L)
Day 0 (0 Hours)	-Control	6		8a		6		13				
Date: 12/11/07	Blank		8.2		22.5		0.18		8.3			
Sample ID: CO71201166a	100		8.4		22.7		0.60		7.978			
Dilutions (Tech): AMM												
WQ* Time: 1:50												
Technician: NS												
24 Hours	Control	1	—	9A	—	6	—	11	—			
Date: 12/12/07	Blank		9.1		22.9		0.69023		8.1			
WQ Time: 12:45	100		9.0		22.5		0.67		7.8			
Replicate: WQ SURV												
Technician: AEL												
48 Hours	Control	6		11		5		10				
Date: 12/13/07	Blank		8.6		23.0		0.23		8.3			
WQ Time: 1:40	100		8.4		23.2		0.65		8.1			
Replicate: SUM												
Technician: NS												
72 Hours	Control	1		8A		6		13				
Date: 12/14/07	Blank		8.7		21.9		0.25		8.1			
WQ Time: 1:44	100		8.8		22.0		0.47		8.0			
Replicate: SUM												
Technician: AEL												
96 Hours	Control	6		6		5		10				
Date: 12/15/07	Blank		8.5		21.5		0.29		8.4			
WQ Time: 1:40	100		8.3		21.4		0.80		8.2			
Replicate: WQ SURV												
Technician: CB												

*water quality performed on stock dilutions on Day 0.

Start Time:	1725 AL
End Time:	1615 9/16
Supplier:	Aquatic Indicators
Organism Batch:	A-19211 Age: 9 days

Dilution Water Batch:	DMW 362
Hobo Temp. No.:	119280
Test Location:	rm 5
Test Acceptability: X ≥ 90% Survival in Control	

- ① WC 12/11/07 NS
- ② WC 12/12/07 AEL



Hyalella 96-Hour Acute Toxicity Test

BIO078

Weston Test ID: C071201.1678	Client: County of SD + Copermittices	Client Sample ID: AHC MLS EDTA 3.0mg/L
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Survival Data									
Conc.	Rep	24 Hours		48 Hours		72 Hours		96 Hours	
		Date: 12/12/07		Date: 12/13/07		Date: 12/14/07		Date: 12/15/07	
		Time: 1040		Time: 1503		Time: 1120		End Time: 1615	
		Tech: AM		Tech: EB		Tech: AM		Tech: EB	
		# Alive	# Dead	# Alive	# Dead	# Alive	# Dead	# Alive	# Dead
Control	1								
	2								
	3								
	4								
blank	1	5	0	5	0	5	0	5	0
	2	5	0	5	0	5	0	5	0
	3	5	0	5	0	5	0	5	0
	4	5	0	5	0	5	0	5	0
100	1	5	0	5	0	4	1	1	2 (1NB)
	2	5	0	5	0	4	1	2	2
	3	5	0	5	0	5	0	3	2
	4	5	0	5	0	5	0	4	1
	1								
	2								
	3								
	4								
	1								
	2								
	3								
	4								

FEEDING INFORMATION					
Day 0 (0 Hours)			48 Hours		
Date: 12/11/07	Time: 1800	Initials: EB	Date: 12/13/07	Time: 1515	Initials: NS



Hyalella 96-Hour Acute Toxicity Test

BIO078

Client:	County of SD + Coopermittees
Project:	TIE
Client Sample ID:	AHC-MLS STS 10mg/L
WESTON Test ID:	0712011678
Species:	Hyalella azteca

Date Received:	12/11/07
Date Test Started:	12/11/07
Date Test Ended:	12/15/07
Study Director:	A. Margolis
# Organisms/Chamber:	5

	Conc.	meter #	D.O. (mg/L)	meter #	Temp (°C)	meter #	Cond. (mS/cm)	meter #	pH	Hardness (mg/L CaCO ₃)	Alkalinity (mg/L CaCO ₃)	Chlorine (mg/L)
Day 0 (0 Hours)	Control	6		8a		6		13				
Date: 12/11/07	Blank		8.0		22.5		0.18		8.4			
Sample ID: 07120116a	100		8.3		22.3		0.60		7.9			
Dilutions (Tech): am												
WQ* Time:												
Technician: NS												
24 Hours	Control	1	—	9a	—	6	—	13	—			
Date: 12/12/07	Blank		9.2		22.0		0.23		8.0			
WQ Time: 1242	100		8.9		22.0		0.64		7.8			
Replicate: WQ SURR												
Technician: AEL												
48 Hours	Control	6		11		5		10				
Date: 12/13/07	Blank		7.8		23.0		0.25		8.3			
WQ Time: 1155	100		7.7		23.0		0.66		8.0			
Replicate: SURR												
Technician: NS												
72 Hours	Control	1		8a		6		13				
Date: 12/14/07	Blank		8.4		21.6		0.26		8.2			
WQ Time: 1221	100		8.5		21.6		0.82		8.4			
Replicate: SURR												
Technician: AEL												
96 Hours	Control	6		6		5		10				
Date: 12/15/07	Blank		7.9		21.7		0.29		8.4			
WQ Time: 1545	100		7.6		22.6		0.89		8.2			
Replicate: WQ SURR												
Technician: AB												

*water quality performed on stock dilutions on Day 0.

Start Time:	1735 AL
End Time:	1559 EB
Supplier:	Aquatic Indicators
Organism Batch:	AI 9211 Age: 9 days

Dilution Water Batch:	DMW 362
Hobo Temp. No.:	119280
Test Location:	rm 5
Test Acceptability: X ≥ 90% Survival in Control	

OIE 12/14/07 AEL



Hyaella 96-Hour Acute Toxicity Test

BIO078

Weston Test ID: 0071216-0071201.1678 <i>am</i>	Client: County of San Copermittes	Client Sample ID: AHC-MLS 55
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Survival Data									
Conc.	Rep	24 Hours		48 Hours		72 Hours		96 Hours	
		Date: 12/12/07		Date: 12/13/07		Date: 12/14/07		Date: 12/15/07	
		Time: 1050		Time: 1440		Time: 1130		End Time: 1559	
		Tech: am		Tech: EB		Tech: am		Tech: EB	
		# Alive	# Dead	# Alive	# Dead	# Alive	# Dead	# Alive	# Dead
Control	1								
	2								
	3								
	4								
blank	1	5	0	5	0	5	0	5	0
	2	5	0	5	0	5	0	5	0
	3	5	0	5	0	5	0	5	0
	4	5	0	5	0	5	0	5	0
100	1	5	0	4	1	4	0	4	0
	2	5	0	5	0	5	0	3	2
	3	5	0	5	0	5	0	4	1
	4	5	0	5	0	5	0	4	1
	1	5	0	5	0	5	0	5	0
	2								
	3								
	4								
	1								
	2								
	3								
	4								
	1								
	2								
	3								
	4								

FEEDING INFORMATION					
Day 0 (0 Hours)			48 Hours		
Date: 12/11/07	Time: 1800	Initials: EB	Date: 12/13/07	Time: 1515	Initials: EB

0 IE 12/14/07-am



Hyalella 96-Hour Acute Toxicity Test

BIO078

Client:	County of San Bernardino
Project:	TIE
Client Sample ID:	AHC-MLS aeration
WESTON Test ID:	0071201.1678
Species:	Hyalella azteca

Date Received:	12/11/07
Date Test Started:	12/11/07
Date Test Ended:	12/15/07
Study Director:	A. Margolis
# Organisms/Chamber:	5

	Conc.	meter #	D.O. (mg/L)	meter #	Temp (°C)	meter #	Cond. (mS/cm)	meter #	pH	Hardness (mg/L CaCO ₃)	Alkalinity (mg/L CaCO ₃)	Chlorine (mg/L)
Day 0 (0 Hours)	Control	6		6a		6		13				
Date: 12/11/07	Blank		7.6		22.5		0.18		8.4			
Sample ID: 0071201.169	100		7.9		23.0		0.55		8.1			
Dilutions (Tech): AM												
WQ* Time: 1545												
Technician: NS												
24 Hours	Control	1		9A		6		13				
Date: 12/12/07	Blank		8.1		23.0		0.22		8.1			
WQ Time: 1327	100		7.9		23.2		0.58		7.9			
Replicate: WQ SURV												
Technician: AEL												
48 Hours	Control	6		11		5		10				
Date: 12/14/07	Blank		8.1		23.2		0.21		8.3			
WQ Time: 1130	100		8.6		23.0		0.56		8.1			
Replicate: SURV												
Technician: NS												
72 Hours	Control	1		8A		6		13				
Date: 12/13/07 12/14/07	Blank		8.5		21.6		0.19		8.0			
WQ Time: 1135 1132	100		8.2		21.8		0.36		7.9			
Replicate: SURV SURV												
Technician: NS AEL												
96 Hours	Control	6		6		5		13				
Date: 12/15/07	Blank		8.4		22.0		0.23		8.4			
WQ Time: 1332 1400	100		8.3		21.6		0.69		8.2			
Replicate: WQ SURV												
Technician: AB												

*water quality performed on stock dilutions on Day 0.

Start Time:	1724 KS
End Time:	1640 AB
Supplier:	Aquatic Indicators
Organism Batch:	BI9211 Age: 9 days

Dilution Water Batch:	DMW 362
Hobo Temp. No.:	119280
Test Location:	rm 5
Test Acceptability: <input checked="" type="checkbox"/> ≥ 90% Survival in Control	

- ① WC 12/12/07 AEL
- ② WC 12/14/07 NS
- ③ WC 12/14/07 AEL



Hyalella 96-Hour Acute Toxicity Test

BIO078

Weston Test ID: 071201.1678	Client: County of SD + Copermitted	Client Sample ID: AHC mls aeration
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Survival Data									
Conc.	Rep	24 Hours		48 Hours		72 Hours		96 Hours	
		Date: 12/12/07		Date: 12/13/07		Date: 12/14/07		Date: 12/15/07	
		Time: 1000		Time: 1323		Time: 1050		End Time: 1640	
		Tech: UM		Tech: EB		Tech: UM		Tech: EB	
		# Alive	# Dead	# Alive	# Dead	# Alive	# Dead	# Alive	# Dead
Control	1								
	2								
	3								
	4								
blank	1	5	0	5	0	5	0	5	0
	2	5	0	5	0	5	0	5	0
	3	5	0	5	0	5	0	5	0
	4	5/5	0/0	5/5	0/0	5/5	0/0	5/5	0/0
100	1	5	0	4	1	4	0	3	1
	2	5	0	5	0	5	0	5	0
	3	5	0	5	0	5	0	5	0
	4	5/5	0/0	5/5	0/0	5/5	0/0	3/3	2/2
	1								
	2								
	3								
	4								
	1								
	2								
	3								
	4								

FEEDING INFORMATION					
Day 0 (0 Hours)			48 Hours		
Date: 12/11/07	Time: 1800	Initials: EB	Date: 12/13/07	Time: 1515	Initials: NS



Hyalella 96-Hour Acute Toxicity Test

BIO078

Client:	County of SD + Copermittes
Project:	TIE
Client Sample ID:	AHC-MLS Carboxylesterase
WESTON Test ID:	C071201.1678
Species:	Hyalella azteca

Date Received:	12/11/07
Date Test Started:	12/11/07
Date Test Ended:	12/15/07
Study Director:	A. Margolis
# Organisms/Chamber:	5

	Conc.	meter #	D.O. (mg/L)	meter #	Temp (°C)	meter #	Cond. (mS/cm)	meter #	pH	Hardness (mg/L CaCO ₃)	Alkalinity (mg/L CaCO ₃)	Chlorine (mg/L)
Day 0 (0 Hours)	Control	6		8A		6		13				
Date: 12/11/07	Blank		8.4		22.5		0.18		8.4			
Sample ID: C071201.169	100		8.9		22.5		0.60		7.8			
Dilutions (Tech): AM												
WQ* Time: 1540												
Technician: NS												
24 Hours	Control	1	—	9A	—	6	0.22	13	—			
Date: 12/12/07	Blank		8.7		22.9		0.70		8.0			
WQ Time: 1330	100		8.3		22.8		0.70		7.8			
Replicate: WQ SURF												
Technician: AEL												
48 Hours	Control	6		11		5		10				
Date: 12/13/07	Blank		8.4		23.5		0.23		8.3			
WQ Time: 1140	100		8.1		23.9		0.68		8.0			
Replicate: SURF												
Technician: NS												
72 Hours	Control	1		8A		6		13				
Date: 12/14/07	Blank		8.9		21.6		0.20		8.2			
WQ Time: 1148	100		8.6		22.0		0.73		8.0			
Replicate: SURF												
Technician: AEL												
96 Hours	Control	6		6		5		10				
Date: 12/15/07	Blank		8.3		21.7		0.27		8.4			
WQ Time: 1515	100		8.0		21.9		0.73		8.1			
Replicate: WQ SURF												
Technician: AS												

*water quality performed on stock dilutions on Day 0.

Start Time:	1705 AL
End Time:	1545 AC
Supplier:	Aquatic Indicators
Organism Batch:	AJ 9211 Age: 9 days

Dilution Water Batch:	DMW 362
Hobo Temp. No.:	119280
Test Location:	rm5
Test Acceptability: X ≥ 90% Survival in Control	

① WC AEL 12/12/07



Hyaella 96-Hour Acute Toxicity Test

BIO078

Weston Test ID: 0071201.1678	Client: County of San Bernardino	Client Sample ID: AHC-MLS Carboxylesterase
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Survival Data									
Conc.	Rep	24 Hours		48 Hours		72 Hours		96 Hours	
		Date: 12/12/07		Date: 12/13/07		Date: 12/14/07		Date: 12/14/07	
		Time: 1135		Time: 1401		Time: 1210		End Time: 1545	
		Tech: am		Tech: EB		Tech: am		Tech: AL	
		# Alive	# Dead	# Alive	# Dead	# Alive	# Dead	# Alive	# Dead
Control	1								
	2								
	3								
	4								
blank	1	5	0	5	0	5	0	5	0
	2	5	0	5	0	5	0	5	0
	3	5	0	5	0	5	0	5	0
	4	5	0	5	0	5	0	5	0
100	1	5	0	4	0 (INB)	5	0 PB	4	1
	2	5	0	4	0 (INB)	5	0 PB	3	2
	3	5	0	3	1 (INB)	4	0 PB	3	1
	4	5	0	5	0 (INB)	5	0 PB	3	2
	1								
	2								
	3								
	4								
	1								
	2								
	3								
	4								

FEEDING INFORMATION					
Day 0 (0 Hours)			48 Hours		
Date: 12/11/07	Time: 1800	Initials: EB	Date: 12/13/07	Time: 1515	Initials: MS

0 WC 12/12/07 am



Hyalella 96-Hour Acute Toxicity Test

BIO078

Client:	County of SB + Copermittes
Project:	TIE
Client Sample ID:	AHC-MLS BSA
WESTON Test ID:	CO71201.1678
Species:	Hyalella azteca

Date Received:	12/11/07
Date Test Started:	12/11/07
Date Test Ended:	12/15/07
Study Director:	A. Margolis
# Organisms/Chamber:	5

	Conc.	meter #	D.O. (mg/L)	meter #	Temp (°C)	meter #	Cond. (mS/cm)	meter #	pH	Hardness (mg/L CaCO ₃)	Alkalinity (mg/L CaCO ₃)	Chlorine (mg/L)
Day 0 (0 Hours)	-Control	6		8a		6		13				
Date: 12/11/07	BLANK		8.6		22.3		0.18		8.4			
Sample ID: CO71201.16a	100		9.4		22.5		0.60		7.8			
Dilutions (Tech): CUM												
WQ* Time: 1535												
Technician: NS												
24 Hours	Control	1	—	9A	—	6	—	13	—			
Date: 12/12/07	Blank		9.1		23.0		0.23		7.9			
WQ Time: 1347	100		7.3		23.0		0.72		7.6			
Replicate: WQ SURR												
Technician: AEL												
48 Hours	Control	6		11		5		10				
Date: 12/13/07	Blank		8.2		23.0		0.23		7.4			
WQ Time: 1145	100		6.5		23.5		0.74		7.8			
Replicate: SURR												
Technician: NS												
72 Hours	Control	1		8A		6		13				
Date: 12/14/07	Blank		8.0		22.0		0.26		8.0			
WQ Time: 1156	100		7.9		23.0		0.75		7.8			
Replicate: SURR												
Technician: AEL												
96 Hours	Control	6		6		5		10				
Date: 12/15/07	Blank		7.8		22.0		0.29		8.3			
WQ Time: 1520	100		6.9		22.5		0.90		8.0			
Replicate: WQ SURR												
Technician: EB												

*water quality performed on stock dilutions on Day 0.

Start Time:	1710 EB
End Time:	1620 AL
Supplier:	Aquatic Indicators
Organism Batch:	AI 9211 Age: 9 days

Dilution Water Batch:	DMW 302
Hobo Temp. No.:	119280
Test Location:	rm 5
Test Acceptability:	X ≥ 90% Survival in Control

Weston Test ID: C071201.1678	Client: County of SD + Coopermittees	Client Sample ID: AHC-MLS BSA
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Survival Data									
Conc.	Rep	24 Hours		48 Hours		72 Hours		96 Hours	
		Date: 12/12/07		Date: 12/13/07		Date: 12/14/07		Date: 12/15/07	
		Time: 1145		Time: 1341		Time: 1230		End Time: 1420 1620	
		Tech: am		Tech: 9D		Tech: am		Tech: AL	
		# Alive	# Dead	# Alive	# Dead	# Alive	# Dead	# Alive	# Dead
Control	1								
	2								
	3								
	4								
blank	1	5	0	5	0	4	1	3	1
	2	5	0	5	0	5	0 am	3	2
	3	5	0	5	0	5	0 am	2	3
	4	5	0	5	0	5	0 am	2	3
100	1	5	0	4	1	4	0 am	3	0 (1NB)
	2	5	0	3	(2NB) 0	5	0 2FB	4	1
	3	4	0 NB	3	0 (1NB)	1	2	0	1
	4	5	0	5	0	5	0 1NB	4	2
	1								
	2								
	3								
	4								
	1								
	2								
	3								
	4								
	1								
	2								
	3								
	4								

FEEDING INFORMATION					
Day 0 (0 Hours)			48 Hours		
Date: 12/11/07	Time: 1800	Initials: EB	Date: 12/13/07	Time: 1515	Initials: NS

- 0 fuzzy material in chamber 12/14/07 am
- 0 IE 12/14/07 am
- 0 IE 12/15/07 AL



Hyalella 96-Hour Acute Toxicity Test

BIO078

Client:	County of San Coperniticos
Project:	TIE
Client Sample ID:	AHC-MLS MeOH eluate
WESTON Test ID:	C07201.1678
Species:	Hyalella azteca

Date Received:	12/11/07
Date Test Started:	12/12/07
Date Test Ended:	12/16/07
Study Director:	A. Margolis
# Organisms/Chamber:	5

	Conc.	meter #	D.O. (mg/L)	meter #	Temp (°C)	meter #	Cond. (mS/cm)	meter #	pH	Hardness (mg/L CaCO ₃)	Alkalinity (mg/L CaCO ₃)	Chlorine (mg/L)
Day 0 (0 Hours) Date: 12/12/07 Sample ID: C07201.166 Dilutions (Tech): Am WQ* Time: 1515 Technician: Am	Control	11	9.6	9A	22.5	6	0.20	13	8.1			
	Blank		9.6		22.4		0.20		8.2			
	2X		9.4		22.5		0.20		8.2			
24 Hours Date: 12/13/07 WQ Time: 1530 Replicate: SWCC Technician: NS	Control	1	6.6	8A	22.5	6	0.21	13	8.4			
	Blank		6.8		22.1		0.21		8.4			
	2X		6.9		22.2		0.21		8.4			
48 Hours Date: 12/14/07 WQ Time: 1135 Replicate: SWCC Technician: NS	Control	6	7.2	11	23.9	5	0.21	10	8.3			
	Blank		8.1		23.5		0.25		8.5			
	2X		7.1		23.9		0.20		8.3			
72 Hours Date: 12/15/07 WQ Time: 1300 Replicate: WQ SWW Technician: GB	Control	6	7.7	6	23.2	5	0.22	10	8.3			
	Blank		8.0		23.1		0.26		8.4			
	2X		7.0		23.3		0.21		8.3			
96 Hours Date: 12/16/07 WQ Time: 1640 Replicate: WQ SWW Technician: GB	Control	6	7.6	6	22.1	5	0.22	10	8.3			
	Blank		8.0		22.9		0.26		8.5			
	2X		7.0		22.8		0.21		8.3			

*water quality performed on stock dilutions on Day 0.

Start Time:	1445	Am
End Time:	1645	GB
Supplier:	Aquatic Indicators	
Organism Batch:	PI 9211	Age: 10 days

Dilution Water Batch:	DMW 362
Hobo Temp. No.:	119280
Test Location:	rm 5
Test Acceptability: <input checked="" type="checkbox"/> ≥ 90% Survival in Control	

① W 12/13/07 NS (pH 8.4)



Hyalella 96-Hour Acute Toxicity Test

BIO078

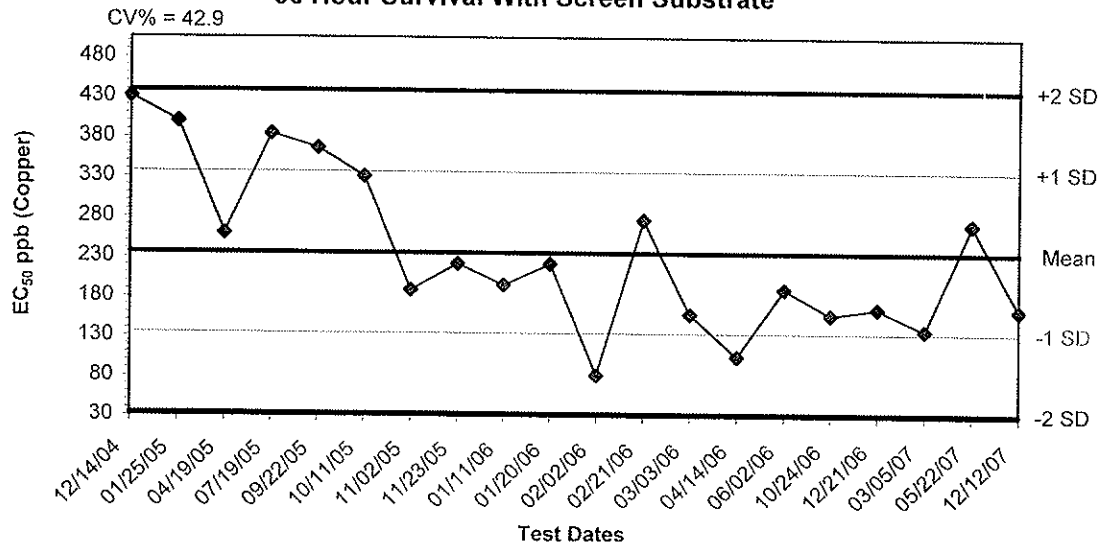
Weston Test ID: C0712.01.1678	Client: County of SD + Copermitts	Client Sample ID: AHC-MLS MeOH eluate
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Survival Data											
Conc.	Rep	24 Hours		48 Hours		72 Hours		96 Hours			
		Date: 12/13/07	Date: 12/14/07	Date: 12/15/07	Date: 12/16/07	Time: 1315	Time: 1145	Time: 1237	Time: 1645	Tech: EB	Tech: NS
		# Alive	# Dead	# Alive	# Dead	# Alive	# Dead	# Alive	# Dead		
Control	1	5	0	5	0	5	0	5	0		
	2	5	0	5	0	5	0	5	0		
	3	5	0	5	0	5	0	5	0		
	4/5	5/5	0/0	5/5	0/0	5/5	0/0	5/5	0/0		
Blank	1	5	0	5	0	5	0	4	1		
	2	5	0	5	0	5	0	5	0		
	3	5	0	5	0	5	0	5	0		
	4/5	5/5	0/0	5/5	0/0	5/5	0/0	5/5	0/0		
2X	1	5	0	5	0	5	0	4	1		
	2	5	0	5	0	5	0	5	0		
	3	5	0	5	0	5	0	5	0		
	4/5	5/5	0/0	5/5	0/0	5/5	0/0	5/5	0/0		
	1										
	2										
	3										
	4										
	1										
	2										
	3										
	4										

FEEDING INFORMATION					
Day 0 (0 Hours)			48 Hours		
Date: 12/12/07	Time: 1515	Initials: Am	Date: 12/14/07	Time: 1345	Initials: Am

① IE 12/14/07 EB

***Hyalella azteca* Reference Toxicant Control Chart:
96 Hour Survival With Screen Substrate**



Dates	Values	Mean	-1 SD	-2 SD	+1 SD	+2 SD
12/14/04	431.4190	235.9937	134.6394	33.2851	337.3480	438.7023
01/25/05	400.1000	235.9937	134.6394	33.2851	337.3480	438.7023
04/19/05	260.3740	235.9937	134.6394	33.2851	337.3480	438.7023
07/19/05	384.3600	235.9937	134.6394	33.2851	337.3480	438.7023
09/22/05	366.6700	235.9937	134.6394	33.2851	337.3480	438.7023
10/11/05	331.3500	235.9937	134.6394	33.2851	337.3480	438.7023
11/02/05	189.4130	235.9937	134.6394	33.2851	337.3480	438.7023
11/23/05	222.6600	235.9937	134.6394	33.2851	337.3480	438.7023
01/11/06	195.6220	235.9937	134.6394	33.2851	337.3480	438.7023
01/20/06	222.6030	235.9937	134.6394	33.2851	337.3480	438.7023
02/02/06	82.0310	235.9937	134.6394	33.2851	337.3480	438.7023
02/21/06	277.3900	235.9937	134.6394	33.2851	337.3480	438.7023
03/03/06	159.2020	235.9937	134.6394	33.2851	337.3480	438.7023
04/14/06	105.6500	235.9937	134.6394	33.2851	337.3480	438.7023
06/02/06	191.1800	235.9937	134.6394	33.2851	337.3480	438.7023
10/24/06	158.1360	235.9937	134.6394	33.2851	337.3480	438.7023
12/21/06	166.4600	235.9937	134.6394	33.2851	337.3480	438.7023
03/05/07	138.8230	235.9937	134.6394	33.2851	337.3480	438.7023
05/22/07	272.2640	235.9937	134.6394	33.2851	337.3480	438.7023
12/12/07	164.1670	235.9937	134.6394	33.2851	337.3480	438.7023

Updated 01/03/08 AM

Hyalella Acute Test-96 Hr Survival

Start Date: 12/12/2007 15:53 Test ID: C060525.203 Sample ID: REF-Ref Toxicant
 End Date: 12/16/2007 16:10 Lab ID: CCA-Weston, Carlsbad Sample Type: CUSO-Copper sulfate
 Sample Date: Protocol: EPA 00-EPA Freshwater Sed Test Species: HA-Hyalella azteca
 Comments:

Conc-ppb	1	2	3	4
Control	1.0000	1.0000	1.0000	1.0000
62.5	1.0000	0.9000	0.7000	1.0000
125	0.8000	0.9000	0.7000	0.7000
250	0.1000	0.1000	0.1000	0.2000
500	0.0000	0.0000	0.0000	0.0000
1000	0.0000	0.0000	0.0000	0.0000

Conc-ppb	Mean	N-Mean	Transform: Untransformed				Rank Sum	1-Tailed Critical	Mean	N-Mean
			Mean	Min	Max	CV%				
Control	1.0000	1.0000	1.0000	1.0000	1.0000	0.000	4	1.0000	0.0000	
62.5	0.9000	0.9000	0.9000	0.7000	1.0000	15.713	4	0.9000	0.1000	
*125	0.7750	0.7750	0.7750	0.7000	0.9000	12.354	4	0.7750	0.2250	
*250	0.1250	0.1250	0.1250	0.1000	0.2000	40.000	4	0.1250	0.8750	
500	0.0000	0.0000	0.0000	0.0000	0.0000	0.000	4	0.0000	1.0000	
1000	0.0000	0.0000	0.0000	0.0000	0.0000	0.000	4	0.0000	1.0000	

Auxiliary Tests

	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.91698	0.844	-0.6802	1.6084
Equality of variance cannot be confirmed				

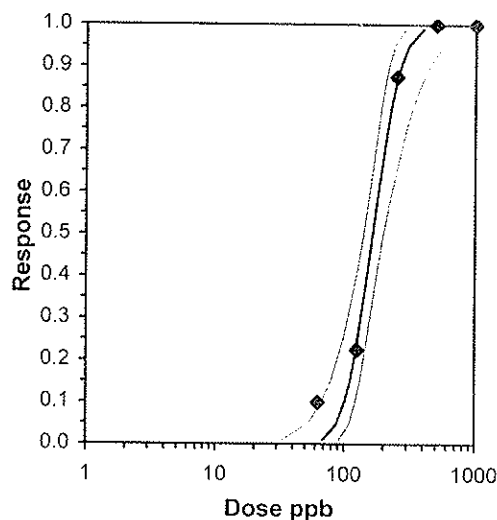
Hypothesis Test (1-tail, 0.05) NOEC LOEC ChV TU

Steel's Many-One Rank Test	62.5	125	88.3883	
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Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	6.12917	1.43753	3.31161	8.94672	0	1.56929	7.81473	0.67	2.21529	0.16315	7
Intercept	-8.5779	3.16123	-14.774	-2.3818							
TSCR											

Point	Probits	ppb	95% Fiducial Limits	
EC01	2.674	68.5067	32.9579	91.4653
EC05	3.355	88.4956	52.3177	110.288
EC10	3.718	101.437	66.6279	122.415
EC15	3.964	111.221	78.1605	131.801
EC20	4.158	119.666	88.4463	140.225
EC25	4.326	127.421	98.0122	148.38
EC40	4.747	149.263	124.159	174.952
EC50	5.000	164.167	140.076	197.403
EC60	5.253	180.56	155.357	226.573
EC75	5.674	211.511	179.668	292.624
EC80	5.842	225.217	189.272	325.721
EC85	6.036	242.318	200.632	369.942
EC90	6.282	265.691	215.349	435.316
EC95	6.645	304.545	238.373	555.91
EC99	7.326	393.405	286.719	884.639



Test: HA-Hyalella Acute Test · Test ID: C060525.203 ·
 Species: HA-Hyalella azteca · Protocol: EPA 00-EPA Freshwater Sediment (Mod) ·
 Sample ID: REF-Ref Toxicant · Sample Type: CUSO-Copper sulfate ·
 Start Date: 12/12/2007 15:53 · End Date: 12/16/2007 16:10 Lab ID: CCA-Weston, Carlsbad .

Pos	ID	Rep	Group	Start	24 Hr	48 Hr	72 Hr	96 Hr	Notes
	1	1	Control	10				10	
	2	2	Control	10				10	
	3	3	Control	10				10	
	4	4	Control	10				10	
	5	1	62.500	10				10	
	6	2	62.500	10				9	
	7	3	62.500	10				7	
	8	4	62.500	10				10	
	9	1	125.000	10				8	
	10	2	125.000	10				9	
	11	3	125.000	10				7	
	12	4	125.000	10				7	
	13	1	250.000	10				1	
	14	2	250.000	10				1	
	15	3	250.000	10				1	
	16	4	250.000	10				2	
	17	1	500.000	10				0	
	18	2	500.000	10				0	
	19	3	500.000	10				0	
	20	4	500.000	10				0	
	21	1	1000.000	10				0	
	22	2	1000.000	10				0	
	23	3	1000.000	10				0	
	24	4	1000.000	10				0	

Comments:



96-Hour *Hyaella*
Reference Toxicant Test

BIO078

Test ID: <i>CO60525.203</i>	Associated Test(s): <i>S.D. Stormwater TIE</i>	Study Director: <i>J. Hansen</i>
Organism Batch: <i>AZ 9211</i>	Location: <i>Room 5</i>	Replicates: <i>4</i>
Start Time: <i>1553</i>	Initials: <i>AL/HH</i>	End Time: <i>1610</i>
		No. of Organisms: <i>10</i>
		Initials: <i>EB</i>

Toxicant: Copper Sulfate (0.509gCu/LCuSO ₄)	Toxicant Lot Number: <i>1605565</i>	Toxicant Stock Preparation Date: <i>7/20/07</i>				
Serial Dilute by 1/2 to obtain concentrations of 1000, 500, 250, 125, and 62.5 ppb.						
Date Prepared	Day	Target Conc.	A. Toxicant (target) B. Toxicant (actual)	A. Diluent (target) B. Diluent (actual)	Dilution Water Batch	Tech.
<i>12/12/07</i>	<i>0</i>	<i>1000 ppb</i>	(A) <i>3.932 mL</i> (B) <i>3.9319 mL</i>	(A) <i>2000 mL</i> (B) <i>2000.0 mL</i>	<i>DMW 262</i>	<i>EA</i>

Day 0 Water Quality Data						
Stock						
Date: <i>12/12/07</i>	Time: <i>1315</i>		Initials: <i>AL</i>			
	Control	62.5	125	250	500	1000
D.O. (mg/L)	<i>8.4</i>	<i>8.4</i>	<i>8.5</i>	<i>8.6</i>	<i>8.6</i>	<i>8.6</i>
Temp. (°C)	<i>23.7</i>	<i>23.6</i>	<i>23.7</i>	<i>23.4</i>	<i>23.5</i>	<i>24.1</i>
Conductivity	<i>0.19</i>	<i>0.19</i>	<i>0.19</i>	<i>0.19</i>	<i>0.19</i>	<i>0.20</i>
pH	<i>8.2</i>	<i>8.3</i>	<i>8.3</i>	<i>8.3</i>	<i>8.2</i>	<i>8.2</i>

Day 4 (96-Hour) Water Quality Data						
Date: <i>12/16/07</i>	Time: <i>1640</i>		Replicate: <i>1</i>	Initials: <i>EB</i>		
	Control	62.5	125	250	500	1000
D.O. (mg/L)	<i>8.0</i>	<i>7.9</i>	<i>7.9</i>	<i>8.0</i>	<i>8.0</i> →	
Temp. (°C)	<i>22.1</i>	<i>22.2</i>	<i>22.0</i>	<i>21.9</i>		
Conductivity	<i>0.20</i>	<i>0.20</i>	<i>0.20</i>	<i>0.20</i>		
pH	<i>8.3</i>	<i>8.3</i>	<i>8.2</i>	<i>8.3</i>		

Pass

Fail

Notes:

① WQ not taken due to animal mortality in all reps. 1/3/08 EB

SURVIVAL DATA

Test ID: C060525203									
Conc. (ppb)	Rep	Day 1		Day 2		Day 3		Day 4	
		Date: 12/13/07		Date: 12/14/07		Date: 12/15/07		Date: 12/16/07	
		Time: 1600		Time: 1145		Time: 1145		End Time: 1610	
		Technician: AL/NS		Technician: JH		Technician: EB		Technician: EB	
		# Alive	# Dead	# Alive	# Dead	# Alive	# Dead	# Alive	# Dead
Control	1	10	0	10	0	10	0	10	0
	2	10	0	10	0	10	0	10	0
	3	10	0	10	0	10	0	10	0
	4	10	0	10	0	10	0	10	0
62.5	1	10	0	10	0	10	0	10	0
	2	10	0	10	0	10	0	9	1
	3	10	0	10	0	10	0	7	3
	4	10	0	10	0	10	0	10	0
125	1	10	0	9	1	8	1	8	0
	2	10	0	10	0	10	0	9	1
	3	10	0	9	0 (INB)	9	0	7	2
	4	9	1	9	0	9	0	7	2
250	1	10	0	8	2	4	3 (INB)	1	(A)
	2	10	0	10	0	5	5	1	(A)
	3	10	0	10	0	6	4	1	(5)
	4	10	0	8	2	6	2	2	(4)
500	1	7	3	0	7				
	2	7	3	2	5	0	2		
	3	7	3	2	5	0	2		
	4	6	4	0	6				
1000	1	2	8	0	2				
	2	3	6 (INB)	0	4 (JH)				
	3	0	10						
	4	3	7	1	2	0	1		

Feeding Information*	Day 0	Day 2 (48-Hours)
Date:	12/12/07	12/14/07
Time:	1641	1345
Technician:	AEL	AM

*Organisms should get fed 1 mL wheat grass slurry per test chamber at test initiation and at 48 hours.

0 Found dead body 12/14/07 JH