



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

❖ 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 Gibor

## Introduction

Chronic toxicity screening tests were performed on two samples from the City of Irwindale, 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 the SAWPW 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 tests and TIE were conducted at Nautilus Environmental (Nautilus) in San Diego, California between November 22 and 28, 2016.

## Materials and Methods

### Test Material

Test material consisted of two storm water samples. Collection was conducted under the direction of Mr. Michael Kolbensschlag 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
SAWPW-074A (SAWPW)	11/21/16; 04:10	11/21/16; 18:00
BDW-027A (BDW)	11/21/16; 10:00	

### 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 Period:	11/22/16, 15:00 to 11/28/16 14:25 (SAWPW) 11/22/16, 15:05 to 11/28/16 10:25 (BDW)
Test Organism:	<i>Ceriodaphnia dubia</i> (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 $\geq 80\%$ ; $\geq 60\%$ of surviving females producing 3 or more broods; Mean number of offspring $\geq 15$ 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 SAWPW 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 municipal separate storm sewer system (MS4) permit.

TIE treatments applied to the sample included solid-phase extraction (SPE), filtration (0.45  $\mu\text{m}$ ), 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)	Removes pollutants associated with particles

## Results

### Toxicity Screening

There were no statistically significant effects observed in *C. dubia* survival or reproduction for the BDW sample. There was also no effect in survival of *C. dubia* exposed to the SAWPW sample; however, a 36 percent effect from the lab control was observed in reproduction, which failed according to the TST statistical analysis. A summary of results for the screening tests of the undiluted samples 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	-	-	28.6	-	-
BDW-027A	100	0.0	Pass	29.1	-1.9	Pass
Lab Control	100	-	-	33.9	-	-
SAWPW-074A	100	0.0	Pass	21.6	36.3	Fail

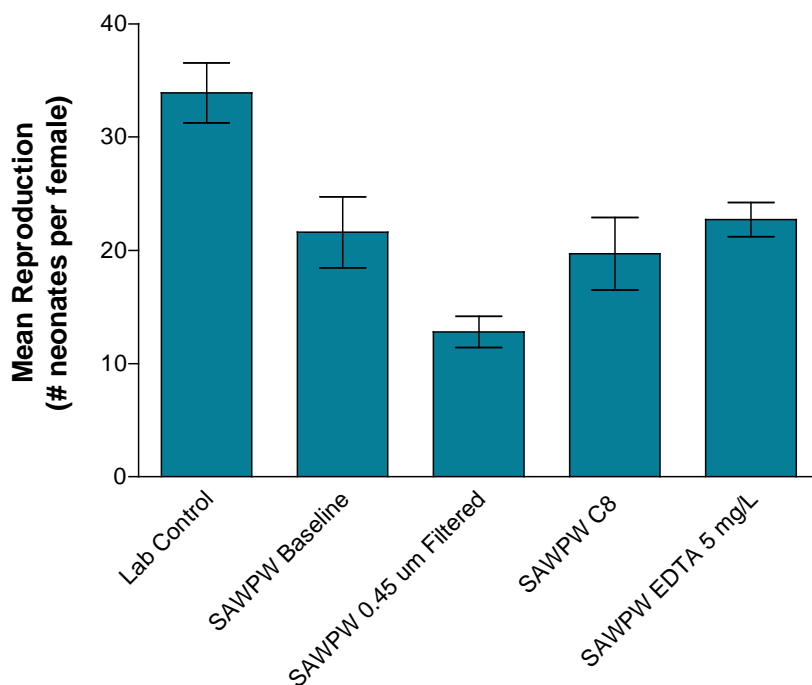
TST analysis is not appropriate for the *C. dubia* chronic survival endpoint because the test design includes only one organism per replicate. Therefore, the result for the chronic survival endpoint is based on percent effect (PE) compared to control calculated as: ((mean response in control - mean response 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 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 100 percent in the laboratory and method controls, the SAWPW baseline sample, and all sample treatments. Since there were no survival effects, the remainder of this discussion will focus on the reproductive endpoint.

The average number of offspring produced in the unmanipulated baseline SAWPW sample was 21.6, compared to 33.9 in the concurrent lab control; a 36.3 percent effect. Although the percent effect was below the 50 percent effect TIE trigger in the permit, the test was conducted concurrently with the screen to proactively address toxicity at this site observed in a previous monitoring event. None of the TIE treatments performed removed toxicity in the sample, resulting in an inconclusive TIE study. Further studies will need to be conducted if toxicity is observed at this site in future monitoring events. Mean reproduction results for TIE treatments of the SAWPW 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 SAWPW sample collected November 21, 2016 (mean  $\pm$  standard error).**

## Quality Assurance

The samples were received under appropriate conditions and the tests were initiated within 36 hours of collection. 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. All 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 ( $\mu\text{g/L}$ copper)	$LC_{50}/EC_{50}$ ( $\mu\text{g/L}$ copper)	Historical $LC_{50}/EC_{50}$ $\pm 2$ SD ( $\mu\text{g/L}$ copper)
Survival	50	61.9	$61.7 \pm 15.3$
Reproduction	50	71.8	$59.5 \pm 24.7$

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  $LC_{50}/EC_{50} \pm 2$  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. 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**  
**Test Initiation Date: 11/22/16**  
**Sample Collection Date: 11/21/16**  
**Sample: City of Irwindale BDW**  
**7-day Chronic *Ceriodaphnia* Survival and Reproduction**

Sample (%)	Number Alive (neonates)	Mean Survival (%)	Number of Young (neonates)	Mean Number Per Adult (neonates)	Percent Effect
Lab Control	1	100	29	28.6	--
	1		18		
	1		38		
	1		17		
	1		27		
	1		32		
	1		34		
	1		33		
	LIP		LIP		
	1		29		
100	1	100	29	29.1	-1.9
	1		16		
	1		24		
	1		28		
	1		33		
	1		37		
	1		36		
	1		34		
	1		18		
	1		36		

LIP = organism lost in progress of testing.

## Freshwater Chronic Bioassay

## Water Quality Measurements

Client: AEI Casc/City of Irwindale

Test Species: *C. dubia*

Sample ID: BDW

Start Date/Time: 11/22/2016

1505

Test No: 1611-5228

End Date/Time: 11/28/16

1025

Concentration	Lab Control							
Day	0	1	2	3	4	5	6	7
Initial								
pH	8.20	8.20	8.00	8.22	8.25	8.22	Ⓢ	
DO (mg/L)	7.4	8.0	7.9	8.1	8.0	7.5		
Cond. (µmhos/cm)	193	190	191	191	190	192		
Temp (°C)	24.7	24.0	24.2	24.3	24.1	24.5		
Final								
pH		8.13	8.15	8.22	8.24	8.16	8.25	—
DO (mg/L)		8.1	7.9	8.3	8.3	7.8	8.7	—
Temp (°C)		24.3	24.9	24.2	24.1	24.3	24.9	—

Concentration	BDW (100%)							
Day	0	1	2	3	4	5	6	7
Initial								
pH	7.68	7.65	7.51	7.52	7.32	7.42	Ⓢ	
DO (mg/L)	8.0	8.4	8.3	8.6	8.9	7.9		
Cond. (µmhos/cm)	107	105	106	106	106	109		
Temp (°C)	24.0	24.0	24.3	24.7	24.4	24.6		
Final								
pH		7.74	7.82	7.91	7.98	7.86	7.83	—
DO (mg/L)		8.29	7.7	8.3	8.3	7.7	8.5	—
Temp (°C)		24.3	24.9	24.2	24.1	24.3	24.9	—

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:	KB	JH	ACS	MM	MM	ML	—	
Final:		ACS	ACS	AG	NHE	ACS	JH	—
Dilutions made by:	PP, DBU, PA	CH	ACS	NHE	NHE	ACS	—	
Sample Used (A, B, C):	A	A	A	A	A	A	—	

Comments: Ⓢ 0.18 ACS 11/23/16 Ⓢ 0.18 JH 11/28/16 Ⓢ Test terminated, no initial readings taken

Animal Source/Date Received: Internal / NA

Animal Age at Initiation: &lt;24h

Sample Log-in Numbers: A: 16-1285

B: —

C: —

QC Check: KB 12/12/16

Final Review: —

## Freshwater Chronic Bioassay

## Daphnid Survival and Reproduction Datasheet

Test Species: *C. dubia*

Client/Sample ID: AEI Casc/ City of Irwindale - BDW

Start Date/Time: 11/22/2016 1505

Test Number: 1611-5228

End Date/Time: 11/28/16 1025

Conc.	Rep	Rand #	Daily Reproduction/ Survival								Total	QC
			1	2	3	4	5	6	7	8		
LC	1	17	0	0	0	6	11	12			29	
	2	1	0	0	0	6	12	0			18	
	3	15	0	0	0	7	12	19			38	
	4	5	0	0	0	6	11	0			17	
	5	18	0	0	0	0	12	15			27	
	6	7	0	0	0	5	10	17			32	
	7	2	0	0	3	0	14	17			34	16
	8	9	0	0	0	6	15	12			33	
	9	4	LIP	-	-	-	-	-	-	-	LIP	EL
	10	8	0	0	0	5	10	14			29	
Tech:			ALS	ALS	ALC	NHE	MLR	ALS				3W

Mean = 28.6  
(Excl. LIP)

Conc.	Rep	Rand #	Daily Reproduction/ Survival								Total	QC
			1	2	3	4	5	6	7	8		
BDW (100%)	1	11	0	0	0	0	15	0			29	
	2	3	0	0	0	3	13	0			16	
	3	10	0	0	0	8	14	0			24	0
	4	12	0	0	0	0	11	14			28	
	5	6	0	0	40	3	12	18			33	
	6	13	0	0	4	0	11	22			37	
	7	16	0	0	4	0	11	21			36	
	8	20	0	0	5	0	12	17			34	
	9	14	0	0	0	5	13	0			18	
	10	19	0	0	0	7	13	16			36	

Conc.	Rep	Rand #	Daily Reproduction/ Survival								Total	QC
			1	2	3	4	5	6	7	8		
	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:	137									5
Cup:	19	26	21	25	27	28	29	30	34	35
Rand # QC:	AD			KFP			AD			KFP 0130 CH
Verified By:										
Initiated By:										
QC'd By:										

Time Fed/Test Solution Renewed (day):

(0) 1505 (1) 1245 (2) 1130 (3) 1050 (4) 1030 (5) 1400 (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 (Excl. ALS 11/28/16)

QC Check: KB 12/9/16

Final Review: AC 1/21/17

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

Treatment	Number Alive (neonates)	Mean Survival (%)	Number of Young (neonates)	Mean Number Per Adult (neonates)
0.45 $\mu$ m 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	

AEI-CASC

Test Initiation Date: 11/22/16

Sample Collection Date: 11/21/16

Sample: City of Irwindale SAWPW

Chronic *Ceriodaphnia* Survival and Reproduction

Treatment (100%)	Number Alive (neonates)	Mean Survival (%)	Number of Young (neonates)	Mean Number Per Adult (neonates)	Percent Effect
Lab Control	1	100	40	33.9	--
	1		12		
	1		35		
	1		35		
	1		42		
	1		38		
	1		36		
	1		30		
	1		36		
	1		35		
Baseline	1	100	16	21.6	36.3
	1		4		
	1		19		
	1		13		
	1		22		
	1		35		
	1		28		
	1		16		
	1		34		
	1		29		
Filtered	1	100	6	12.8	62.2
	1		10		
	1		12		
	1		14		
	1		22		
	1		15		
	1		9		
	1		11		
	1		14		
	1		15		
C8	1	100	15	19.7	41.9
	1		7		
	1		14		
	1		16		
	1		41		
	1		23		
	1		14		
	1		16		
	1		33		
	1		18		
EDTA	1	100	19	22.7	33.04
	1		14		
	1		25		
	1		27		
	1		19		
	1		31		
	1		22		
	1		23		
	1		22		
	1		25		

## Freshwater Chronic Bioassay

## Water Quality Measurements

Client: AEI-CASC

Test Species: *C. dubia*

Sample ID: TIE Method Controls

Start Date/Time: 11/22/2016

Test No: 1611-5229, 5231

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)	195	201	193	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	8.69	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)	190	202	194	197	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	8	AD	EG	AD	AD	EG	
Final:		AD	AD	EG	AD	AD	EG	—
Dilutions made by:	PA	8	AC	EG	AD	AD	EG	
Sample Used (A, B, C):	—	—	—	—	—	—	—	

Comments: RAD 018 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 Irwindale  
 Sample ID: SAWPW  
 Test No: 1611-8229

Test Species: *C. dubia*  
 Start Date/Time: 11/22/16 1500  
 End Date/Time: 11/25/16 1425

Concentration	Lab Control							
Day	0	1	2	3	4	5	6	7
	Initial							
pH	8.15	8.09	8.02	8.16	8.10	7.91	8.08	
DO (mg/L)	7.4	7.9	8.2	8.3	9.0	8.4	9.0	
Cond. (µmhos/cm)	191	194	198	198	198	196	194	
Temp (°C)	25.0	25.4	25.4	24.8	25.9	25.3	24.6	
	Final							
pH		8.03	8.14	8.16	8.25	8.5	8.15	-
DO (mg/L)		8.0	8.2	7.8	8.1	8.4	8.5	-
Temp (°C)		24.9	24.0	23.7	24.3	24.2	24.1	-

Concentration	100% C8 SPE							
Day	0	1	2	3	4	5	6	7
	Initial							
pH	7.18	7.21	7.20	7.12	7.04	7.09	7.62	
DO (mg/L)	7.7	7.8	6.3	7.2	6.5	6.4	8.5	
Cond. (µmhos/cm)	92	90	90	87	90	90	89	
Temp (°C)	25.0	25.3	25.2	25.5	25.8	25.0	24.2	
	Final							
pH		7.30	7.17	7.64	7.76	7.65	7.67	-
DO (mg/L)		6.3	7.9	7.7	8.1	8.3	8.4	-
Temp (°C)		24.9	24.0	23.7	24.3	24.2	24.1	-

Concentration	100% Baseline							
Day	0	1	2	3	4	5	6	7
	Initial							
pH	7.27	7.14	7.20	7.08	7.03	7.13	6.99	
DO (mg/L)	7.8	7.7	7.6	7.9	7.8	7.6	7.6	
Cond. (µmhos/cm)	89	89	89	88	90	90	91	
Temp (°C)	24.1	25.3	25.3	25.2	25.8	25.0	24.9	
	Final							
pH		7.57	7.66	7.70	7.73	7.67	7.69	-
DO (mg/L)		7.9	8.0	7.9	8.0	8.3	8.4	-
Temp (°C)		24.9	24.0	23.7	24.3	24.2	24.1	-

Concentration	100% EDTA 5 mg/L							
Day	0	1	2	3	4	5	6	7
	Initial							
pH	7.36	7.10	7.20	7.13	6.97	7.11	6.96	
DO (mg/L)	7.7	7.8	7.3	7.5	7.1	7.5	8.2	
Cond. (µmhos/cm)	88	88	90	88	89	91	89	
Temp (°C)	24.1	24.9	25.0	24.8	25.7	24.9	24.9	
	Final							
pH		7.49	7.64	7.65	7.70	7.56	7.59	-
DO (mg/L)		7.3	7.9	7.8	8.0	8.2	8.3	-
Temp (°C)		24.9	24.0	23.7	24.3	24.2	24.1	-

Concentration	100% Filtered							
Day	0	1	2	3	4	5	6	7
	Initial							
pH	7.21	7.04	7.11	7.10	6.99	7.07	6.99	
DO (mg/L)	7.6	7.9	8.9	9.0	9.0	9.0	8.9	
Cond. (µmhos/cm)	99	104	102	103	97	93	89	
Temp (°C)	24.4	25.2	25.2	25.5	25.7	24.9	24.7	
	Final							
pH		7.64	7.74	7.71	7.78	7.62	7.58	-
DO (mg/L)		7.6	8.0	7.8	8.0	8.2	8.3	-
Temp (°C)		24.9	24.0	23.7	24.3	24.2	24.1	-

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)								

Animal Source/Date Received: Internal NA  
 Animal Age at Initiation: < 24h  
 Sample Log-in Numbers: A: 16-1284 C: —  
 B: —

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

Comments: @ EG Q15 11/22/16 @ EG Q15 11/23/16

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/ TIE Method Controls

Start Date/Time: 11/22/2016 1500

Test Number: 1611-5229, 5231

End Date/Time: 11/28/16 1455

Conc.	Rep	Daily Reproduction/ Survival								Total	QC
		1	2	3	4	5	6	7	8		
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 AD EG											JH

Conc.	Rep	Daily Reproduction/ Survival								Total	QC
		1	2	3	4	5	6	7	8		
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	12	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								Total	QC
		1	2	3	4	5	6	7	8		
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			137						
Cup:	1	3	6	10	11	12	18	22	25	
Rand # QC:	NA			NA						
Verified By:				AP						
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

① CH Q18 11/22/16 ② AD Q18 11/26/16

③ EG 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 Irwindale SAWPW

Start Date/Time: 11/22/2016 1500

Test No: 1611-5229

End Date/Time: 11/28/16 1425

Conc.	Rep	Daily Reproduction/ Survival								Total	QC
		1	2	3	4	5	6	7	8		
LC	1	0	0	0	6	14	20			40	
	2	0	0	0	5	14	15			34	
	3	0	0	0	7	12	16			35	
	4	0	0	0	7	12	22			42	
	5	0	0	0	6	12	20			38	
	6	0	0	0	6	14	16			36	
	7	0	0	0	7	15	15			30	
	8	0	0	0	7	10	21			38	
	9	0	0	0	7	12	19			38	
	10	0	0	0	7	12	19			38	
Tech: AC AD EL AD AD AL		Mean neonates/surviving female (for TAC): 33.9									34

Conc.	Rep	Daily Reproduction/ Survival								Total	QC
		1	2	3	4	5	6	7	8		
100 C8	1	0	0	0	4	11	0			15	
	2	0	0	0	4	9	0			13	
	3	0	0	0	4	9	0			13	
	4	0	0	0	4	12	0			16	
	5	0	0	5	12	24				41	24
	6	0	0	5	10	10				23	
	7	0	0	0	10	0				14	
	8	0	0	0	11	0				16	
	9	0	0	0	10	17				27	
	10	0	0	0	10	7				17	

Conc.	Rep	Daily Reproduction/ Survival								Total	QC
		1	2	3	4	5	6	7	8		
100%	1	0	0	0	3	13	0			16	
	2	0	0	0	3	10	0			13	
	3	0	0	0	3	10	0			13	
	4	0	0	0	3	10	0			13	
	5	0	0	0	3	10	18			31	
	6	0	0	0	3	10	20			33	
	7	0	0	0	3	10	17			28	
	8	0	0	0	3	10	17			28	
	9	0	0	0	3	10	17			34	
	10	0	0	0	3	10	17			29	

Conc.	Rep	Daily Reproduction/ Survival								Total	QC
		1	2	3	4	5	6	7	8		
100 EDTA	1	0	0	0	4	7	8			19	8
	2	0	0	0	4	10	12			26	
	3	0	0	0	4	12	13			29	
	4	0	0	0	4	12	14			30	
	5	0	0	2	10	17				29	
	6	0	0	2	10	19				31	
	7	0	0	2	10	14				26	
	8	0	0	0	10	13				23	
	9	0	0	2	10	16				28	
	10	0	0	2	10	13				25	

Conc.	Rep	Daily Reproduction/ Survival								Total	QC
		1	2	3	4	5	6	7	8		
100 Filt	1	0	0	0	5	0				5	
	2	0	0	0	5	0				5	
	3	0	0	0	5	0				5	
	4	0	0	0	5	0				5	
	5	0	0	0	5	0				5	
	6	0	0	0	5	0				5	
	7	0	0	0	5	0				5	
	8	0	0	0	5	0				5	
	9	0	0	0	5	0				5	
	10	0	0	0	5	0				5	

Conc.	Rep	Daily Reproduction/ Survival								Total	QC
		1	2	3	4	5	6	7	8		
	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:	136									
Cup:	1	3	6	9	10	11	12	18	22	25
Rand # QC:	NR	Verified By:	KFF	Initiated By:	AD	QC'd By:	AD			

Time Fed/Test Solution Renewed (day): (0) 1500 (1) 1635 (2) 1450 (3) 1345 (4) 1415 (5) 1710 (6) 1425 (7) —

Comments: (A) CHQ 11/22/16 (B) EG Q15 11/25/16

QC Check: 16312/12/16

Final Review: 1/20/17

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

# TST Summary Sheet

<b>Lab Name</b>	Nautilus Environmental	<b>Client Name</b>	AEI-CASC
<b>Test ID</b>	City of Irwindale, SAWPW	<b>Test Species</b>	<i>C. dubia</i> (water flea)
<b>Test Date</b>	11/22/2016	<b>Test Type</b>	Chronic
<b>Test Duration</b>	6 days	<b>Endpoint</b>	Reproduction
<b>Critical Conc.</b>	100		

Statistic	Control	Critical Concentration
Mean of Raw Data	33.90	21.60
Mean used in Calculation (non-transformed)	33.90	21.60
Variance used in Calculation (non-transformed)	69.656	98.044
Standard Deviation of Raw Data	8.346	9.902
CV of Raw Data	0.246	0.458
n	10	10

## Mean % Effect at Critical Conc.

36.28

Calculated t-value	Degrees of Freedom	Table t-value	Percent Difference
-1.0326	15	0.8662	

## 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.)
	40		16
	12		4
	35		19
	35		13
	42		22
	38		35
	36		28
	30		16
	36		34
	35		29

## **Appendix B**

### **Sample Check-In Information**

Nautilus Environmental  
4340 Vandever Avenue  
San Diego, CA 92120

Client: AEI-CASC

Tests Performed: C. dubia Chronic

Project: City of Irwindale

Test ID No.(s): 1611-S228 + S229

Sample ID:	1) <u>SAWPLW</u>	2) <u>BDW</u>	3	4)
Log-in No. (16-xxxx):	<u>1284</u>	<u>1285</u>		
Sample Collection Date & Time:	<u>11/21/16 0410</u>	<u>11/21/16 1800</u>		
Sample Receipt Date & Time:	<u>11/21/16 1800</u>	<u>11/21/16 1800</u>		
Number of Containers & Container Type:	<u>1, 10L cubi</u>	<u>1, 10L cubi</u>		
Approx. Total Volume Received (L):	<u>10 L</u>	<u>10 L</u>		
Check-in Temp (°C)	<u>11.5</u>	<u>13.0</u>		
Temperature OK? <sup>1</sup>	<u>Y</u> N	<u>Y</u> N	Y N	Y N
DO (mg/L)	<u>9.1</u>	<u>9.9</u>		
pH (units)	<u>7.32</u>	<u>7.59</u>		
Conductivity (µS/cm)	<u>89</u>	<u>103</u>		
Salinity (ppt)	<u>0.1</u>	<u>0.1</u>		
Alkalinity (mg/L) <sup>2</sup>	<u>25</u>	<u>51</u>		
Hardness (mg/L) <sup>2,3</sup>	<u>21</u>	<u>34</u>		
Total Chlorine (mg/L)	<u>NM</u>	<u>NM</u>		
Technician Initials	<u>AD/EG</u>	<u>AD/EG</u>		

**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 <sup>1</sup> Temperature for sample must be 0-6°C if received >24 hours past collection time.

<sup>2</sup> mg/L as CaCO<sub>3</sub>, <sup>3</sup> Measured for freshwater samples only, NA = Not Applicable

Additional Comments

NM = not measured

QC Check: KB 12/12/16

**Sample Check-In Information**

**Sample Descriptions:**

1) light yellow, clear, no odor, light debris  
2) light yellow, clear, no odor, no debris  
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<sub>2</sub> Adjustment? Y N

	1	2	3	4	5	6
Initial Free Cl <sub>2</sub> :						
STS added:						
Final Free Cl <sub>2</sub> :						

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

NH<sub>3</sub> Other: \_\_\_\_\_

Tech Initials \_\_\_\_\_

Final Review: AC 1/20/17

## **Appendix C**

### **Chain-of-Custody Form**



## Chain of Custody

Date 11-21-16 Page 1 of 1

Sample Collection By:							
<div style="float:left; width:30%;"><b>Report to:</b> <b>Company</b> CASC Engineering and Consulting <b>Address</b> 2740 W. Magnolia Blvd, Suite 102 <b>City/State/Zip</b> Burbank, CA 91505 <b>Contact</b> Michael Kolbenschlager <b>Phone</b> 818-800-5775 <b>Email</b> mkolbenschlader@cascinc.com</div> <div style="float:right; width:30%;"><b>Invoice To:</b> <b>Company</b> _____ <b>Address</b> _____ <b>City/State/Zip</b> _____ <b>Contact</b> _____ <b>Phone</b> _____ <b>Email</b> _____</div> <div style="clear:both;"></div>							
SAMPLE ID	DATE	TIME	MATRIX	CONTAINER TYPE	NO. OF CONTAINERS	COMMENTS	
SAW PW - 074 A	"11/21/16"	0410	SW	10L CUBE	1		
BDW - 027 A	"11/21/16"	1000	SW	10L CUBE	1		
PROJECT INFORMATION	SAMPLE RECEIPT			RELINQUISHED BY (CLIENT)		RELINQUISHED BY (COURIER)	
Client:	Total No. of Containers	2	(Signature) [Handwritten Signature]	(Time) 8:00	(Signature)	(Time)	
PO No.: IRWINDALE	Received Good Condition?	[Initials]	(Printed Name) Alejandro Escobar	(Date) 11/21/2016	(Printed Name)	(Date)	
Shipped Via: CASC	Matches Test Schedule?	[Initials]	(Company) CASC Engineering		(Company)		
SPECIAL INSTRUCTIONS/COMMENTS: IRWINDALE SW OUTFALL MONITORING			RECEIVED BY (COURIER)		RECEIVED BY (LABORATORY)		
(Signature)			(Time)		(Signature)		(Time)
(Printed Name)			(Date)		(Printed Name)		(Date)
(Company)					(Company)		
(Signature)			(Time)		(Signature)		(Time)
(Printed Name)			(Date)		(Printed Name)		(Date)
(Company)					(Company)		

**DISTRIBUTION:** WHITE - Nautilus Environmental, COLOR - Originator

Nautilus ID: 16-1284 and -1285

## **Appendix D**

### **Qualifier Code Glossary**

### 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  $\leq 110\%$
- 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 &amp; Reproduction Test

Nautilus Environmental (CA)

Test Type: Reproduction and Survival

Organism: Ceriodaphnia dubia (Water Flea)

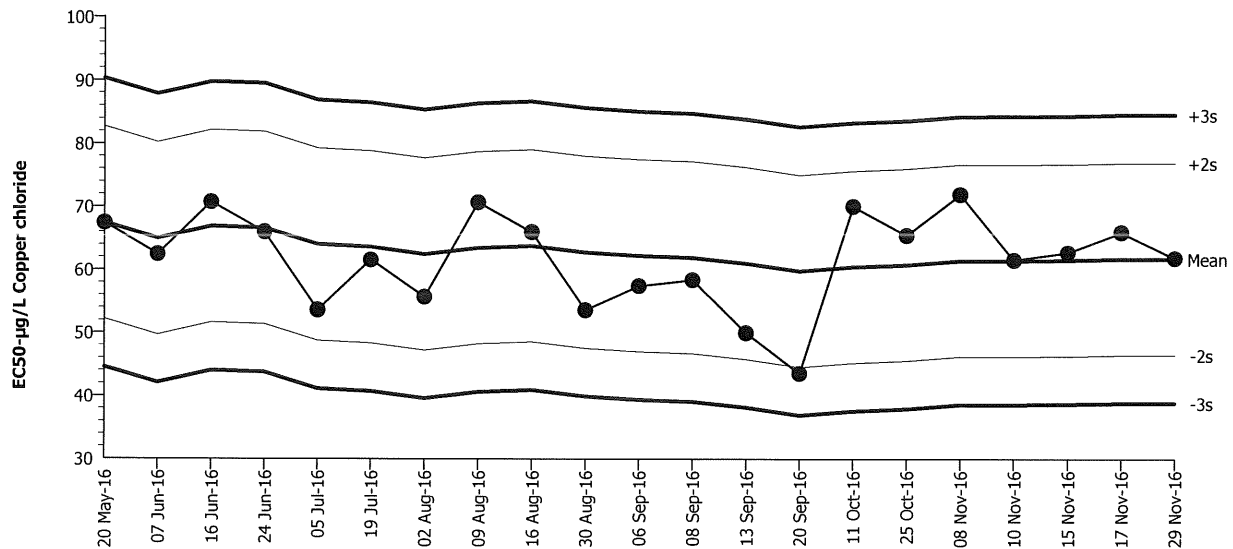
Material: Copper chloride

Protocol: EPA/821/R-02-013 (2002)

Endpoint: 3 Brood Survival

Source: Reference Toxicant-REF

## Ceriodaphnia 3 Brood Survival &amp; Reproduction Test



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

## Ceriodaphnia 3 Brood Survival &amp; Reproduction Test

Nautilus Environmental (CA)

Test Type: Reproduction and Survival

Organism: Ceriodaphnia dubia (Water Flea)

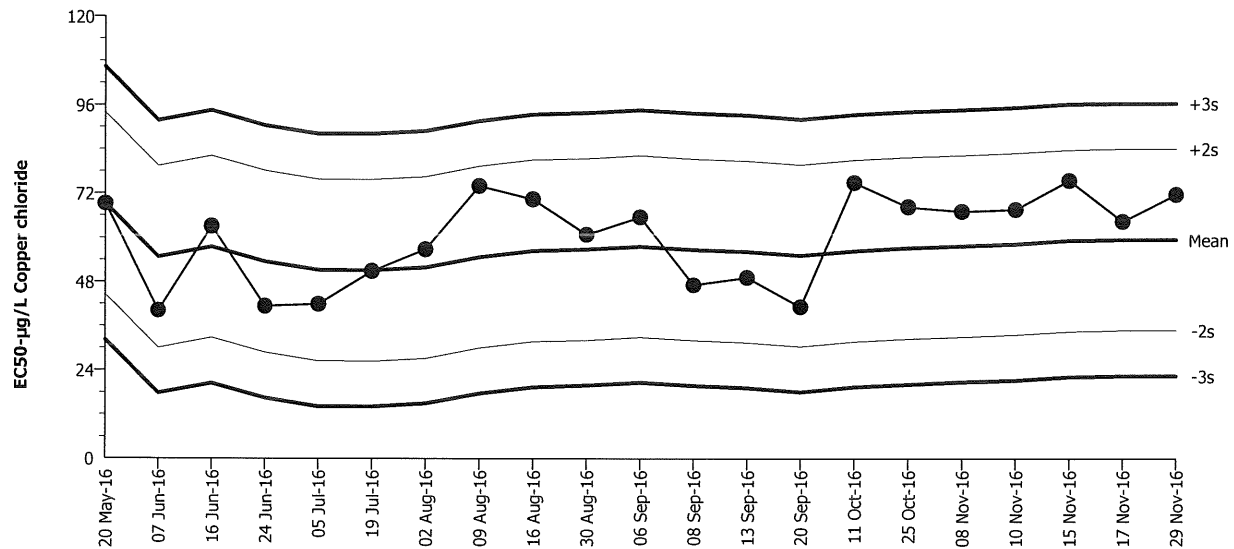
Material: Copper chloride

Protocol: EPA/821/R-02-013 (2002)

Endpoint: 3 Brood Reproduction

Source: Reference Toxicant-REF

## Ceriodaphnia 3 Brood Survival &amp; Reproduction Test



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