Nautilus Environmental

Results of Wet Weather Chronic Toxicity Testing for the City of Irwindale

❖ Sample Collection Date: December 16, 2016

Prepared for: AEI-CASC Consulting
2740 W. Magnolia Blvd., Suite 102
Burbank, CA 91505

Prepared by: Nautilus Environmental
4340 Vandever Avenue
San Diego, CA 92120
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Report Submitted: February 14, 2017

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 Cibor

Introduction

Bioassay testing was performed on one sample from the City of Irwindale, California to evaluate the toxicity of storm water runoff to the water flea (*Ceriodaphnia dubia*). 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 December 16, 2016. The toxicity screening test was conducted at Nautilus Environmental (Nautilus) in San Diego, California between December 17 and 23, 2016.

Materials and Methods

Test Material

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

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
SGR-077	12/16/16; 04:30	12/16/16; 17:10

Chronic Toxicity Methods

Testing was conducted in accordance with methods published in USEPA (2002). 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 analysis was performed using the Comprehensive Environmental Toxicity Information System™ (CETIS) software by Tidepool Scientific Software. 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 *C. dubia* Test Specifications

Test Start Date, Time:	12/17/16, 16:00
Test End Date, Time:	12/23/16, 15:00
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; 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:	CETIS™, version 1.8.7.20/ TST Analysis

Results

Water flea survival was 100 percent in both the lab control and the SGR-077 sample. However, mean water flea reproduction in the sample was reduced relative to the lab control. The reproduction endpoint failed according to the TST analysis with a 34 percent effect from control. A summary of results is presented in Table 3. 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 Appendices B and C, respectively.

Table 3. 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	-	-	24.2	-	-
SGR-077	100	0.0	Pass	16.0	34	Fail

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 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

Quality Assurance

The sample was received under appropriate conditions and the tests were initiated within the required 36-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 4. All test acceptability criteria were met. Additionally, the median lethal and median effect concentrations (LC_{50}/EC_{50}) were within two standard deviations (SD) from the historical mean, indicating that the test organisms exhibited typical sensitivity to copper as that historically observed in the Nautilus laboratory. The survival and reproduction control charts for the past 20 reference toxicant tests conducted at Nautilus are presented in Appendix E.

Table 4. 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	25	50.0	60.8 ± 16.1
Reproduction	25	55.3	60.1 ± 23.8

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. 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.

Appendix A

Raw Data and Statistical Summaries

CETIS Summary Report

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

Batch ID:	04-8633-1950	**Test Type:**	Reproduction and Survival	**Analyst:**	
Start Date:	17 Dec-16 16:00	**Protocol:**	EPA/821/R-02-013 (2002)	**Diluent:**	Not Applicable
Ending Date:	23 Dec-16 15:00	**Species:**	Ceriodaphnia dubia	**Brine:**	Not Applicable
Duration:	5d 23h	**Source:**	In-House Culture	**Age:**	<24h

Sample ID:	04-3130-5454	**Code:**	16-1410	**Client:**	Aei-Casc Consulting
Sample Date:	16 Dec-16 04:30	**Material:**	Stormwater	**Project:**	
Receive Date:	16 Dec-16 17:10	**Source:**	Aei-Casc		
Sample Age:	35h (6 °C)	**Station:**	City of Irwindale (SGR-077)		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
06-6973-2355	3 Brood Reproduction	<100	100	NA	9.45%	>1	TST-Welch's t Test
10-9587-1400	3 Brood Survival	100	>100	NA	NA	1	Fisher Exact Test

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
06-6973-2355	3 Brood Reproduction	Control Resp	24.2	15 - NL	Yes	Passes Acceptability Criteria
10-9587-1400	3 Brood Survival	Control Resp	1	0.8 - NL	Yes	Passes Acceptability Criteria
06-6973-2355	3 Brood Reproduction	PMSD	0.09446	0.13 - 0.47	Yes	Below Acceptability Criteria (1)

3 Brood Reproduction Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	10	24.2	19.22	29.18	12	33	2.2	6.957	28.75%	0.0%
100		10	16	11.32	20.68	9	31	2.071	6.549	40.93%	33.88%

3 Brood Survival Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	10	1	1	1	1	1	0	0	0.0%	0.0%
100		10	1	1	1	1	1	0	0	0.0%	0.0%

3 Brood Reproduction 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	Lab Control	26	16	30	31	26	33	17	25	26	12
100		17	11	12	31	23	9	15	16	14	12

3 Brood Survival 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	Lab Control	1	1	1	1	1	1	1	1	1	1
100		1	1	1	1	1	1	1	1	1	1

(1) PMSD test acceptability limits do not apply to TST method.

CETIS Analytical Report

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

Analysis ID: 06-6973-2355 **Endpoint:** 3 Brood Reproduction **CETIS Version:** CETISv1.8.7
Analyzed: 18 Jan-17 13:06 **Analysis:** Parametric Bioequivalence-Two Sample **Official Results:** Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	TST b	PMSD	Test Result
Untransformed	NA	C*b < T	NA	NA	0.75	9.45%	Fails 3 brood reproduction

TST-Welch's t Test

Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:20%)
Lab Control		100	-0.812	0.8633	2.286	17	0.7860	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	336.2	336.2	1	7.366	0.0142	Significant Effect
Error	821.6	45.64444	18			
Total	1157.8		19			

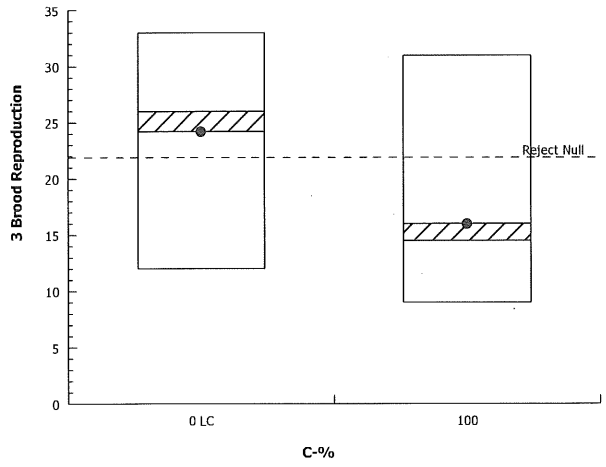
Distributional Tests

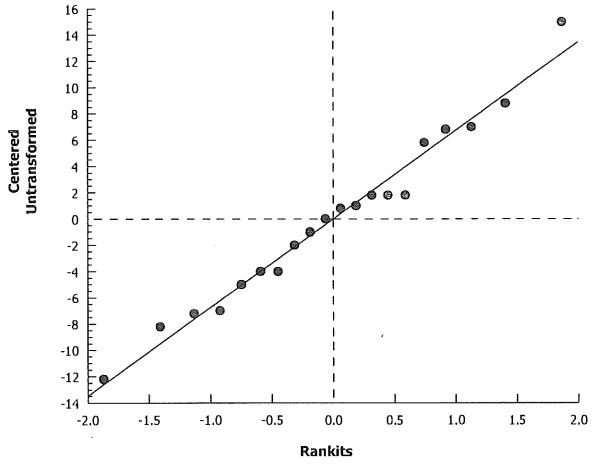
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Variance Ratio F	1.128	6.541	0.8600	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9818	0.866	0.9552	Normal Distribution

3 Brood Reproduction Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	10	24.2	19.22	29.18	26	12	33	2.2	28.75%	0.0%
100		10	16	11.32	20.68	14.5	9	31	2.071	40.93%	33.88%

Graphics





CETIS Analytical Report

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

Analysis ID:	10-9587-1400	**Endpoint:**	3 Brood Survival	**CETIS Version:**	CETISv1.8.7
Analyzed:	18 Jan-17 13:06	**Analysis:**	Single 2x2 Contingency Table	**Official Results:**	Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	Test Result
Untransformed		C > T	NA	NA	Passes 3 brood survival

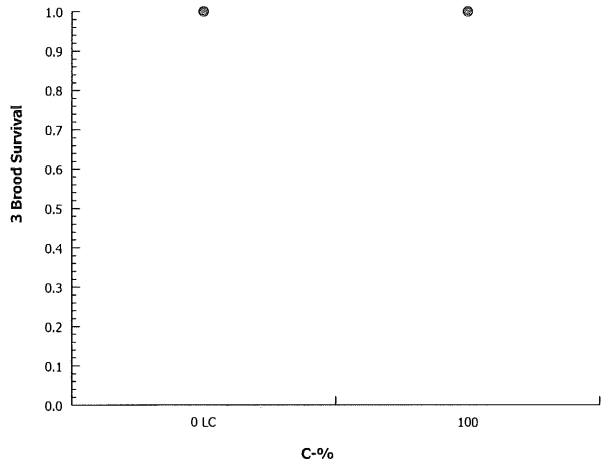
Fisher Exact Test

Control	vs	C-%	Test Stat	P-Value	P-Type	Decision(α:5%)
Lab Control		100	1	1.0000	Exact	Non-Significant Effect

Data Summary

C-%	Control Type	NR	R	NR + R	Prop NR	Prop R	%Effect
0	Lab Control	10	0	10	1	0	0.0%
100		10	0	10	1	0	0.0%

Graphics



Freshwater Chronic Bioassay | **Daphnid Survival and Reproduction Datasheet**

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

Client/Sample ID: AEI-CASC/ City of Irwindale - SGR-077 **Start Date/Time:** 12/17/2016 1600

Test Number: 1612-S178 **End Date/Time:** 12/24/2016 ~~1315~~ 1500

EG QA 12/23/16 23 MM QA 12/23/16

Conc.	Rep	Rand #	Daily Reproduction/ Survival 1	2	3	4	5	6	7	8	Total	QC
LC	1	26	0	0	0	6	2	18			26	
	2	2	0	0	0	6	10	0			16	
	3	17	0	0	(A)34	10	16	0			30	
	4	12	0	0	(A)34	11	0	16			31	
	5	28	0	0	(A)~~4~~3	8	0	15			26	
	6	21	0	0	0	6	12	15			33	
	7	14	0	0	0	6	11	0			17	
	8	3	0	0	0	4	8	13			25	
	9	11	0	0	0	4	10	12			26	
	10	6	0	0	0	6	0	6			12	
		Tech:	CH	ACS	CH	ACS	EG	EG				

Mean neonates/surviving female (for TAC): 27.2

Conc.	Rep	Rand #	Daily Reproduction/ Survival 1	2	3	4	5	6	7	8	Total	QC
100% SGR-077	1	30	0	0	0	5	0	12			17	
	2	1	0	0	0	5	6	0			11	
	3	18	0	0	3	9	0	0			12	
	4	10	0	0	3	0	11	17			31	
	5	4	0	0	0	6	0	17			23	
	6	8	0	0	0	6	3	0			9	
	7	7	0	0	0	5	0	10			15	
	8	5	0	0	0	1	8	7			16	
	9	25	0	0	0	5	8	(D)1			14	
	10	16	0	0	0	5	7	0			12	

Conc.	Rep	Rand #	Daily Reproduction/ Survival 1	2	3	4	5	6	7	8	Total	QC
	1	24										
	2	13										
	3	15										
	4	20										
	5	29										
	6	27										
	7	19										
	8	9										
	9	22										
	10	23										

Conc.	Rep	Rand #	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:	148	→	→	→	→	→	→	→	→	→
Cup:	1	15	24	29	31	33	35	56	60	23

Rand # QC: ACS Verified By: AD Initiated By: AC QC'd By: AC

Time Fed/Test Solution Renewed (day):

(0) 1600 (1) 1240 (2) 1315 (3) 1040 (4) 1350 (5) 1310 (6) — (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 (A)CH QA 12/20/16 (B)QA NHE 12/23/16

QC Check: KB 1/10/17 Final Review: PFP 1/19/17

Freshwater Chronic Bioassay **Water Quality Measurements**

Client: AEI-CASC/ City of Irwindale Test Species: *C. dubia*

Sample ID: SGR-077 Start Date/Time: 12/17/2016 1600

Test No: 1612-S179 End Date/Time: 12/24/2016 ~~1315~~ 1500

EG Q18 12/23/16 23 MM Q18 12/23/16

Concentration	Q1 Lab Control							
Day	0	1	2	3	4	5	6	7
Initial								
pH	8.23	8.01	8.01	8.08	8.16	8.23	8.16	
DO (mg/L)	7.8	8.4	8.0	7.8	8.0	7.8	7.9	
Cond. (µmhos/cm)	219	200	193	217	189	193	192	
Temp (°C)	24.5	24.6	24.4	24.1	25.8	25.6	24.5	
Final								
pH		8.26	7.91	8.06	8.15	8.13	8.18	—
DO (mg/L)		8.4	7.8	7.5	7.7	~~7.9~~ 8.3	8.2	—
Temp (°C)		24.1	23.2	25.3	24.5	~~25.6~~ @24.3	25.1	—

Concentration	SGR-077 100%							
Day	0	1	2	3	4	5	6	7
Initial								
pH	7.14	6.75	6.90	6.71	6.97	7.07	7.05	
DO (mg/L)	8.5	8.7	8.9	8.8	8.7	8.3	8.0	
Cond. (µmhos/cm)	17	21	17	20	17	18	17	
Temp (°C)	24.7	24.6	24.3	24.8	25.9	25.8	25.8	
Final								
pH		7.32	7.20	7.30	7.53	7.46	7.34	—
DO (mg/L)		8.6	7.9	7.5	7.8	8.3	8.1	—
Temp (°C)		24.1	23.2	25.3	24.5	24.3	25.1	—

Concentration	~~100%~~ Q18 KB 1/18/17							
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)								

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)								

	0	1	2	3	4	5	6	7
Analysts: Initial:	KFP	RH	EG	MM	ACJ	AL	MM	
Final:		AC	AD	MM	ACJ	EG	MM	—
Dilutions made by:	~~KB~~ EG	KB	MM	EG	EG 03C MM	AL 03C MM	MM	
Sample Used (A, B, C):	A	A	A	A	A	A	A	

Comments: @EG Q18 12/22/16

Animal Source/Date Received: Internal/N/A Animal Age at Initiation: 24hr

Sample Log-in Numbers: A: 16-1413 B: — C: —

QC Check: ~~16-1413~~ Q18 KB 1/18/17 Final Review: KFP 1/19/17

Appendix B

Sample Check-In Information

Nautilus Environmental
4340 Vandever Avenue
San Diego, CA 92120

Sample Check-In Information

Client: AEI-CASC

Tests Performed: Chronic Ceriodaphnia

Project: Irvindale SW outfall Monitoring

Test ID No.(s): 1612-S178

	1)	2)	3)	4)	5)	6)
Sample ID:	SGR-077					
Log-in No. (16-xxxx):	1410					
Sample Collection Date & Time:	12/16/16 0430					
Sample Receipt Date & Time:	12/16/16 1710					
Number of Containers & Container Type:	1, 4L cubi					
Approx. Total Volume Received (L):	~4L					
Check-in Temp (°C)	6.0					
Temperature OK? [1]	(Y) N	Y N	Y N	Y N	Y N	Y N
DO (mg/L)	10.5					
pH (units)	6.91					
Conductivity (µS/cm)	24.0					
Salinity (ppt)	0.0					
Alkalinity (mg/L) [2]	15					
Hardness (mg/L) [2,3]	11					
Total Chlorine (mg/L)	—					
Technician Initials	EH					

Freshwater Tests:

Control/Dilution Water Source: (8:2) Culligan Other: _____ Alkalinity: 88 Hardness: 90

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 _____

QC Check: KB 1/18/17

Sample Descriptions:

1) colorless, clear, odorless, light debris
2) _____
3) _____
4) _____
5) _____
6) _____

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: KFP 1/19/17

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 12/16/16 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: CHRONIC TOX - C. DUBIA	Receipt Temperature (°C)
1	SGR-077	12/16/16	0430	SW	4L CUBE	1		X	6.0
2									
3									
4									
5									
6									
7									
8									
9									
10									

PROJECT INFORMATION		SAMPLE RECEIPT	
Client:		Total No. of Containers	1
PO No.:	IRWINDALE	Received Good Condition?	Y
Shipped Via:	CASC	Matches Test Schedule?	Y

SPECIAL INSTRUCTIONS/COMMENTS:

IRWINDALE SW OUTFALL MONITORING

RELINQUISHED BY (CLIENT)

(Signature) (Time): 1710
(Printed Name) (Date): Alejandro Escobar 12/16/2016
(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): 1710
(Printed Name) (Date): ERIC GREEN 12/16/16
(Company): Nautilus

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

DISTRIBUTION: WHITE - Nautilus Environmental, COLOR - Originator

Log in # 16-1410

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.

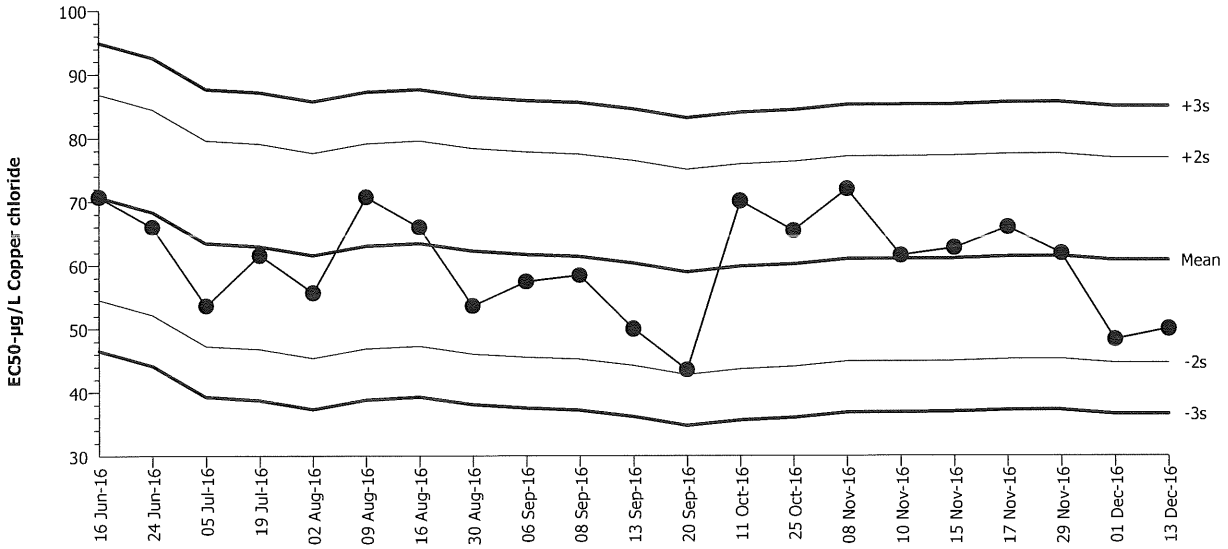
Appendix E

Reference Toxicant Test Control Charts

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

Ceriodaphnia 3 Brood Survival & Reproduction Test



Mean:	60.76	**Count:**	20	**-2s Warning Limit:**	44.63	**-3s Action Limit:**	36.57
Sigma:	8.062	**CV:**	13.30%	**+2s Warning Limit:**	76.88	**+3s Action Limit:**	84.94

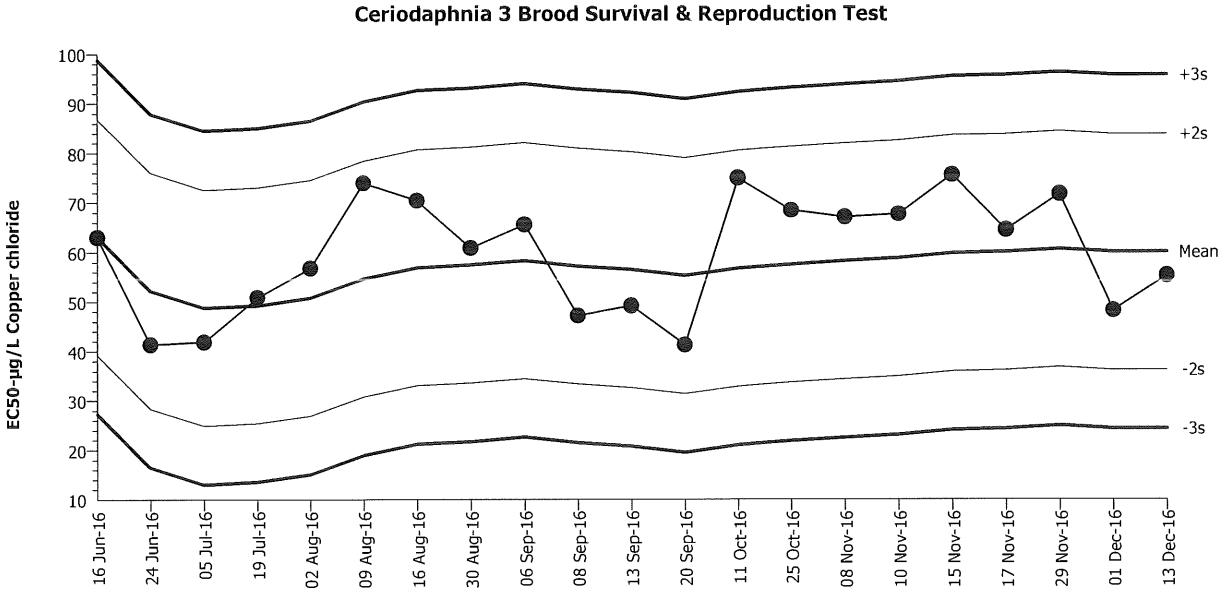
Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2016	Jun	16	15:30	70.71	9.951	1.234			15-7926-0172	08-5371-7888
2			24	15:30	65.98	5.215	0.6469			19-4835-1616	02-4637-9012
3		Jul	5	13:55	53.59	-7.171	-0.8895			12-6488-9449	05-8649-5314
4			19	15:20	61.56	0.7972	0.09889			06-0553-1257	05-4294-1200
5		Aug	2	15:10	55.67	-5.087	-0.6309			05-8463-3888	02-0772-0545
6			9	16:35	70.71	9.951	1.234			02-2043-2312	09-2151-2849
7			16	15:10	65.98	5.215	0.6469			16-6661-1547	09-7320-9096
8			30	14:45	53.59	-7.171	-0.8895			21-3933-6233	20-7271-4257
9		Sep	6	13:00	57.43	-3.325	-0.4124			16-0048-4604	14-7082-6301
10			8	14:45	58.4	-2.356	-0.2922			08-7783-2081	08-2568-2652
11			13	16:15	50	-10.76	-1.335			01-7282-6786	19-0309-4236
12			20	15:50	43.53	-17.23	-2.137	(-)		13-8609-6471	11-8818-0657
13		Oct	11	15:10	70.11	9.348	1.16			04-3012-9943	05-9910-0987
14			25	16:30	65.47	4.709	0.5841			15-0850-1329	15-0536-7821
15		Nov	8	17:10	71.98	11.22	1.392			16-3937-5405	08-9684-9575
16			10	16:00	61.56	0.7972	0.09889			01-8907-8090	07-4905-3451
17			15	16:00	62.75	1.986	0.2463			00-9759-3840	14-6616-4887
18			17	16:15	65.98	5.215	0.6469			13-5459-1721	07-0502-7123
19			29	15:15	61.87	1.109	0.1376			03-7140-9966	07-7956-0623
20		Dec	1	11:00	48.3	-12.46	-1.546			04-2024-5023	15-2732-3440
21			13	15:00	50	-10.76	-1.335			04-2596-7095	14-9254-5691

CETIS QC Plot

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:	60.06	**Count:**	20	**-2s Warning Limit:**	36.24	**-3s Action Limit:**	24.33
Sigma:	11.91	**CV:**	19.80%	**+2s Warning Limit:**	83.88	**+3s Action Limit:**	95.79

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2016	Jun	16	15:30	63.03	2.969	0.2493			15-7926-0172	10-8786-2437
2			24	15:30	41.39	-18.67	-1.567			19-4835-1616	02-7331-8754
3		Jul	5	13:55	41.9	-18.16	-1.525			12-6488-9449	09-6581-0357
4			19	15:20	50.89	-9.173	-0.7702			06-0553-1257	14-9290-2274
5		Aug	2	15:10	56.81	-3.253	-0.2731			05-8463-3888	20-6109-2329
6			9	16:35	74.04	13.98	1.174			02-2043-2312	07-0596-3744
7			16	15:10	70.45	10.39	0.8728			16-6661-1547	16-2952-1624
8			30	14:45	60.83	0.7689	0.06456			21-3933-6233	10-9745-5543
9		Sep	6	13:00	65.61	5.549	0.4659			16-0048-4604	10-8825-9903
10			8	14:45	47.21	-12.85	-1.079			08-7783-2081	05-6176-8657
11			13	16:15	49.22	-10.84	-0.9103			01-7282-6786	05-2923-3442
12			20	15:50	41.28	-18.78	-1.577			13-8609-6471	20-3000-6091
13		Oct	11	15:10	75	14.94	1.254			04-3012-9943	18-3006-0109
14			25	16:30	68.46	8.399	0.7052			15-0850-1329	11-4242-3200
15		Nov	8	17:10	67.13	7.074	0.5939			16-3937-5405	10-4040-1441
16			10	16:00	67.71	7.652	0.6425			01-8907-8090	05-2922-7513
17			15	16:00	75.65	15.59	1.309			00-9759-3840	00-1740-9039
18			17	16:15	64.53	4.472	0.3755			13-5459-1721	13-1287-3479
19			29	15:15	71.78	11.72	0.9839			03-7140-9966	18-6371-9337
20		Dec	1	11:00	48.27	-11.79	-0.9898			04-2024-5023	06-1750-9717
21			13	15:00	55.32	-4.737	-0.3978			04-2596-7095	10-6182-0772