Nautilus Environmental

Results of Wet Weather Chronic Toxicity Testing for the City of El Monte

❖ Sample Collection Date: November 21, 2016

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Data Quality Assurance:

- Nautilus Environmental is accredited in accordance with NELAP by the State of Oregon Environmental Laboratory Accreditation Program (Certificate No. 4053). It is also certified by the State of California Water Resources Control Board Environmental Laboratory Accreditation Program (Certificate No. 1802) and the State of Washington Department of Ecology (Lab ID C552). Specific fields of testing applicable to each accreditation are available upon request.
- All data have been reviewed and verified.
- All test results have met minimum test acceptability criteria under their respective EPA protocols, unless otherwise noted in this report.
- All test results have met internal Quality Assurance Program requirements.

Results verified by: Adrienne Cibar

Introduction

A chronic toxicity screening test was performed on a sample from the City of El Monte, California to evaluate the impact of storm water runoff to the water flea (*Ceriodaphnia dubia*). Due to reproductive effects observed to *C. dubia* in a previous storm sample collected from this location, a toxicity identification evaluation (TIE) test was conducted concurrently with the screening test. Sampling and testing was conducted to satisfy requirements in the Los Angeles Region MS4 permit (Order No. R4-2012-0175). The sample was collected during a storm event that occurred on November 21, 2016. The toxicity screening test was conducted at Nautilus Environmental (Nautilus) in San Diego, California between November 22 and 28, 2016.

Materials and Methods

Test Material

Test material consisted of one storm water sample. Collection was conducted under the direction of Mr. Michael Kolbenschlag of AEI-CASC Consulting (AEI-CASC). The sample was collected by AEI-CASC personnel and hand delivered to Nautilus.

Upon arrival at Nautilus, an aliquot of the sample was drawn and water quality parameters of pH, dissolved oxygen (DO), conductivity, salinity, alkalinity, hardness, and temperature were measured and recorded. The sample was stored in the dark at 4°C until used for testing. A summary of the sample collection and receipt times is provided in Table 1.

Table 1. Sample Collection and Receipt Times

Sample ID	Sample Collection Date; Time	Sample Receipt Date; Time
Outfall #6 (LL)	11/21/16; 01:00	11/21/16; 18:00

Chronic Toxicity Screen Methods

Toxicity testing was conducted in accordance with methods published in USEPA 2002. General test specifications are summarized in Table 2.

In accordance with permit requirements, chronic toxicity test biological endpoint data was analyzed using the Test of Significant Toxicity (TST) t-test approach specified in the USEPA NPDES TST Implementation Document (USEPA 2010). For this monitoring program, the critical chronic in-stream waste concentration (IWC) is set at 100 percent sample (i.e. no dilution). A pass/fail result is reported per the TST method comparing the 100 percent sample to the lab control. The TST method applies a modified t-test that takes into account both the statistical power of the test and magnitude of biological effects in determining the presence of a response; results are reported as “Pass” if a sample is considered non-toxic according to the TST calculation, or “Fail” if considered toxic according to TST.

Table 2. Chronic Water Flea Test Specifications

Test Start Date, Time:	11/22/16, 15:00
Test End Date, Time:	11/28/16, 14:25
Test Organism:	*Ceriodaphnia dubia* (water flea)
Test Organism Source; Age:	In-house culture; < 24 hours
Lab Control Water:	EPA diluted mineral water (80% Nanopure DI and 20% Perrier®)
Test Concentrations:	100 percent sample[a]; lab control
Endpoints/Protocol:	Survival and Reproduction/ EPA/821/R-02-013, USEPA 2002
Acceptability Criteria:	Mean control survival ≥ 80%; ≥ 60% of surviving females producing 3 or more broods. Mean number of offspring ≥ 15 per surviving female.
Statistical Analysis Software/ Analysis:	USEPA TST Calculator Tool v. 1.8. The reference toxicant test was analyzed using the Comprehensive Environmental Toxicity Information System™ (CETIS) software by Tidepool Scientific Software.

Toxicity Identification Evaluation (TIE) Methods

Select Phase 1 TIE treatments were conducted on the sample concurrently with the toxicity screening test. The TIE treatments were initiated due to toxicity observed in a previous storm water sample collected at this location. The TIE was initiated concurrently with the screening test in order to proactively target the cause of toxicity; often if TIE treatments are performed after the results from the initial 7-day screen are reported, the toxic signal in the sample has been lost or diminished and the TIE data are not useful. This targeted TIE approach is based on previous experience with storm water samples collected from urban areas, and other monitoring guidance based on the MS4 permit.

TIE treatments applied to the sample included solid-phase extraction (SPE), filtration (0.45 μm), and ethylenediaminetetraacetic acid (EDTA) addition treatments targeting non-polar organic, particulates, and metals, respectively. Phase 1 TIE procedures followed methods outlined in USEPA TIE guidance manuals (USEPA 1991 and 1992). The targeted TIE sample manipulations and which compounds they address are outlined in Table 3.

Table 3. TIE Sample Manipulation Procedures and Factors Addressed by TIE Procedures

Procedure	Compounds/ Confounding Factors Addressed
Baseline	None. Used for treatment effectiveness comparison
C8 Column Solid-Phase Extraction (SPE)	Removes non-polar organics and some cationic metals
EDTA addition (5 mg/L)	Reduces toxicity of cationic metals by chelation
Filtration (0.45 μm)	Pollutants associated with particles

Results

Toxicity Screening

There was no statistically significant effects observed in *C. dubia* survival for the Outfall #6 sample; however, a 41 percent effect from the lab control was observed in reproduction, which failed according to the TST statistical analysis. A summary of test results for the undiluted sample is presented in Table 4. Complete statistical analyses and raw bench datasheets are provided in Appendix A. Water quality measurements recorded at sample check-in and a copy of the chain of custody form and are provided in Appendix B and C, respectively.

Table 4. Summary of *C. dubia* Survival and Reproduction Results

Sample ID (100% sample)	Mean Percent Survival	PE (%)	TST Result (Pass/Fail)	Mean Reproduction (# neonates)	PE (%)	TST Result (Pass/Fail)
Lab Control	100	-	-	28.4	-	-
Outfall #6 (LL)	90.0	10	Pass	16.7	41.2	Fail

N/A = not applicable

TST analysis is not appropriate for the *C. dubia* chronic survival endpoint because the test design includes only one organism per replicate. Therefore, the result for the chronic survival endpoint is based on percent effect (PE) compared to control calculated as: ((mean response in control - mean response in sample)/mean response in control) *100. A negative PE indicates better organism performance in the sample compared to that in the control. For the 7-day survival endpoint, the TST result is considered a Pass (non-toxic) if PE <25 and a Fail (toxic) if PE ≥ 25.

TST: Pass = sample is non-toxic according to the TST calculation; Fail = sample is toxic according to the TST calculation

TIE Results

Survival was between 90 and 100 percent in all controls and sample treatments. Since there was no statistically significant effect in survival, the remainder of this discussion will focus on the reproductive endpoint.

The average number of offspring produced in the unmanipulated baseline Outfall #6 LL sample was 15.7, compared to 28.4 in the lab control; a 41.2 percent effect. Mean reproduction in the filtered sample was similar to the baseline indicating that the decrease in reproduction in the sample was not caused by particulate matter. The C8 SPE treatment improved reproduction somewhat, and EDTA addition completely removed toxicity in the sample, increasing the average *C. dubia* reproduction in the treated sample nearly equal to that in the lab control. This indicates that divalent cationic metals such as copper and zinc are the likely cause for reduced reproduction of *C. dubia* exposed to the sample. These metals are common constituents in urban storm water runoff. Mean reproduction results for TIE treatments of the Outfall #6 sample are shown in Figure 1 and are also available in Appendix D.

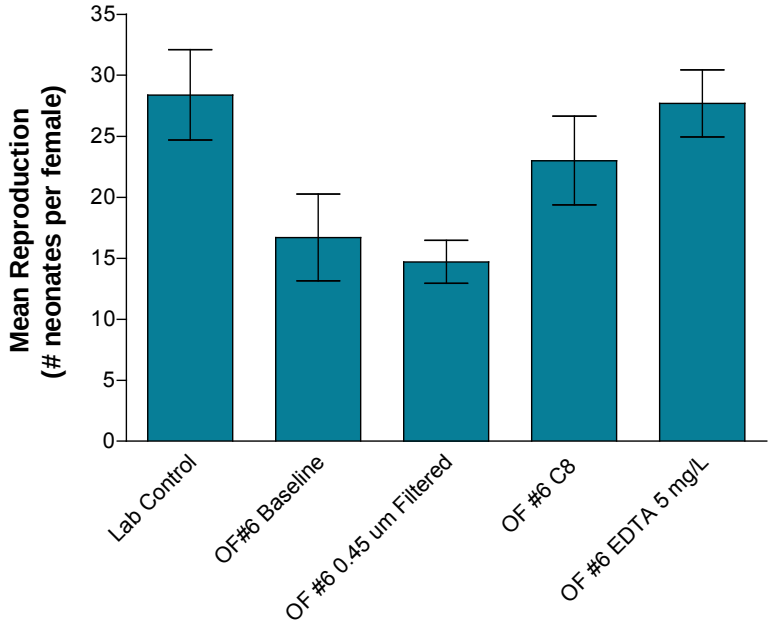


Figure 1. Percent *C. dubia* reproduction results in baseline and TIE treated Outfall (OF) #6 sample collected November 21, 2016 (mean ± standard error).

Quality Assurance

The sample was received under appropriate conditions and the test was initiated 38 hours after sample collection; within the maximum allowable 72-hour holding time. Mean control responses met minimum acceptability criteria. Appropriate alpha levels were used for statistical analyses according to the TST Implementation Document guidelines (USEPA 2010). A list of laboratory qualifier codes used on raw data sheets is available in Appendix D.

Results for the monthly reference toxicant test used to monitor laboratory performance and test organism sensitivity are summarized in Table 5. A ll test acceptability criteria were met. Additionally, the median lethal concentration (LC_{50}) for survival and the median effect concentration (EC_{50}) for reproduction were within two standard deviations of the mean; this indicates that the test organisms exhibited typical sensitivity to copper as that historically observed in the Nautilus laboratory. The reference toxicant test control charts for the past 20 reference toxicant tests conducted at Nautilus are presented in Appendix E.

Table 5. Summary of Statistical Results for the Reference Toxicant Test

Test Endpoint	NOEC (µg/L copper)	LC_{50}/EC_{50} (µg/L copper)	Historical LC_{50}/EC_{50} ± 2 SD (µg/L copper)
Survival	50	61.9	61.7 ± 15.3
Reproduction	50	71.8	59.5 ± 24.7

NOEC = the highest concentration tested that results in no observed effect.

LC_{50}/EC_{50} = concentration expected to cause mortality or an adverse effect to 50 percent of the test organisms.

Historical LC_{50}/EC_{50} ± 2 SD = the mean LC_{50} or EC_{50} from the previous 20 tests performed by Nautilus, plus or minus two standard deviations.

References

Tidepool Scientific Software. 2000-2013. CETIS Comprehensive Environmental Toxicity Information System Software, Version 1.8.7.20.

USEPA. 1991. Methods for Aquatic Toxicity Identification Evaluation - Phase I Toxicity Characterization Procedures, 2nd Edition, EPA/600/6-91/003 February 1991.

USEPA. 1992. Toxicity Identification Evaluation - Characterization of Chronically Toxic Effluents, Phase I. EPA/600/6-91/005F May 1992.

USEPA. 2000. Understanding and Accounting for Method Variability in Whole Effluent Toxicity Applications under the National Pollutant Discharge Elimination System, (EPA-R-00-003). USEPA Office of Wastewater Management, Washington DC. June 2000.

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). USEPA Office of Water, Washington DC.

USEPA. 2010. National Pollutant Discharge Elimination System Test of Significant Toxicity Implementation Document. EPA/833/R-10/003. June 2010.

USEPA, TST Spreadsheet Tool. Version 1.8

Appendix A

Raw Data and Statistical Summaries

AEI-CASC/ City of El Monte
Chronic *Ceriodaphnia* Survival and Reproduction TIE
Sample: LL, Outfall #6
Sample Collection Date: 11/21/16
Test Initiation Date: 11/22/16

Treatment (100%)	Number Alive (neonates)	Mean Survival (%)	Number of Young (neonates)	Mean Number Per Adult (neonates)	Percent Effect
Lab Control	1 1 1 1 1 1 1 1 1 1	100	8 23 39 14 36 39 18 34 40 33	28.4	--
100% Baseline	0 1 1 1 1 1 1 1 1 1	90.0	0 7 14 8 26 29 26 4 29 24	16.7	41.2
100% 0.45 μm Filtered	1 1 1 1 1 1 1 1 1 1	100	18 7 17 5 19 23 11 15 15 17	14.7	48.2
100% C8 SPE	1 1 1 1 1 1 1 1 1 1	100	34 5 17 17 38 35 9 18 32 25	23.0	19.0
100% EDTA 5 mg/L	1 1 1 1 1 0 1 1 1 1	90.0	36 25 31 26 29 5 31 28 35 31	27.7	2.46

AEI-CASC/ City of El Monte
Chronic *Ceriodaphnia* Survival and Reproduction TIE
Method Contols
Test Initiation Date: 11/22/16

Treatment	Number Alive (neonates)	Mean Survival (%)	Number of Young (neonates)	Mean Number Per Adult (neonates)
0.45 μm Filtration Control	1	100	40	36.4
	1		32	
	1		34	
	1		35	
	1		44	
	1		38	
	1		37	
	1		31	
	1		39	
	1		34	
C8 SPE Control	1	100	3	32.4
	1		34	
	1		34	
	1		41	
	1		41	
	1		32	
	1		34	
	1		32	
	1		42	
	1		31	
EDTA 5 mg/L Control	1	100	35	31.0
	1		17	
	1		30	
	1		30	
	1		37	
	1		33	
	1		31	
	1		33	
	1		34	
	1		30	

Freshwater Chronic Bioassay

Daphnid Survival and Reproduction Datasheet

Test Species: ***C. dubia***

Client/Sample ID: AEI-CASC/ TIE Method Controls

Start Date/Time: 11/22/2016 1500

Test Number: 1611-S229, S231

End Date/Time: 11/28/16 1455

Conc.	Rep	Daily Reproduction/ Survival 1	2	3	4	5	6	7	8	Total	QC
Filt Cont	1	0	0	0	8	14	18			40	
	2	0	0	0	6	10	16			32	
	3	0	0	0	5	14	15			34	
	4	0	0	0	7	13	15			35	
	5	0	0	7	0	15	22			44	22
	6	0	0	6	11	0	21			38	
	7	0	0	0	5	14	18			37	
	8	0	0	0	4	11	16			31	
	9	0	0	7	0	12	20			39	
	10	0	0	5	0	11	18			34	
	Tech:	AC	AD	AC	AD	AP	EQ				JH

Conc.	Rep	Daily Reproduction/ Survival 1	2	3	4	5	6	7	8	Total	QC
C8 Cont	1	0	0	0	3	0	Ⓒ10			3	
	2	0	0	0	5	9	20			34	
	3	0	0	0	6	12	16			34	
	4	0	0	0	7	14	20			41	
	5	0	0	6	Ⓑ20	12	23			41	
	6	0	0	4	12	0	16			32	
	7	0	0	0	5	10	19			34	
	8	0	0	0	6	10	16			32	16
	9	0	0	7	0	15	20			42	
	10	0	0	0	5	9	17			31	

Conc.	Rep	Daily Reproduction/ Survival 1	2	3	4	5	6	7	8	Total	QC
EDTA Cont	1	0	0	0	5	11	19			35	
	2	0	0	0	2	9	6			17	6
	3	0	0	0	7	13	10			30	
	4	0	0	0	4	12	14			30	
	5	0	0	4	15	0	18			37	
	6	0	0	5	11	0	17			33	
	7	0	0	0	4	11	16			31	
	8	0	0	0	4	13	16			33	
	9	0	0	6	0	9	19			34	
	10	0	0	0	4	10	16			30	

Neonates for each replicate were blocked across concentrations at test initiation

Rep:	1	2	3	4	5	6	7	8	9	10
Board:	136			~~LIP~~ Ⓐ						→
Cup:	1	3	6	Ⓐ7 54	10	11	12	18	22	25
Rand # QC:	NA	Verified By:		√ KB	Initiated By:		AP	QC'd By:		CH

Time Fed/Test Solution Renewed (day):

(0) 1500 (1) 1720 (2) 1425 (3) 1325 (4) 1610 (5) 1745 (6) ~~1455~~ Ⓒ (7) —

Notes: d = dead; M = male; LIP = lost in progress; B = 4th brood (only the first 3 broods are included in total)

* = dead neonates observed, but only live neonate counts recorded Ⓐ CH Q18 11/22/16 Ⓑ AD Q18 11/26/16 Ⓒ EQ Q18 11/28/16

QC Check: KB 12/12/16

Final Review: AC 1/20/17

Freshwater Chronic Bioassay

Daphnid Survival and Reproduction Datasheet

Test Species: *C. dubia*

Client/Sample ID: AEI-CASC/ City of El Monte LL

Start Date/Time: 11/22/2016 1500

Test No: 1611-5231

End Date/Time: 11/28/16 1425

Conc.	Rep	Daily Reproduction/ Survival 1	2	3	4	5	6	7	8	Total	QC
LC	1	0	0	0	6	0	2			8	
	2	0	0	0	1	9	13			23	
	3	0	0	0	6	14	19			39	
	4	0	0	0	6	8	0			14	
	5	0	0	7	0	10	19			36	
	6	0	0	5	0	12	22			39	
	7	0	0	0	7	9	2#			18	
	8	0	0	0	6	13	15		34 ~~14~~	~~14~~ (E)	
	9	0	0	7	0	14	19			40	19
	10	0	0	6	0	12	15			33	
	Tech:	AC	AD	AC	AD	AD	EG				JH

Mean neonates/surviving female (for TAC): 29.4

Conc.	Rep	Daily Reproduction/ Survival 1	2	3	4	5	6	7	8	Total	QC
100 C8	1	0	0	0	5	13	16			34	
	2	0	0	0	2	3	0			5	
	3	0	0	0	7	10	0			17	
	4	0	0	0	4	13	0			17	
	5	0	0	0	6*	14	8			28	
	6	0	0	0	5	12	18			35	
	7	0	0	0	4	5	0			9	
	8	0	0	0	3	13	0			16	
	9	0	0	0	5	9	18			32	
	10	0	0	0	3	10	15			25	15

Conc.	Rep	Daily Reproduction/ Survival 1	2	3	4	5	6	7	8	Total	QC
100%	1	0	0	d/d	-	-	-	-		0/d	
	2	0	0	0	3	4 (C)	0			7	
	3	0	0	0	4	10	0			14	
	4	0	0	0	6	8	0			8	
	5	0	0	3 (B)	0	11	12			26	
	6	0	0	0	4	10	15		29 ~~19~~	~~19~~ (E)	
	7	0	0	0	4	9	13			26	13
	8	0	0	0	4	0 ~~(12)~~	0			4	
	9	0	0	0	4	12 (D)	13			29	
	10	0	0	0	4	8	12			24	

Conc.	Rep	Daily Reproduction/ Survival 1	2	3	4	5	6	7	8	Total	QC
100 EDTA	1	0	0	0	5	15	16			36	16
	2	0	0	0	3	7	15			25	
	3	0	0	0	6	12	13			31	
	4	0	0	0	5	3	16			26	
	5	0	0	4	8	0	17			29	
	6	0	0	4	d/d	—	—			5/0	
	7	0	0	0	5	11	15			31	
	8	0	0	0	4	10	14			28	
	9	0	0	2	0	13	20			35	
	10	0	0	3	0	11	17			31	

Conc.	Rep	Daily Reproduction/ Survival 1	2	3	4	5	6	7	8	Total	QC
100 Filt	1	0	0	0	2	16	0			18	
	2	0	0	0	1	6	0			7	
	3	0	0	0	5	12	0			17	
	4	0	0	0	0	5	0			5	0
	5	0	0	0	6	13	0			19	
	6	0	0	0	7	16	6			23	
	7	0	0	0	5	6	0			11	
	8	0	0	0	5	10	0			15	
	9	0	0	0	2	13	0			15	
	10	0	0	0	5	8	4			17	

Conc.	Rep	Daily Reproduction/ Survival 1	2	3	4	5	6	7	8	Total	QC
	1										
	2										
	3										
	4										
	5										
	6										
	7										
	8										
	9										
	10										

Neonates for each replicate were blocked across concentrations at test initiation

Rep:	1	2	3	4	5	6	7	8	9	10
Board:	126			~~13~~						→
Cup:	1	3	6	~~8~~ 54	10	11	12	18	22	25
Rand # QC:	NA	Verified By:	VKP	Initiated By:	AD	QC'd By:	CH			

Notes: d = dead; M = male; LIP = lost in progress;
B = 4th brood (only the first 3 broods are included in total)
* = dead neonates observed, but only live neonate counts recorded

Time Fed/Test Solution Renewed (day): (0) 1500 (1) ~~1700~~ (2) 1435 (3) 1345 (4) 1545 (5) 1635 (6) ~~1715~~ (P) (7) _____

Comments: (A) CH Q18 11/22/16 (B) adult and neonates twitching on bottom. (C) MD Q18 11/27/16 (D) EG Q18 11/28/16 (E) Q18 AC 1/20/17

QC Check: KB 12/12/16

Final Review: AC 1/20/17

Freshwater Chronic Bioassay

Water Quality Measurements

Client: AEI-CASC

Test Species: *C. dubia*

Sample ID: TIE Method Controls

Start Date/Time: 11/22/2016 1500

Test No: 1611-S229, S231

End Date/Time: 11/28/16 1455

Concentration	0.45 um Filtration Control							
Day	0	1	2	3	4	5	6	7
Initial								
pH	7.90	8.00	7.94	8.12	7.94	7.95	8.10	
DO (mg/L)	6.7	8.0	8.6	8.5	8.8	8.9	9.0	
Cond. (µmhos/cm)	1952	1193	192	192	193	193	194	
Temp (°C)	24.6	25.7	25.6	24.5	25.8	26.0	24.7	
Final								
pH		8.08	8.22	8.09	8.18	8.16	8.20	—
DO (mg/L)		7.7	8.1	7.8	7.9	8.2	8.5	—
Temp (°C)		25.3	25.9	24.8	24.6	25.5	25.3	—

Concentration	C8 SPE Control							
Day	0	1	2	3	4	5	6	7
Initial								
pH	7.95	8.03	7.93	8.13	8.05	8.00	8.07	
DO (mg/L)	7.4	8.4	8.8	8.6	8.6	8.7	9.0	
Cond. (µmhos/cm)	~~1902~~	202	194	197	198	197	199	194
Temp (°C)	25.9	25.7	25.6	24.3	25.8	25.9	24.9	
Final								
pH		8.09	8.12	8.00	8.03	8.08	8.03	—
DO (mg/L)		7.8	7.6	7.4	7.3	8.0	8.1	—
Temp (°C)		25.3	25.9	24.8	24.6	25.5	25.3	—

Concentration	EDTA 5 mg/L Control							
Day	0	1	2	3	4	5	6	7
Initial								
pH	7.98	8.02	7.97	8.08	8.01	8.00	8.06	
DO (mg/L)	7.7	7.8	8.3	8.0	8.4	8.5	8.7	
Cond. (µmhos/cm)	191	193	192	198	194	194	191	
Temp (°C)	24.7	25.5	25.5	24.6	25.3	26.0	25.2	
Final								
pH		8.01	8.23	8.19	8.20	8.18	8.24	—
DO (mg/L)		7.6	8.0	7.8	7.7	8.0	8.5	—
Temp (°C)		25.3	25.9	24.8	24.6	25.5	25.3	—

	0	1	2	3	4	5	6	7
Analysts: Initial:	AD	[illegible]	AD	EG	AD	AD	EG	
Final:		AD	AD	EG	AD	AD	EG	—
Dilutions made by:	PA	[illegible]	AC	EG	AD	AD	EG	
Sample Used (A, B, C):	—	—	—	—	—	—	—	

Comments: RAD 0/8 11/22/16

Animal Source/Date Received: Internal / NA

Animal Age at Initiation: <24h

Sample Log-in Numbers: A: — B: — C: —

QC Check: KB 12/2/16

Final Review: AC 1/20/17

Freshwater Chronic Bioassay

Water Quality Measurements

Client: AEI-CASC/City of El Monte

Sample ID: LL

Test No: 1611-S231

Test Species: *C. dubia*

Start Date/Time: 11/22/16 1500

End Date/Time: 11/28/16 1425

Lab Control								
Day	0	1	2	3	4	5	6	7
Initial								
pH	8.17	8.07	7.99	8.06	8.00	8.00	8.01	
DO (mg/L)	7.4	8.0	8.2	8.3	9.0	8.5	9.0	
Cond. (µmhos/cm)	190	194	194	197	199	195	193	
Temp (°C)	25.7	25.2	25.5	24.0	25.3	24.0	24.8	
Final								
pH		8.05	8.19	8.17	8.25	8.11	(C)	—
DO (mg/L)		7.6	8.1	8.2	8.0	8.2		—
Temp (°C)		24.7	24.1	24.0	24.2	25.3	25.0	—

100% C8 SPE								
Day	0	1	2	3	4	5	6	7
Initial								
pH	(A) 7.24 ~~6.97~~	7.30	7.19	7.02	7.15	7.03	6.91	
DO (mg/L)	7.5	8.0	7.6	8.2	8.3	6.2	7.4	
Cond. (µmhos/cm)	~~117~~ 113	113	114	113	112	112	108	
Temp (°C)	25.2	25.4	25.7	24.5	24.8	25.4	24.6	
Final								
pH		7.44	7.68	7.60	7.66	7.62	(C)	—
DO (mg/L)		7.2	8.1	7.9	8.1	8.2		—
Temp (°C)		24.7	24.1	24.0	24.2	25.3	25.0	—

100% Baseline								
Day	0	1	2	3	4	5	6	7
Initial								
pH	7.20	7.26	7.17	7.01	7.07	7.03	6.98	
DO (mg/L)	7.6	7.8	7.4	7.9	7.7	7.7	8.2	
Cond. (µmhos/cm)	111	113	113	112	114	114	113	
Temp (°C)	24.1	25.5	26.0	24.6	25.1	25.9	25.3	
Final								
pH		7.53	7.69	7.63	7.66	7.54	(C)	—
DO (mg/L)		7.7	8.1	7.7	8.0	8.2		—
Temp (°C)		24.7	24.1	24.0	24.2	25.3	25.0	—

100% EDTA 5 mg/L								
Day	0	1	2	3	4	5	6	7
Initial								
pH	(A) 7.14 ~~7.05~~	7.18	7.11	7.01	7.08	7.02	6.93	
DO (mg/L)	7.5	7.8	7.5	7.4	7.4	6.8	7.9	
Cond. (µmhos/cm)	110	112	112	113	114	114	114	
Temp (°C)	24.2	25.0	25.9	25.1	24.9	25.5	24.6	
Final								
pH		7.50	7.64	7.59	7.65	7.60	7.44	—
DO (mg/L)		7.5	8.0	7.8	8.0	8.2	8.4	—
Temp (°C)		24.7	24.1	24.0	24.2	25.3	25.0	—

100% Filtered								
Day	0	1	2	3	4	5	6	7
Initial								
pH	(A) 7.17 ~~6.95~~	7.19	7.08	7.05	6.97	6.96	7.03	
DO (mg/L)	7.5	8.4	8.6	8.9	8.9	8.9	8.4	
Cond. (µmhos/cm)	(A) ~~118~~ 115	115	120	120	115	112	113	
Temp (°C)	25.0	25.2	26.0	24.3	24.8	25.8	24.6	
Final								
pH		7.57	7.65	7.62	7.72	7.54	(C)	—
DO (mg/L)		7.5	8.0	7.9	8.0	8.1		—
Temp (°C)		24.7	24.1	24.0	24.2	25.3	25.0	—

Concentration								
Day	0	1	2	3	4	5	6	7
Initial								
pH								
DO (mg/L)								
Cond. (µmhos/cm)								
Temp (°C)								
Final								
pH								
DO (mg/L)								
Temp (°C)								

Analysts:	0	1	2	3	4	5	6	7
Initial:	AD	[illegible]	AD	EG	AD	AD	EG	
Final:		AD	AD	EG	AD	AD	EG	—
Dilutions made by:	PA	[illegible]	AC	EG	[illegible]	AD	EG	
Sample Used (A, B, C):	A	A	A	A	A	A	A	

Animal Source/Date Received: Internal NA

Animal Age at Initiation: <24h

Sample Log-in Numbers: A: 16-12883 (B)

B: —

C: —

Comments: (A) EG Q18 11/22/16 (B) Q18 NHE 11/26/16 (C) Tech error: readings not measured (D) Q18 KB 12/12/16

QC Check: KB 12/12/16

Final Review: AC 1/20/17

TST Summary Sheet

Lab Name	Nautilus Environmental	**Client Name**	AEI-CASC
Test ID	City of El Monte LL, Outfall #6	**Test Species**	*C. dubia (water flea)*
Test Date	11/22/2016	**Test Type**	Chronic
Test Duration	6 days	**Endpoint**	Reproduction
Critical Conc.	100		

Statistic	Control	Critical Concentration
Mean of Raw Data	28.40	16.70
Mean used in Calculation (non-transformed)	28.40	16.70
Variance used in Calculation (non-transformed)	136.711	127.344
Standard Deviation of Raw Data	11.692	11.285
CV of Raw Data	0.412	0.676
n	10	10

Mean % Effect at Critical Conc.

41.20

Calculated t-value	Degrees of Freedom	Table t-value	Percent Difference
-1.0178	16	0.8647	

Results

Fail Sample is Toxic

Raw Data

Control Data		Critical Concentration Data	
No. of Organisms Exposed or Counted	Response (Final Count, Weight, Length, etc.)	No. of Organisms Exposed or Counted	Response (Final Count, Weight, Length, etc.)
	8		0
	23		7
	39		14
	14		8
	36		26
	39		29
	18		26
	34		4
	40		29
	33		24

Entry: KB QC: AC 1/22/17

Appendix B

Sample Check-In Information

Nautilus Environmental
4340 Vandever Avenue
San Diego, CA 92120

Sample Check-In Information

Client: AET-CASC Tests Performed: C. dubia chronic

Project: City of El Monte Test ID No.(s): 1611-S231

	1)	2)	3	4)
Sample ID:	Outfall #6			
Log-in No. (16-xxxx):	1283			
Sample Collection Date & Time:	11/21/16 0100			
Sample Receipt Date & Time:	11/21/16 1800			
Number of Containers & Container Type:	1, 10L cubi			
Approx. Total Volume Received (L):	10L			
Check-in Temp (°C)	12.5			
Temperature OK? [1]	(Y) N	Y N	Y N	Y N
DO (mg/L)	8.7			
pH (units)	7.23			
Conductivity (µS/cm)	110			
Salinity (ppt)	0.1			
Alkalinity (mg/L) [2]	28			
Hardness (mg/L) [2,3]	26			
Total Chlorine (mg/L)	NM			
Technician Initials	AD/EG			

Freshwater Tests:

Control/Dilution Water Source: (8:2) Culligan Other: _____ Alkalinity: 80 Hardness: 83

Additional Control? Y N = _____ Alkalinity: _____ Hardness: _____

Marine Tests:

Control/Dilution Water Source: LAB SW ART SW Other: _____ Alkalinity: _____ Salinity: _____

Additional Control? Y N = _____ Alkalinity: _____ Salinity: _____

Sample Salted w/ artificial salt? Y N If yes, target ppt and source? _____

Sample salted w/brine? Y N If yes, target ppt? _____

Notes [1] Temperature for sample must be 0-6°C if received >24 hours past collection time.

[2] mg/L as CaCO3, [3] Measured for freshwater samples only, NA = Not Applicable

Additional Comments NM = not measured

QC Check: KB 12/12/16

Sample Descriptions:

1) Light yellow, clear, no oda, light debris

2) _____

3) _____

4) _____

COC Complete? (Y) N

Filtration? Y (N)

Pore Size: _____

Organisms or Debris

pH Adjustment? Y (N)

	1	2	3	4	5	6
Initial pH:						
Amount of HCl added:						
Final pH:						

Cl_2 Adjustment? Y (N)

	1	2	3	4	5	6
Initial Free Cl_2:						
STS added:						
Final Free Cl_2:						

Sample Aeration? Y (N)

	1	2	3	4	5	6
Initial D.O.						
Duration & Rate						
Final D.O.						

Subsamples For Additional Chemistry Required? Y (N)

NH3 Other _____

Tech Initials _____

Final Review: AC 1/20/17

Appendix C

Chain-of-Custody Form

Nautilus Environmental

4340 Vandever Ave.
San Diego, CA 92120
Phone 858.587.7333
Fax 858.587.3961

Chain of Custody

Date 11-21-16 Page 1 of 1

Sample Collection By:

Report to:

Company	CASC Engineering and Consulting
Address	2740 W. Magnolia Blvd, Suite 102
City/State/Zip	Burbank, CA 91505
Contact	Michael Kolbenschlag
Phone	818-800-5775
Email	mkolbenschlad@cascinc.com

Invoice To:

Company	(same)
Address	
City/State/Zip	
Contact	
Phone	
Email	

	SAMPLE ID	DATE	TIME	MATRIX	CONTAINER TYPE	NO. OF CONTAINERS	COMMENTS	ANALYSES REQUIRED: TIE	Ceriodaphnia chronic test									Receipt Temperature (°C)
1	OUTFALL #6 (LL)	11/21/16	0100	SW	10L CUBE	1		X										12.5
2																		
3																		
4																		
5																		
6																		
7																		
8																		
9																		
10																		

PROJECT INFORMATION

Client:	
PO No.:	EL MONTE
Shipped Via:	CASC

SAMPLE RECEIPT

Total No. of Containers	1
Received Good Condition?	Y
Matches Test Schedule?	Y

SPECIAL INSTRUCTIONS/COMMENTS:

EL MONTE SW OUTFALL MONITORING

RELINQUISHED BY (CLIENT)

(Signature) (Time) 1800
(Printed Name) (Date) Alejandro Escobar 11/21/2016
(Company) CASC Engineering

RELINQUISHED BY (COURIER)

(Signature) (Time)
(Printed Name) (Date)
(Company)

RECEIVED BY (COURIER)

(Signature) (Time)
(Printed Name) (Date)
(Company)

RECEIVED BY (LABORATORY)

(Signature) (Time) 1800
(Printed Name) (Date) ERIC GREEN 11/21/16
(Company) NAUTILUS ID: 16-1283

Additional costs may be required for sample disposal or storage. Payment net 30 unless otherwise contracted.

DISTRIBUTION: WHITE - Nautilus Environmental, COLOR - Originator

Appendix D

Qualifier Code Glossary

Nautilus Environmental

Glossary of Qualifier Codes:

Q1 - Temperatures out of recommended range; corrective action taken and recorded in Test Temperature Correction Log

Q2 - Temperatures out of recommended range; no action taken, test terminated same day

Q3 - Sample aerated prior to initiation or renewal due to dissolved oxygen (D.O.) levels below 6.0 mg/L

Q4 - Test aerated; D.O. levels dropped below 4.0 mg/L

Q5 - Test initiated with aeration due to an anticipated drop in D.O.

Q6 - Airline obstructed or fell out of replicate and replaced; drop in D.O. occurred

Q7 - Salinity out of recommended range

Q8 - Spilled test chamber/ Unable to recover test organism(s)

Q9 - Inadequate sample volume remaining, 50% renewal performed

Q10 - Inadequate sample volume remaining, no renewal performed

Q11 - Sample out of holding time; refer to QA section of report

Q12 - Replicate(s) not initiated; excluded from data analysis

Q13 - Survival counts not recorded due to poor visibility or heavy debris

Q14 - D.O. percent saturation was checked and was ≤ 110%

Q15 - Did not meet minimum test acceptability criteria. Refer to QA section of report.

Q16 - Percent minimum significant difference (PMSD) was below the lower bound limit for acceptability. This indicates that statistics may be over-sensitive in detecting a difference from the control due to low variability in the data set.

Q17 - Percent minimum significant difference (PMSD) was above the upper bound limit for acceptability. This indicates that statistics may be under-sensitive in detecting a difference from the control due to high variability in the data set.

Q18 - Incorrect Entry

Q19 - Illegible Entry

Q20 - Miscalculation

Q21 - Other (provide reason in comments section)

Q22 - Greater than 10% mortality observed upon receipt and/or in holding prior to test initiation. Organisms acclimated to test conditions at Nautilus and ultimately deemed fit to use for testing.

Q23 - Test organisms received at a temperature greater than 3°C outside the recommended test temperature range. However, due to age-specific protocol requirements and/or sample holding time constraints, the organisms were used to initiate tests upon the day of arrival. Organisms were acclimated to the appropriate test conditions upon receipt and prior to test initiation.

Q24 - Test organisms received at salinity greater than 3 ppt outside of the recommended test salinity range. However, due to age-specific protocol requirements and/or sample holding time constraints, the organisms were used to initiate tests upon the day of arrival. Organisms were acclimated to the appropriate test conditions upon receipt and prior to test initiation.

Appendix E

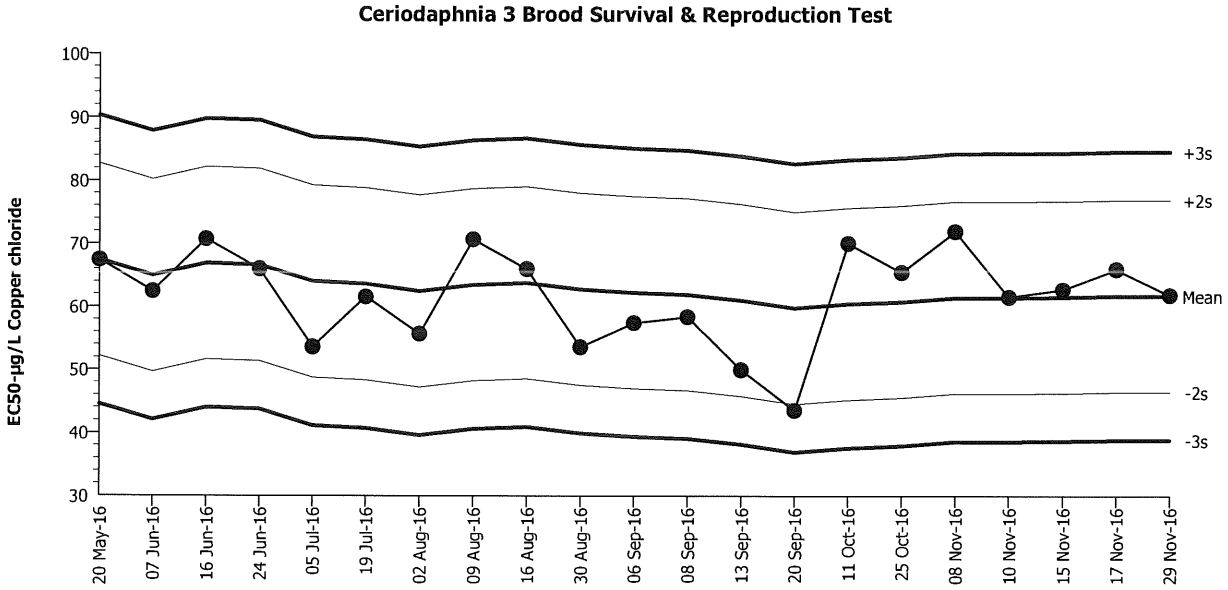
Reference Toxicant Test Control Charts

CETIS QC Plot

Report Date: 21 Dec-16 15:13 (1 of 1)

Ceriodaphnia 3 Brood Survival & Reproduction Test — **Nautilus Environmental (CA)**

Test Type:	Reproduction and Survival	**Organism:**	Ceriodaphnia dubia (Water Flea)	**Material:**	Copper chloride
Protocol:	EPA/821/R-02-013 (2002)	**Endpoint:**	3 Brood Survival	**Source:**	Reference Toxicant-REF



Mean:	61.74	**Count:**	20	**-2s Warning Limit:**	46.48	**-3s Action Limit:**	38.85
Sigma:	7.63	**CV:**	12.40%	**+2s Warning Limit:**	77	**+3s Action Limit:**	84.63

Quality Control Data

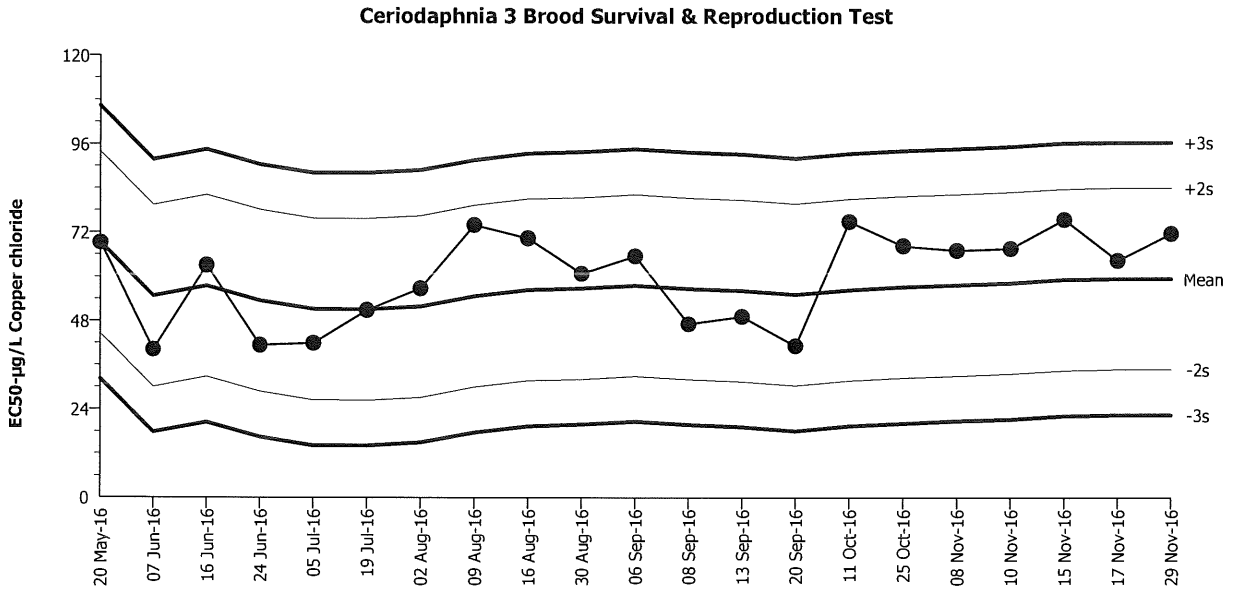
Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2016	May	20	14:30	67.41	5.67	0.7431			16-8010-2708	03-9057-6318
2		Jun	7	16:15	62.43	0.6901	0.09044			17-5156-5919	08-4916-2844
3			16	15:30	70.71	8.971	1.176			15-7926-0172	08-5371-7888
4			24	15:30	65.98	4.235	0.5551			19-4835-1616	02-4637-9012
5		Jul	5	13:55	53.59	-8.151	-1.068			12-6488-9449	05-8649-5314
6			19	15:20	61.56	-0.1828	-0.02396			06-0553-1257	05-4294-1200
7		Aug	2	15:10	55.67	-6.067	-0.7951			05-8463-3888	02-0772-0545
8			9	16:35	70.71	8.971	1.176			02-2043-2312	09-2151-2849
9			16	15:10	65.98	4.235	0.5551			16-6661-1547	09-7320-9096
10			30	14:45	53.59	-8.151	-1.068			21-3933-6233	20-7271-4257
11		Sep	6	13:00	57.43	-4.305	-0.5642			16-0048-4604	14-7082-6301
12			8	14:45	58.4	-3.336	-0.4372			08-7783-2081	08-2568-2652
13			13	16:15	50	-11.74	-1.539			01-7282-6786	19-0309-4236
14			20	15:50	43.53	-18.21	-2.387	(-)		13-8609-6471	11-8818-0657
15		Oct	11	15:10	70.11	8.368	1.097			04-3012-9943	05-9910-0987
16			25	16:30	65.47	3.729	0.4888			15-0850-1329	15-0536-7821
17		Nov	8	17:10	71.98	10.24	1.342			16-3937-5405	08-9684-9575
18			10	16:00	61.56	-0.1828	-0.02396			01-8907-8090	07-4905-3451
19			15	16:00	62.75	1.006	0.1318			00-9759-3840	14-6616-4887
20			17	16:15	65.98	4.235	0.5551			13-5459-1721	07-0502-7123
21			29	15:15	61.87	0.1291	0.01692			03-7140-9966	07-7956-0623

CETIS QC Plot

Report Date: 21 Dec-16 15:13 (1 of 1)

Ceriodaphnia 3 Brood Survival & Reproduction Test — **Nautilus Environmental (CA)**

Test Type:	Reproduction and Survival	**Organism:**	Ceriodaphnia dubia (Water Flea)	**Material:**	Copper chloride
Protocol:	EPA/821/R-02-013 (2002)	**Endpoint:**	3 Brood Reproduction	**Source:**	Reference Toxicant-REF



Mean:	59.53	**Count:**	20	**-2s Warning Limit:**	34.85	**-3s Action Limit:**	22.51
Sigma:	12.34	**CV:**	20.70%	**+2s Warning Limit:**	84.21	**+3s Action Limit:**	96.55

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2016	May	20	14:30	69.24	9.706	0.7866			16-8010-2708	15-9807-1703
2		Jun	7	16:15	40.17	-19.36	-1.569			17-5156-5919	18-1234-5449
3			16	15:30	63.03	3.499	0.2836			15-7926-0172	10-8786-2437
4			24	15:30	41.39	-18.14	-1.47			19-4835-1616	02-7331-8754
5		Jul	5	13:55	41.9	-17.63	-1.428			12-6488-9449	09-6581-0357
6			19	15:20	50.89	-8.643	-0.7004			06-0553-1257	14-9290-2274
7		Aug	2	15:10	56.81	-2.723	-0.2206			05-8463-3888	20-6109-2329
8			9	16:35	74.04	14.51	1.176			02-2043-2312	07-0596-3744
9			16	15:10	70.45	10.92	0.8853			16-6661-1547	16-2952-1624
10			30	14:45	60.83	1.299	0.1053			21-3933-6233	10-9745-5543
11		Sep	6	13:00	65.61	6.079	0.4926			16-0048-4604	10-8825-9903
12			8	14:45	47.21	-12.32	-0.9987			08-7783-2081	05-6176-8657
13			13	16:15	49.22	-10.31	-0.8356			01-7282-6786	05-2923-3442
14			20	15:50	41.28	-18.25	-1.479			13-8609-6471	20-3000-6091
15		Oct	11	15:10	75	15.47	1.254			04-3012-9943	18-3006-0109
16			25	16:30	68.46	8.929	0.7236			15-0850-1329	11-4242-3200
17		Nov	8	17:10	67.13	7.604	0.6162			16-3937-5405	10-4040-1441
18			10	16:00	67.71	8.182	0.6631			01-8907-8090	05-2922-7513
19			15	16:00	75.65	16.12	1.306			00-9759-3840	00-1740-9039
20			17	16:15	64.53	5.002	0.4054			13-5459-1721	13-1287-3479
21			29	15:15	71.78	12.25	0.9926			03-7140-9966	18-6371-9337