

# Acute Toxicity Test Results for ADC Wet Weather Water Quality Monitoring

Monitoring Period: February 2021

Prepared for: Cardno  
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Submitted: March 17, 2021

**Data Quality Assurance:**

- Enthalpy Analytical (formerly 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 Department of Health Services Environmental Laboratory Accreditation Program (Certificate No. 1802) and the State of Washington Department of Ecology (Lab ID C552).
- 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 results have met internal Quality Assurance Program requirements, unless otherwise noted in this report.

**Data Verified by:**  Laboratory Director

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## Introduction

Three samples were collected during a storm event for the ADC Kekaha Water Quality Monitoring. Samples were submitted by Cardno-GS. Testing was conducted at the Enthalpy Analytical Laboratory in San Diego, California. Pacific topsmelt (*Atherinops affinis*), inland silverside (*Menidia beryllina*), and mysid shrimp (*Americamysis bahia*) 96-hour acute survival tests were used for the DW-1/WW-1 sample received at a salinity level greater than 1 part per thousand (ppt). Fathead minnow (*Pimephales promelas*), water flea (*Ceriodaphnia dubia*), and freshwater amphipod (*Hyaella azteca*) 96-hour acute survival tests were used for the WW-2 and WW-3 samples, which were received below 1 ppt salinity.

## Materials and Methods

### Sample Information

Client:	Cardno-GS
Project Name:	ADC Kekaha Water Quality Monitoring
Sample IDs:	DW-1/WW-1, WW-2, WW-3
Sample Collection Dates, Times <sup>a</sup> :	2/20/2021, 14:00 to 19:45
Sample Receipt Dates, Times:	2/23/2021, 08:50
Sample Material:	Stormwater sample
Sampling Method:	Grab

<sup>a</sup> Collection times adjusted to Pacific Standard Time from Hawaii Standard Time.

### Water Quality Parameters Measured upon Sample Receipt

Sample ID	pH	DO (mg/L)	Temp. (°C)	Cond. (µS/cm)	Salinity (ppt)	Alkalinity (mg/L as CaCO <sub>3</sub> )	Hardness (mg/L as CaCO <sub>3</sub> )	Total Chlorine (mg/L)
DW-1/WW-1	7.46	9.7	2.4	2,500	1.4	105	366	0.05
WW-2	5.83 <sup>a</sup>	9.5	2.9	513	0.2	7	50	<0.02
WW-3	5.79	9.4	3.3	90	0.1	13	34	nm

nm = not measured; sample too dark and opaque to measure on colorimeter.

<sup>a</sup> The pH of WW-2 was adjusted daily with sodium hydroxide to bring pH to USEPA required range of 6-9 to toxicity testing for freshwater tests. The pH of sample WW-3 was above 6.0 when raised to test temperature and did not require manipulation for any tests.

## Acute Toxicity Test Methods

Testing was conducted in accordance with methods published in US Environmental Protection Agency (USEPA) guidance (2002). Test specifications for all marine tests are summarized in Table 1, and test specifications for freshwater tests are summarized in Table 2.

**Table 1. 96-hr Acute Survival Test Specifications - Marine Organisms**

Pacific topsmelt test: 2/23, 15:25 to 2/27, 14:55	Species: <i>Atherinops affinis</i> . Source & Age: Aquatic Biosystems (Ft. Collins, CO), 13 days
Inland silverside test: 2/23, 16:40 to 2/27, 14:45	Species: <i>Menidia beryllina</i> . Source & Age: Aquatic Biosystems (Ft. Collins, CO), 8 days
Mysid shrimp test: 2/23, 15:35 to 2/27, 14:30	Species: <i>Americamysis bahia</i> . Source & Age: Aquatic Biosystems (Ft. Collins, CO), 3 days
Protocol Used:	Acute Manual (EPA/821/R-02/012), EPA 2002
Test Acceptability Criteria:	Control mean survival $\geq$ 90%
Test Concentration:	100% sample (DW-1/WW-1)
Sample Manipulation:	Artificial salts (Instant Ocean®) were added to bring the salinity of the sample to 30 $\pm$ 1 parts per thousand (ppt)
Lab Control Water:	20- $\mu$ m filtered seawater (Source: Scripps Institution of Oceanography [SIO] Intake); diluted to 30 ppt with deionized water
Salt Control:	Salt Control – 30 ppt artificial saltwater (Instant Ocean®)

**Table 2. 96-hr Acute Survival Test Specifications – Freshwater Organisms**

Fathead minnow test: 2/23, 13:50 to 2/27, 15:45	Species: <i>Pimephales promelas</i> . Source & Age: Aquatic Biosystems (Ft. Collins, CO), 6 days
Water flea test: 2/23, 13:40 to 2/27, 12:20	Species: <i>Ceriodaphnia dubia</i> . Source & Age: Internal culture, < 24 hours
FW amphipod test <sup>a</sup> : 2/24, 17:20 to 2/28, 15:20	Species: <i>Hyalella azteca</i> . Source & Age Aquatic Research Organisms (Hampton, NH), 13 days
Protocol Used:	Acute Manual (EPA/821/R-02/012), EPA 2002
Test Acceptability Criteria:	Control mean survival $\geq$ 90%
Test Concentration:	100% sample (WW-2 and WW-3)
Sample Manipulation:	WW-2 sample pH was adjusted with NaOH to above 6 (EPA required range for toxicity testing is 6-9). No adjustments were made to sample WW-3.
Lab Control Water:	Diluted mineral water (per EPA protocol)

<sup>a</sup> The freshwater amphipod test was initiated out of holding time due to an organism shipping delay; see QA section.

Statistical analyses were conducted using EPA flowchart specifications as outlined in the test guidance manual (USEPA 2002). Organism performance in the sample was compared to that observed in the concurrent artificial salt control. Results were used to calculate whether a statistically significant effect was observed between the control and sample result. Comprehensive Environmental Toxicity Information System™ (CETIS) software by Tidepool Scientific Software, version 1.8.7.20.

## Results

No statistically significant effects were observed to any of the marine species exposed the DW-1/WW-1 sample compared to the respective artificial salt control. The artificial salt control for the inland silverside resulted in 75 percent mean survival, below the test acceptability criterion (TAC) of 90 percent (see QA section for further details). The lab control for this species resulted in 87.5 percent mean survival, which is still below TAC. The DW-1/WW-1 sample (82.5 percent survival) also resulted in no statistically significant effect compared to the lab control. A summary of results for the marine species tests is presented in Table 3.

The freshwater lab controls for the fathead minnow and water flea test met TAC. However, the lab control for the freshwater amphipod test was below TAC (see QA section). The control for the amphipod test resulted in a mean survival of 67.5 percent while samples WW-2 and WW-3 resulted in 92.5 and 87.5 percent survival, respectively. None of the freshwater species tested resulted in statistically significant mortality, with the exception of the water fleas in the WW-3 sample test. Water flea mean survival in the WW-3 sample was 10 percent, compared to 100 percent in the lab control. The WW-3 sample was very opaque and turbid with a significant amount of fine silt present in the sample. The water flea species is known to have difficulty in these types of samples due to easy clogging of their filtering apparatus which is used for feeding. Further testing with this sample would need to be conducted to confirm whether the reduced survival of this species was due to a toxicant or a physical barrier due to turbidity. A summary of results for the freshwater tests is presented in Table 4.

Raw datasheets and complete statistical summaries for all tests are provided in Appendix A. Sample receipt information is provided in Appendix B, and a copy of the chain of custody form is presented in Appendix C.

**Table 3. Summary of Marine 96-hr Acute Survival Results**

Sample ID	Species	Salt Control Result	100% Sample Result	Statistically Significant Effect? (Yes/No)	Percent Effect
DW-1/WW-1	Pacific topsmelt	90.0	100	No	-11
	Inland silverside	75.0 <sup>a</sup>	82.5	No	-10
	Mysid shrimp	90.0	95.0	No	-5.6

<sup>a</sup> The control did not meet minimum test acceptability criterion; see QA section.

Percent effect from control is calculated as: ((mean response in salt control - mean response in undiluted sample)/mean response in salt control) \*100. A negative value results when organism performance in the sample is greater than that in the salt control.

**Table 4. Summary of Freshwater 96-hr Acute Survival Results**

Sample ID	Species	Lab Control Result	100% Sample Result	Statistically Significant Effect? (Yes/No)	Percent Effect
WW-2	Fathead minnow	97.5	93.3	No	4.3
	Water flea	100	100	No	0.0
	Freshwater amphipod	67.5 <sup>a</sup>	92.5	No	-37
WW-3	Fathead minnow	97.5	90.0	No	7.7
	Water flea	100	10.0*	Yes	90
	Freshwater amphipod	67.5 <sup>a</sup>	87.5	No	-30

<sup>a</sup> The control did not meet minimum test acceptability criterion; see QA section.

\*Values with an asterisk indicate a statistically significant reduction from the lab control.

Percent effect from control is calculated as: ((mean response in lab control - mean response in undiluted sample)/mean response in lab control) \*100. A negative value results when organism performance in the sample is greater than that in the lab control.

## Quality Assurance

The samples were received via overnight delivery service three days after collection. The samples were received slightly below the range of 0-6 degrees Celsius (°C) and had some ice crystals in the sample containers. The client was immediately notified and requested to proceed with testing. The samples were left at room temperature to completely thaw and were then homogenized before being poured out to measure water quality (including temperature) and for test preparation. The freshwater amphipod test was initiated outside of the 72-hour maximum allowable holding time (the samples were approximately 99 hours past collection) due to the initial order of test organisms being lost in transit. All other tests were initiated within the maximum allowable holding time of 72 hours.

Mean control responses met minimum acceptability criteria for all tests, except for the inland silverside and freshwater amphipod discussed below. Fish, mysid, and amphipod tests were initiated with continuous, light aeration in all sample replicates and the lab control to maintain adequate dissolved oxygen (DO) levels. DO was maintained at appropriate levels for the duration of all tests. Minor QA issues that were unlikely to have any bearing on the final test data, such as slight temperature deviations, are noted on the datasheets and a list of laboratory qualifier codes can be found in Appendix D.

The inland silverside test had a mean survival of 87.5 percent in the lab control and 75 percent in the artificial salt control, which is below the TAC of 90 percent. The lab control for the reference toxicant test initiated the following day resulted in 95 percent mean survival, indicating that the control failure for the DW-1/WW-1 test was likely due to the limited acclimation time allowed in order to initiate the sample test within 72 hours.

The freshwater amphipod lab control had a mean survival of 67.5 percent, which is both below the

minimum acceptability criterion of 90 percent for mean control response. The lab control in the reference toxicant test for this species was 55 percent, indicating that the batch of organisms was not optimal for testing. The organisms were received during a severe ice storm in much of the country and though they are shipped in insulated boxes, excess shipping stress is suspected for this batch of organisms, which were received from an east coast supplier, whereas all other test species were either from an internal culture, or received from Colorado.

**Reference Toxicant Testing**

Results for reference toxicant testing used to monitor laboratory performance and test organism sensitivity are summarized in Table 5. The mean control response for the freshwater amphipod reference toxicant test was below the minimum test acceptability criteria of 90 percent. The amphipod mean control survival was 55 percent; the test showed a dose response and had sensitivity results that were within historical means. The reference toxicant tests for all other species tested met all acceptability criteria. Additionally, the median effect concentration value for these tests was within two standard deviations of the historical mean for all species tested, indicating typical organism sensitivity to copper. The control chart for the previous 20 reference toxicant tests is presented in Appendix E.

**Table 5. Summary of 96-hr Acute Survival Reference Toxicant Test Results**

<b>Species</b>	<b>NOEC (µg/L copper)</b>	<b>LC<sub>50</sub> (µg/L copper)</b>	<b>Historical LC<sub>50</sub> ± 2 SD (µg/L copper)</b>	<b>CV (%)</b>
Pacific Topsmelt	50	107	174 ± 119	34.2
Inland Silverside	100	218	183 ± 95.8	26.1
Mysid Shrimp	200	283	244 ± 70.9	14.5
Fathead Minnow	15	37.5	82.0 ± 62.4	38.0
Water Flea	10	12.8	21.1 ± 9.96	23.6
Freshwater Amphipod	100	106	132 ± 124	46.9

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

LC<sub>50</sub> = concentration expected to cause a lethal effect to 50 percent of the test organisms

Historical LC<sub>50</sub> ± 2 SD = the mean LC<sub>50</sub> from the previous 20 tests performed by Enthalpy, plus or minus two standard deviations

CV = Coefficient of Variation

## **References**

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

USEPA. 2002. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, Fifth Edition (EPA/821/R-02/012). US EPA Office of Water, Washington, DC.

## **Appendix A**

### **Raw Data and Statistical Summaries**

**CETIS Summary Report**

Report Date: 03 Mar-21 09:48 (p 1 of 1)  
 Test Code: 2102-S172 | 05-1763-8751

**Pacific Topsmelt 96-h Acute Survival Test** **Nautilus Environmental (CA)**

Batch ID: 08-9898-2385	Test Type: Survival (96h)	Analyst:
Start Date: 23 Feb-21 15:25	Protocol: EPA/821/R-02-012 (2002)	Diluent: Diluted Natural Seawater
Ending Date: 27 Feb-21 14:55	Species: Atherinops affinis	Brine: Not Applicable
Duration: 96h	Source: Aquatic Biosystems, CO	Age: 13d

Sample ID: 17-1154-7235	Code: 21-0225	Client: Cardno Hawaii
Sample Date: 20 Feb-21 19:45	Material: <i>Effluent Sample Stormwater</i>	Project: ADC Kekaha WQ Monitoring
Receive Date: 23 Feb-21 08:50	Source: Cardno Hawaii	
Sample Age: 68h (2.4 °C)	Station: DW-1/WW-1	

**Comparison Summary**

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
03-4651-5893	96h Survival Rate	100	>100	NA	12.4%	1	Equal Variance t Two-Sample Test

**96h Survival Rate Summary**

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	4	0.9	0.7163	1	0.8	1	0.05774	0.1155	12.83%	0.0%
0	Salt Control	4	0.9	0.7163	1	0.8	1	0.05774	0.1155	12.83%	0.0%
100		4	1	1	1	1	1	0	0	0.0%	-11.11%

**96h Survival Rate Detail**

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Lab Control	0.8	1	1	0.8
0	Salt Control	0.8	1	0.8	1
100		1	1	1	1

*QA AC 3/14/21*

**CETIS Analytical Report**

Report Date: 03 Mar-21 09:48 (p 1 of 1)  
 Test Code: 2102-S172 | 05-1763-8751

**Pacific Topsmelt 96-h Acute Survival Test** Nautilus Environmental (CA)

Analysis ID: 03-4651-5893      Endpoint: 96h Survival Rate      CETIS Version: CETISv1.8.7  
 Analyzed: 03 Mar-21 9:48      Analysis: Parametric-Two Sample      Official Results: Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	Test Result
Angular (Corrected)	NA	C > T	NA	NA	12.4%	Passes 96h survival rate

**Equal Variance t Two-Sample Test**

Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Salt Control		100	-1.732	1.943	0.134	6	0.9330	CDF	Non-Significant Effect

**ANOVA Table**

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.02835395	0.02835395	1	3	0.1340	Non-Significant Effect
Error	0.0567079	0.009451317	6			
Total	0.08506185		7			

**Distributional Tests**

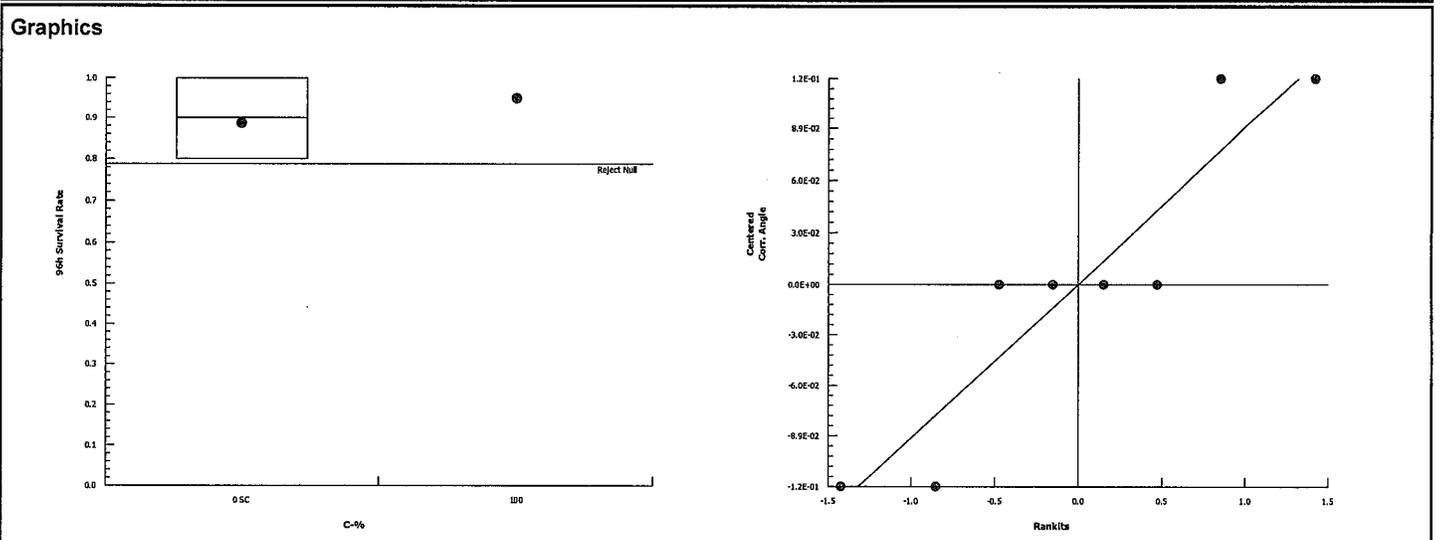
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Distribution	Shapiro-Wilk W Normality	0.8489	0.6451	0.0929	Normal Distribution

**96h Survival Rate Summary**

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Salt Control	4	0.9	0.7163	1	0.9	0.8	1	0.05774	12.83%	0.0%
100		4	1	1	1	1	1	1	0	0.0%	-11.11%

**Angular (Corrected) Transformed Summary**

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Salt Control	4	1.226	1.007	1.445	1.226	1.107	1.345	0.06874	11.21%	0.0%
100		4	1.345	1.345	1.346	1.345	1.345	1.345	0	0.0%	-9.71%



Client: Cardno

Test Species: A. affinis

Sample ID: ADC Kekaha Stormwater Monitoring

Start Date/Time: 2/23/2021 1525

Sample Log-in No.: 21-0225

End Date/Time: 2/27/2021 1455

Test No.: 2102-5172

Tech Initials				
0	24	48	72	96
Counts: DM	DM	TN	DM	DM
Readings: DM	DM	GH	GN	DM
Dilutions made by: GH		TN		

Concentration (%)	Rep	Number of Live Organisms					Salinity (ppt)					Temperature (°C) Q1 Q2 Q3 Q4 Q5					Dissolved Oxygen (mg/L)					pH (units)					
		0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	
Lab Control	A	5	5	4	4	4	30.0	30.4	30.9	30.7	30.6	20.0	19.2	19.7	19.7	19.0	7.5	7.2	7.3	7.3	7.6	8.0	7.9	7.9	7.9	7.9	7.8
	B	5	5	5	5	5		30.4					20.0					7.2					7.8				
	C	5	5	5	5	5																					
	D	5	5	5	5	4																					
Salt Control	A	5	5	5	5	4	30.1	30.5	30.3	30.8	31.0	20.1	19.4	19.5	19.7	19.9	7.3	7.1	7.4	8.4	7.4	8.1	8.05	8.14	8.13	8.08	
	B	5	5	5	5	5		30.5					20.1					7.6	8.0			8.04					
	C	5	4	4	4	4																					
	D	5	5	5	5	5																					
DW-1/WW-31 100%	A	5	5	5	5	5	30.5	30.5	30.4	30.9	31.0	20.8	19.5	19.8	19.8	19.9	7.1	7.1	7.9	7.2	7.3	7.9	8.0	7.88	8.31	8.27	
	B	5	5	5	5	5		30.8					20.2					7.2					8.20				
	C	5	5	5	5	5																					
	D	5	5	5	5	5																					
A																											
B																											
C																											
D																											
A																											
B																											
C																											
D																											

Initial Counts QC'd by: GH  
 Initiated by: DM

Environmental Chamber: C

Animal Source/Date Received: ABS/2/23/21 Age at Initiation: 13 days

Animal Acclimation Qualifiers (circle all that apply): Q22 Q23 / Q24 none  
Q15 RT 2/23/21

Comments: i = initial reading in fresh test solution, f = final reading in test chamber prior to renewal (A) Q15 RT 2/25/21  
 Organisms fed prior to initiation, circle one (y) n ) (A) Q15 PT 2/26/21 (A) Q15 RT 2/23/21

Feeding Times				
0	24	48	72	96
AM:	0845	0850	0920	0905
PM:	1740			

QC Check: ABS 3/2/21

Final Review: AC 3/14/21

**CETIS Summary Report**

Report Date: 14 Mar-21 14:26 (p 1 of 1)  
 Test Code: 2102-S173 | 01-2366-2479

Inland SilverSide 96-h Acute Survival Test	Nautilus Environmental (CA)
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Batch ID: 17-4298-1308	Test Type: Survival (96h)	Analyst:
Start Date: 23 Feb-21 16:40	Protocol: EPA/821/R-02-012 (2002)	Diluent: Diluted Natural Seawater
Ending Date: 27 Feb-21 14:45	Species: Menidia beryllina	Brine: Not Applicable
Duration: 94h	Source: Aquatic Biosystems, CO	Age: 8d

Sample ID: 18-9735-9997	Code: 21-0225	Client: Cardno Hawaii
Sample Date: 20 Feb-21 19:45	Material: Stormwater	Project: ADC Kekaha WQ Monitoring
Receive Date: 23 Feb-21 08:50	Source: Cardno Hawaii	
Sample Age: 69h (2.4 °C)	Station: DW-1/WW-1	

Comparison Summary							
Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
09-0700-8656	96h Survival Rate	100	>100	NA	17.3%	1	Equal Variance t Two-Sample Test
14-0842-3595	96h Survival Rate	100	>100	NA	33.2%	1	Equal Variance t Two-Sample Test

Test Acceptability						
Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
09-0700-8656	96h Survival Rate	Control Resp	0.875	0.9 - NL	Yes	Below Acceptability Criteria
14-0842-3595	96h Survival Rate	Control Resp	0.75	0.9 - NL	Yes	Below Acceptability Criteria <i>P/S</i>

96h Survival Rate Summary											
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	4	0.875	0.6748	1	0.7	1	0.06292	0.1258	14.38%	0.0%
0	Salt Control	4	0.75	0.3712	1	0.4	0.9	0.119	0.238	31.74%	14.29%
100		4	0.825	0.6727	0.9773	0.7	0.9	0.04787	0.09574	11.61%	5.71%

96h Survival Rate Detail					
C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Lab Control	0.7	1	0.9	0.9
0	Salt Control	0.4	0.9	0.8	0.9
100		0.9	0.9	0.7	0.8

**CETIS Analytical Report**

Report Date: 14 Mar-21 14:26 (p 1 of 2)  
 Test Code: 2102-S173 | 01-2366-2479

Inland SilverSide 96-h Acute Survival Test Nautilus Environmental (CA)

Analysis ID: 09-0700-8656      Endpoint: 96h Survival Rate      CETIS Version: CETISv1.8.7  
 Analyzed: 14 Mar-21 14:26      Analysis: Parametric-Two Sample      Official Results: Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	Test Result
Angular (Corrected)	NA	C > T	NA	NA	17.3%	Passes 96h survival rate

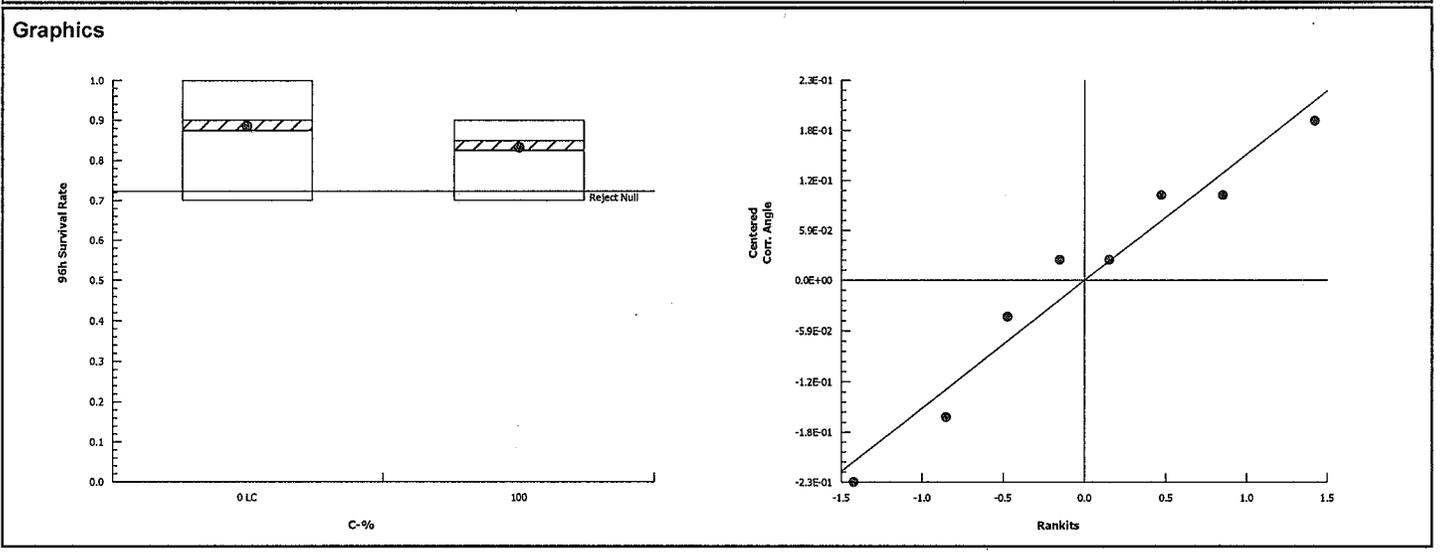
Equal Variance t Two-Sample Test									
Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		100	0.712	1.943	0.208	6	0.2516	CDF	Non-Significant Effect

ANOVA Table						
Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.01161802	0.01161802	1	0.507	0.5032	Non-Significant Effect
Error	0.1374981	0.02291635	6			
Total	0.1491161		7			

Distributional Tests					
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Variance Ratio F	1.945	47.47	0.5985	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9481	0.6451	0.6925	Normal Distribution

96h Survival Rate Summary											
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	4	0.875	0.6748	1	0.9	0.7	1	0.06292	14.38%	0.0%
100		4	0.825	0.6727	0.9773	0.85	0.7	0.9	0.04787	11.61%	5.71%

Angular (Corrected) Transformed Summary											
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	4	1.225	0.9485	1.502	1.249	0.9912	1.412	0.08699	14.2%	0.0%
100		4	1.149	0.9506	1.348	1.178	0.9912	1.249	0.06237	10.86%	6.22%



# CETIS Analytical Report

Report Date: 14 Mar-21 14:26 (p 2 of 2)  
 Test Code: 2102-S173 | 01-2366-2479

**Inland SilverSide 96-h Acute Survival Test** Nautilus Environmental (CA)

Analysis ID: 14-0842-3595      Endpoint: 96h Survival Rate      CETIS Version: CETISv1.8.7  
 Analyzed: 03 Mar-21 9:39      Analysis: Parametric-Two Sample      Official Results: Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	Test Result
Angular (Corrected)	NA	C > T	NA	NA	33.2%	Passes 96h survival rate

**Equal Variance t Two-Sample Test**

Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Salt Control		100	-0.5199	1.943	0.286	6	0.6891	CDF	Non-Significant Effect

**ANOVA Table**

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.01173798	0.01173798	1	0.2703	0.6218	Non-Significant Effect
Error	0.2605956	0.04343261	6			
Total	0.2723336		7			

**Distributional Tests**

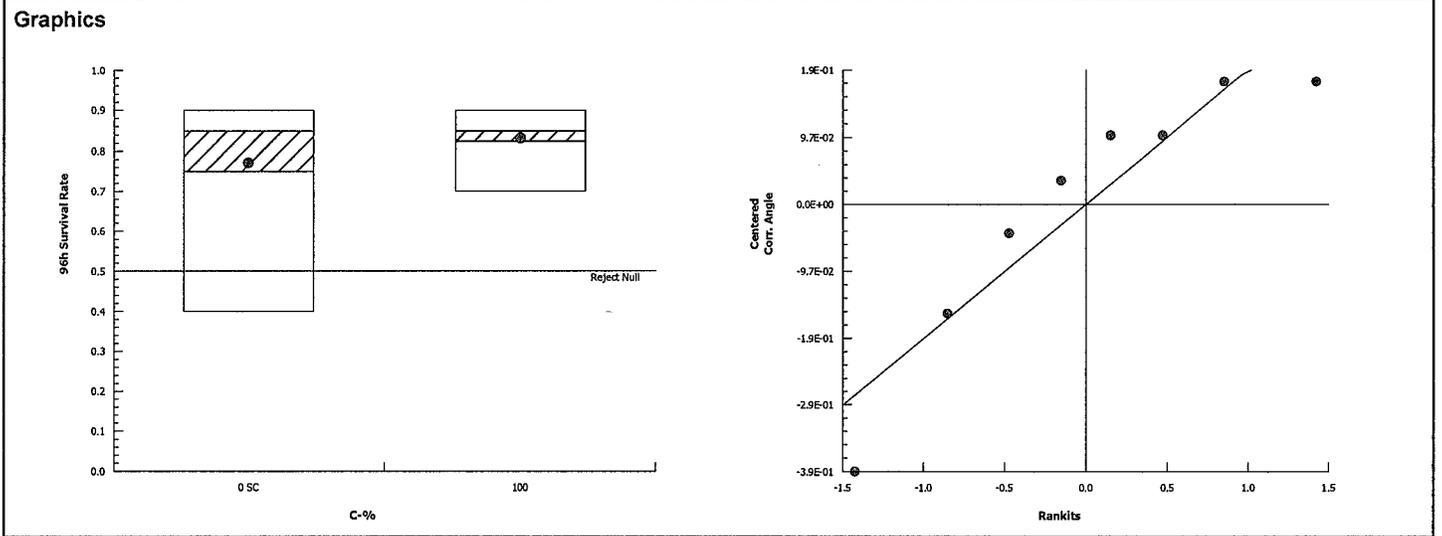
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Variance Ratio F	4.582	47.47	0.2431	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.8687	0.6451	0.1463	Normal Distribution

**96h Survival Rate Summary**

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Salt Control	4	0.75	0.3712	1	0.85	0.4	0.9	0.119	31.74%	0.0%
100		4	0.825	0.6727	0.9773	0.85	0.7	0.9	0.04787	11.61%	-10.0%

**Angular (Corrected) Transformed Summary**

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Salt Control	4	1.072	0.6476	1.497	1.178	0.6847	1.249	0.1335	24.9%	0.0%
100		4	1.149	0.9506	1.348	1.178	0.9912	1.249	0.06237	10.86%	-7.14%



Client: Cardno

Test Species: M. beryllina

Sample ID: ADC Kekaha Stormwater Monitoring

Start Date/Time: 2/23/2021 1640

Sample Log-in No.: 21-0225

End Date/Time: 2/27/2021 1445

Test No.: 2102-5173

Tech Initials				
0	24	48	72	96
GH	DM	DM	DM	DM
DM	DM	GH	GH	DM
GH		DM		

Counts: GH DM DM DM DM

Readings: DM DM GH GH DM

Dilutions made by: GH DM DM

Concentration (%)	Rep	Number of Live Organisms					Salinity (ppt)					Temperature (°C)					Dissolved Oxygen (mg/L)					pH (units)				
		0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96
Lab Control	A	10	7	7	7	7	30.2	30.8	29.4	30.8	31.4	24.3	24.2	25.3	24.7	24.2	6.6	6.4	6.9	6.9	6.6	8.04	8.00	7.94	2.98	7.8
	B	10	10	10	10	10			31.3					24.9					6.7					7.97		
	C	10	9	9	9	9																				
	D	10	9	9	9	9					31.3															
Salt Control	A	10	4	4	4	4	30.1	30.8	30.3	31.3	31.6	24.4	24.5	25.2	24.9	24.1	6.4	6.3	6.6	6.3	6.3	8.11	8.14	8.17	8.19	8.05
	B	10	9	9	9	9			31.4					25.2					6.5					8.15		
	C	10	8	8	8	8																				
	D	10	9	9	9	9																				
DW-1/WW-3 100%	A	10	9	9	9	9	30.1	30.9	30.2	31.3	31.6	25.1	25.0	24.8	25.0	24.2	6.5	6.3	6.7	6.3	6.6	7.8	8.2	7.94	8.32	8.31
	B	10	9	9	9	9			31.6					25.3					6.5					8.29		
	C	10	8	7	7	7																				
	D	10	10	9	9	8																				
	A																									
	B																									
	C																									
	D																									
	A																									
	B																									
	C																									
	D																									
	A																									
	B																									
	C																									
	D																									

Initial Counts QC'd by: GH DM DM

Initiated by: GH

Environmental Chamber: A

Animal Source/Date Received: ABS / 2/23/21

Age at Initiation: 38 8d

Animal Acclimation Qualifiers (circle all that apply): Q22 / Q23 / Q24 / none

Comments: i = initial reading in fresh test solution, f = final reading in test chamber prior to renewal

Organisms fed prior to initiation, circle one (y/n) (y) / n) (A) Q18 GH 2/23/21 (B) Q18 GH 2/25/21  
(C) Q18 GH 2/26/21 (D) Q18 GH 3/2/21

QC Check: AS 3/2/21

Final Review:

Feeding Times				
0	24	48	72	96
AM:	0845	0850	0824	0906
PM:	1740			

AC 3/14/21

**CETIS Summary Report**

Report Date: 03 Mar-21 09:27 (p 1 of 1)  
 Test Code: 2102-S174 | 12-9254-5624

Mysid 96-h Acute Survival Test							Nautilus Environmental (CA)				
Batch ID:	12-9241-1903	Test Type:	Survival (96h)	Analyst:							
Start Date:	23 Feb-21 15:35	Protocol:	EPA/821/R-02-012 (2002)	Diluent:	Diluted Natural Seawater						
Ending Date:	27 Feb-21 14:30	Species:	Americamysis bahia	Brine:	Not Applicable						
Duration:	95h	Source:	Aquatic Biosystems, CO	Age:	3d						
Sample ID:	17-8906-8084	Code:	21-0225	Client:	Cardno Hawaii						
Sample Date:	20 Feb-21 19:45	Material:	Effluent Sample Stormwater	Project:	ADC Kekaha WQ Monitoring						
Receive Date:	23 Feb-21 08:50	Source:	Cardno Hawaii								
Sample Age:	68h (2.4 °C)	Station:	DW-1/WW-1								
Comparison Summary											
Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method				
16-3798-3384	96h Survival Rate	100	>100	NA	16.4%	1	Equal Variance t Two-Sample Test				
Test Acceptability											
Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision					
16-3798-3384	96h Survival Rate	Control Resp	0.9	0.9 - NL	Yes	Passes Acceptability Criteria					
96h Survival Rate Summary											
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	4	0.95	0.7909	1	0.8	1	0.05	0.1	10.53%	0.0%
0	Salt Control	4	0.9	0.7163	1	0.8	1	0.05774	0.1155	12.83%	5.26%
100		4	0.95	0.7909	1	0.8	1	0.05	0.1	10.53%	0.0%
96h Survival Rate Detail											
C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4						
0	Lab Control	1	1	0.8	1						
0	Salt Control	1	1	0.8	0.8						
100		1	1	1	0.8						

Q16 AC 3/14/21

**CETIS Analytical Report**

Report Date: 03 Mar-21 09:27 (p 1 of 1)  
 Test Code: 2102-S174 | 12-9254-5624

<b>Mysid 96-h Acute Survival Test</b>	<b>Nautilus Environmental (CA)</b>
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Analysis ID: 16-3798-3384	Endpoint: 96h Survival Rate	CETIS Version: CETISv1.8.7
Analyzed: 03 Mar-21 9:27	Analysis: Parametric-Two Sample	Official Results: Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	Test Result
Angular (Corrected)	NA	C > T	NA	NA	16.4%	Passes 96h survival rate

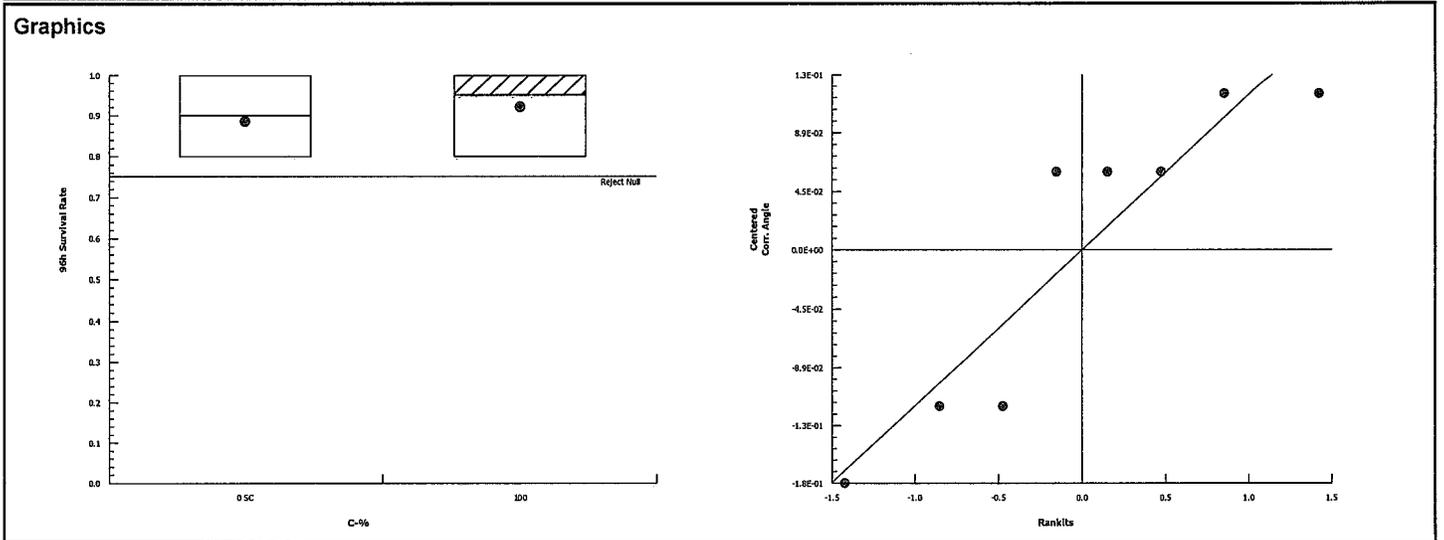
Equal Variance t Two-Sample Test									
Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Salt Control		100	-0.6547	1.943	0.177	6	0.7315	CDF	Non-Significant Effect

ANOVA Table						
Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.007088488	0.007088488	1	0.4286	0.5370	Non-Significant Effect
Error	0.09923882	0.0165398	6			
Total	0.1063273		7			

Distributional Tests					
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Variance Ratio F	1.333	47.47	0.8187	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.8283	0.6451	0.0570	Normal Distribution

96h Survival Rate Summary											
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Salt Control	4	0.9	0.7163	1	0.9	0.8	1	0.05774	12.83%	0.0%
100		4	0.95	0.7909	1	1	0.8	1	0.05	10.53%	-5.56%

Angular (Corrected) Transformed Summary											
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Salt Control	4	1.226	1.007	1.445	1.226	1.107	1.345	0.06874	11.21%	0.0%
100		4	1.286	1.096	1.475	1.345	1.107	1.345	0.05953	9.26%	-4.86%



Client: Cardno

Test Species: A. bahia

Sample ID: ADC Kekaha Stormwater Monitoring

Start Date/Time: 2/23/2021 1535

Sample Log-in No.: 21-0225

End Date/Time: 2/27/2021 1430

Test No.: 2102-8174

Tech Initials				
0	24	48	72	96
GH	RM	DM	DM	DM
DM	DM	GH	GH	DM
GH		FW		

Counts:

Readings:

Dilutions made by:

Concentration (%)	Rep	Number of Live Organisms					Salinity (ppt)					Temperature (°C)					Dissolved Oxygen (mg/L)					pH (units)				
		0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96
Lab Control	A	5	5	5	5	5	30.4	30.8	31.3	30.4	30.5	24.3	24.6	25.3	25.0	24.0	6.7	6.5	6.8	6.2	6.2	7.98	8.03	7.96	8.00	8.00
	B	5	5	5	5	5			30.9					25.3				6.5						8.00		
	C	5	5	5	4	4																				
	D	5	5	5	5	5																				
Salt Control	A	5	5	5	5	5	30.8	31.2	30.3	31.4	31.7	24.7	24.7	25.3	25.1	24.1	6.3	6.4	6.7	6.3	6.4	8.17	8.13	8.18	8.18	8.12
	B	5	5	5	5	5			31.8					25.3				6.4						8.17		
	C	5	5	4	4	4																				
	D	5	4	4	4	4																				
DW-1/WW-20 100%	A	5	5	5	5	5	30.4	30.8	30.4	31.0	31.6	24.8	24.9	25.0	25.0	24.2	6.6	6.4	7.3	6.3	6.4	7.85	8.10	7.88	8.03	8.28
	B	5	5	5	5	5			31.6					25.4				6.4						8.13		
	C	5	5	5	5	5																				
	D	5	5	4	4	4																				
	A																									
	B																									
	C																									
	D																									
	A																									
	B																									
	C																									
	D																									

Initial Counts QC'd by: DM  
 Initiated by: GH

Environmental Chamber: A

Animal Source/Date Received: ATS / 2/23/21 Age at Initiation: 3d

Animal Acclimation Qualifiers (circle all that apply): Q22 / (Q23) / Q24 / none

Comments: i = initial reading in fresh test solution, f = final reading in test chamber prior to renewal @ Q18ATS 3/2/21  
Organisms fed prior to initiation, circle one (y) n )

QC Check: ATS 3/2/21

Final Review:

Feeding Times				
0	24	48	72	96
AM:	0800	0850	0824	0905
PM:	1740	1830	1810	1630

AC 3/14/21

**CETIS Summary Report**

Report Date: 03 Mar-21 10:04 (p 1 of 1)  
 Test Code: 2102-S175 | 13-8567-2692

**Fathead Minnow 96-h Acute Survival Test** **Nautilus Environmental (CA)**

<b>Batch ID:</b> 15-4852-6876	<b>Test Type:</b> Survival (96h)	<b>Analyst:</b>
<b>Start Date:</b> 23 Feb-21 13:50	<b>Protocol:</b> EPA/821/R-02-012 (2002)	<b>Diluent:</b> Diluted Mineral Water (8:2)
<b>Ending Date:</b> 27 Feb-21 15:45	<b>Species:</b> Pimephales promelas	<b>Brine:</b> Not Applicable
<b>Duration:</b> 4d 2h	<b>Source:</b> Aquatic Biosystems, CO	<b>Age:</b> 6d

<b>Sample ID:</b> 11-9545-3973	<b>Code:</b> 21-0226	<b>Client:</b> Cardno Hawaii
<b>Sample Date:</b> 20 Feb-21 14:00	<b>Material:</b> Stormwater	<b>Project:</b> ADC Kekaha WQ Monitoring
<b>Receive Date:</b> 23 Feb-21 08:50	<b>Source:</b> Cardno Hawaii	
<b>Sample Age:</b> 72h (2.9 °C)	<b>Station:</b> WW-2	

**Comparison Summary**

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
09-6027-0742	96h Survival Rate	100	>100	NA	8.4%	1	Equal Variance t Two-Sample Test

**Test Acceptability**

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
09-6027-0742	96h Survival Rate	Control Resp	0.975	0.9 - NL	Yes	Passes Acceptability Criteria

**96h Survival Rate Summary**

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	4	0.975	0.8954	1	0.9	1	0.025	0.05	5.13%	0.0%
100		3	0.9333	0.7899	1	0.9	1	0.03333	0.05774	6.19%	4.27%

**96h Survival Rate Detail**

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Lab Control	1	1	0.9	1
100		0.9	0.9	0.8	1

*QB replicate spilled; excluded from analysis.*

# CETIS Analytical Report

Report Date: 03 Mar-21 10:04 (p 1 of 1)  
 Test Code: 2102-S175 | 13-8567-2692

**Fathead Minnow 96-h Acute Survival Test** **Nautilus Environmental (CA)**

Analysis ID: 09-6027-0742      Endpoint: 96h Survival Rate      CETIS Version: CETISv1.8.7  
 Analyzed: 03 Mar-21 10:04      Analysis: Parametric-Two Sample      Official Results: Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	Test Result
Angular (Corrected)	NA	$\hat{C} > T$	NA	NA	8.4%	Passes 96h survival rate

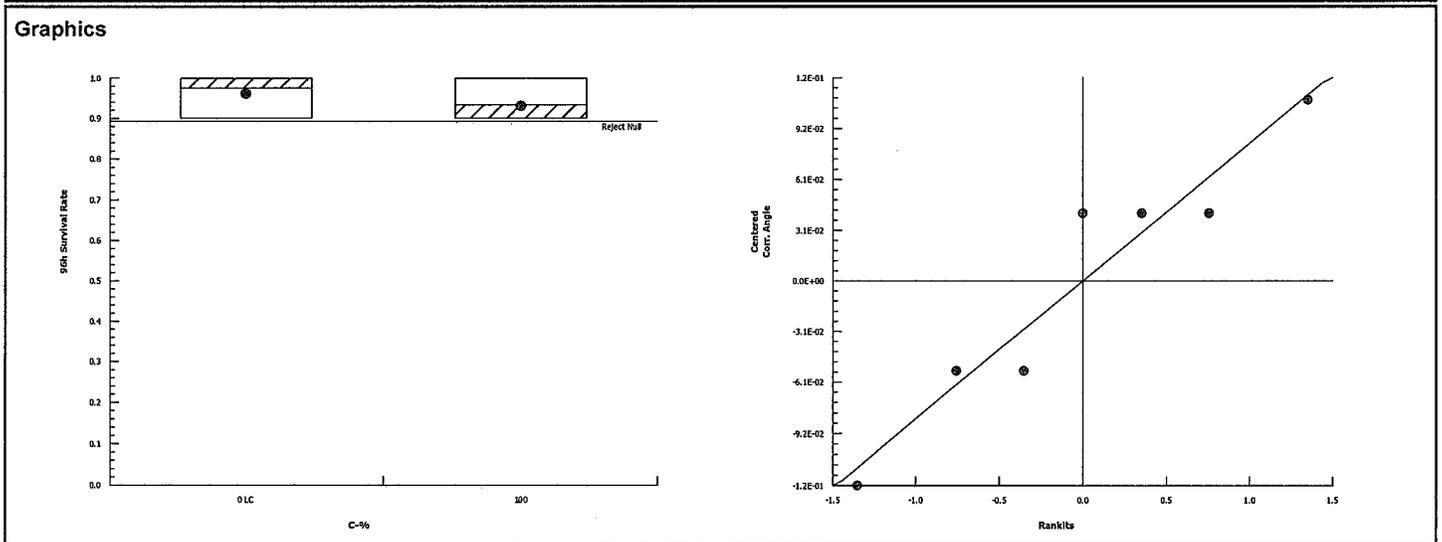
Equal Variance t Two-Sample Test									
Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision( $\alpha:5\%$ )
Lab Control		100	1.025	2.015	0.134	5	0.1762	CDF	Non-Significant Effect

ANOVA Table						
Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision( $\alpha:5\%$ )
Between	0.007904563	0.007904563	1	1.05	0.3524	Non-Significant Effect
Error	0.03762572	0.007525144	5			
Total	0.04553028		6			

Distributional Tests						
Attribute	Test	Test Stat	Critical	P-Value	Decision( $\alpha:1\%$ )	
Variances	Variance Ratio F	1.333	49.8	0.7704	Equal Variances	
Distribution	Shapiro-Wilk W Normality	0.9203	0.5629	0.4717	Normal Distribution	

96h Survival Rate Summary											
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	4	0.975	0.8954	1	1	0.9	1	0.025	5.13%	0.0%
100		3	0.9333	0.7899	1	0.9	0.9	1	0.03333	6.19%	4.27%

Angular (Corrected) Transformed Summary											
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	4	1.371	1.242	1.501	1.412	1.249	1.412	0.04074	5.94%	0.0%
100		3	1.303	1.07	1.537	1.249	1.249	1.412	0.05432	7.22%	4.95%



**CETIS Summary Report**

Report Date: 03 Mar-21 10:07 (p 1 of 1)  
 Test Code: 2102-S178 | 04-4674-5014

**Fathead Minnow 96-h Acute Survival Test** **Nautilus Environmental (CA)**

<b>Batch ID:</b> 09-2651-1067	<b>Test Type:</b> Survival (96h)	<b>Analyst:</b>
<b>Start Date:</b> 23 Feb-21 13:50	<b>Protocol:</b> EPA/821/R-02-012 (2002)	<b>Diluent:</b> Diluted Mineral Water (8:2)
<b>Ending Date:</b> 27 Feb-21 15:45	<b>Species:</b> Pimephales promelas	<b>Brine:</b> Not Applicable
<b>Duration:</b> 4d 2h	<b>Source:</b> Aquatic Biosystems, CO	<b>Age:</b> 6d

<b>Sample ID:</b> 15-9661-4055	<b>Code:</b> 21-0227	<b>Client:</b> Cardno Hawaii
<b>Sample Date:</b> 20 Feb-21 14:30	<b>Material:</b> Stormwater	<b>Project:</b> ADC Kekaha WQ Monitoring
<b>Receive Date:</b> 23 Feb-21 08:50	<b>Source:</b> Cardno Hawaii	
<b>Sample Age:</b> 71h (3.3 °C)	<b>Station:</b> WW-3	

Comparison Summary							
Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
05-1803-6637	96h Survival Rate	100	>100	NA	9.11%	1	Equal Variance t Two-Sample Test

Test Acceptability						
Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
05-1803-6637	96h Survival Rate	Control Resp	0.975	0.9 - NL	Yes	Passes Acceptability Criteria

96h Survival Rate Summary											
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	4	0.975	0.8954	1	0.9	1	0.025	0.05	5.13%	0.0%
100		4	0.9	0.7701	1	0.8	1	0.04082	0.08165	9.07%	7.69%

96h Survival Rate Detail					
C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Lab Control	1	1	0.9	1
100		0.9	1	0.9	0.8

# CETIS Analytical Report

Report Date: 03 Mar-21 10:07 (p 1 of 1)  
 Test Code: 2102-S178 | 04-4674-5014

Fathead Minnow 96-h Acute Survival Test			Nautilus Environmental (CA)		
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Analysis ID: 05-1803-6637	Endpoint: 96h Survival Rate	CETIS Version: CETISv1.8.7
Analyzed: 03 Mar-21 10:07	Analysis: Parametric-Two Sample	Official Results: Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	Test Result
Angular (Corrected)	NA	C > T	NA	NA	9.11%	Passes 96h survival rate

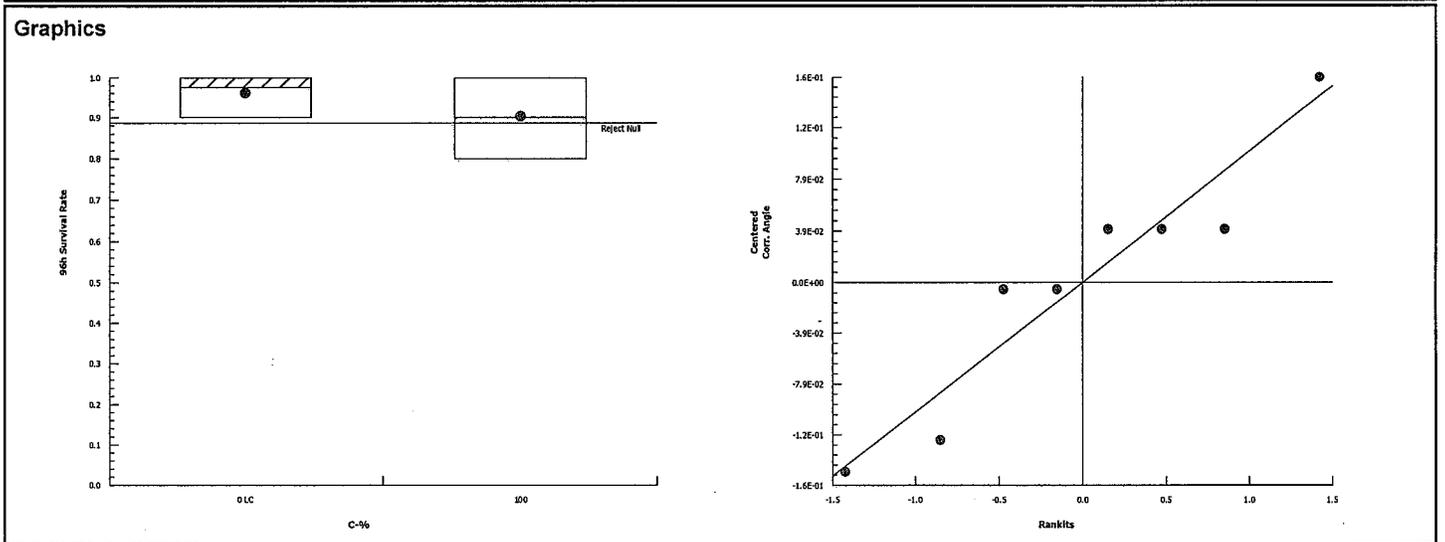
Equal Variance t Two-Sample Test									
Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		100	1.571	1.943	0.145	6	0.0836	CDF	Non-Significant Effect

ANOVA Table						
Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.02735902	0.02735902	1	2.468	0.1672	Non-Significant Effect
Error	0.06650259	0.01108376	6			
Total	0.0938616		7			

Distributional Tests					
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Variance Ratio F	2.339	47.47	0.5035	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9149	0.6451	0.3900	Normal Distribution

96h Survival Rate Summary											
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	4	0.975	0.8954	1	1	0.9	1	0.025	5.13%	0.0%
100		4	0.9	0.7701	1	0.9	0.8	1	0.04082	9.07%	7.69%

Angular (Corrected) Transformed Summary											
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	4	1.371	1.242	1.501	1.412	1.249	1.412	0.04074	5.94%	0.0%
100		4	1.254	1.056	1.453	1.249	1.107	1.412	0.06231	9.94%	8.53%



96-hour Freshwater Acute Bioassay  
 Static-Renewal Conditions  
 DF-006

Water Quality Measurements  
 & Test Organism Survival

Client: Cardno

Test Species: P. promelas

Sample ID: ADC Kehaha Stormwater Monitoring

Start Date/Time: 2/23/2021 1350

Sample Log-in No's.: 21-0226, 21-0227

End Date/Time: 2/27/2021 1545

Test No's.: 2102-5175 + -5174

Tech Initials				
0	24	48	72	96
TN	PT	TN	GH	GH
RT	HH	TN	GH	GH
GH		TN		

Counts:

Readings:

Dilutions made by:

Sample ID (100%)	Rep	Number of Live Organisms					Conductivity (µmhos/cm)					Temperature (°C)					Dissolved Oxygen (mg/L)					pH (units)					
		0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	
Lab Control	A	10	10	10	10	10	187	200	187	197	201	20.5	20.3	19.4	19.9	20.0	8.6	8.6	8.5	9.0	8.9	8.13	8.11	8.05	8.08	8.25	
	B	10	10	10	10	10			211					20.1					8.5					8.09			
	C	10	10	10	9	9																					
	D	10	10	10	10	10											8.14										
WW-2	A	10	9	9	9	9	516	515	516	535	550	20.4	20.2	19.5	20.0	20.0	7.3	8.6	7.3	9.0	8.8	6.00	6.94	6.65	6.88	7.27	
	B	10	9	9	9	9			528					20.2					8.8					6.93			
	C	10	9	9	9	9																					
	D	10	10	10	10	10											8.14										
WW-3	A	10	9	9	9	9	90	108	91	95	99	19.9	20.3	19.4	20.1	20.0	9.8	8.5	9.5	8.9	8.1	6.07	6.51	6.02	6.45	6.47	
	B	10	9	9	9	9			114					19.9					8.9					6.49			
	C	10	9	9	9	9																					
	D	10	9	9	9	9																					

Initial Counts QC'd by: RT  
 Initiated by: TN

Environmental Chamber: C

Animal Source/Date Received: ABS 2/23/21 Age at Initiation: 6 DAY

Animal Acclimation Qualifiers (circle all that apply): Q22 / Q23 / Q24 none

Feeding Times				
0	24	48	72	96
AM:	--	--	05:00	--
PM:	--	--	--	--

Comments: i = initial reading in fresh test solution, f = final reading in test chamber prior to renewal

Organisms fed prior to initiation, circle one (y / n) Q8: 3 fish recovered  
ⓑ TN GH 2/25/21 Ⓒ Q8 GH 2/27/21 2/21/21 RT

QC Check: ACS 3/2/21

Final Review: AC 3/14/21

**CETIS Summary Report**

Report Date: 03 Mar-21 10:19 (p 1 of 1)  
 Test Code: 2102-S176 | 14-4584-8267

**Ceriodaphnia 96-h Acute Survival Test** **Nautilus Environmental (CA)**

Batch ID: 14-2803-4336	Test Type: Survival (96h)	Analyst:
Start Date: 23 Feb-21 13:40	Protocol: EPA/821/R-02-012 (2002)	Diluent: Diluted Mineral Water (8:2)
Ending Date: 27 Feb-21 12:20	Species: Ceriodaphnia dubia	Brine: Not Applicable
Duration: 95h	Source: In-House Culture	Age: <24h

Sample ID: 07-8038-3263	Code: 21-0226	Client: Cardno Hawaii
Sample Date: 20 Feb-21 14:00	Material: Stormwater	Project: ADC Kekaha WQ Monitoring
Receive Date: 23 Feb-21 08:50	Source: Cardno Hawaii	
Sample Age: 72h (2.9 °C)	Station: WW-2	

**Comparison Summary**

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
10-5777-9973	96h Survival Rate	100	>100	NA	NA	1	Wilcoxon Rank Sum Two-Sample Test

**Test Acceptability**

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
10-5777-9973	96h Survival Rate	Control Resp	1	0.9 - NL	Yes	Passes Acceptability Criteria

**96h Survival Rate Summary**

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	4	1	1	1	1	1	0	0	0.0%	0.0%
100		4	1	1	1	1	1	0	0	0.0%	0.0%

**96h Survival Rate Detail**

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Lab Control	1	1	1	1
100		1	1	1	1

# CETIS Analytical Report

Report Date: 03 Mar-21 10:19 (p 1 of 1)  
 Test Code: 2102-S176 | 14-4584-8267

**Ceriodaphnia 96-h Acute Survival Test** **Nautilus Environmental (CA)**

Analysis ID: 10-5777-9973      Endpoint: 96h Survival Rate      CETIS Version: CETISv1.8.7  
 Analyzed: 03 Mar-21 10:19      Analysis: Nonparametric-Two Sample      Official Results: Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	Test Result
Angular (Corrected)	NA	C > T	NA	NA	Passes 96h survival rate

**Wilcoxon Rank Sum Two-Sample Test**

Control	vs	C-%	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		100	18	NA	1	6	1.0000	Exact	Non-Significant Effect

**ANOVA Table**

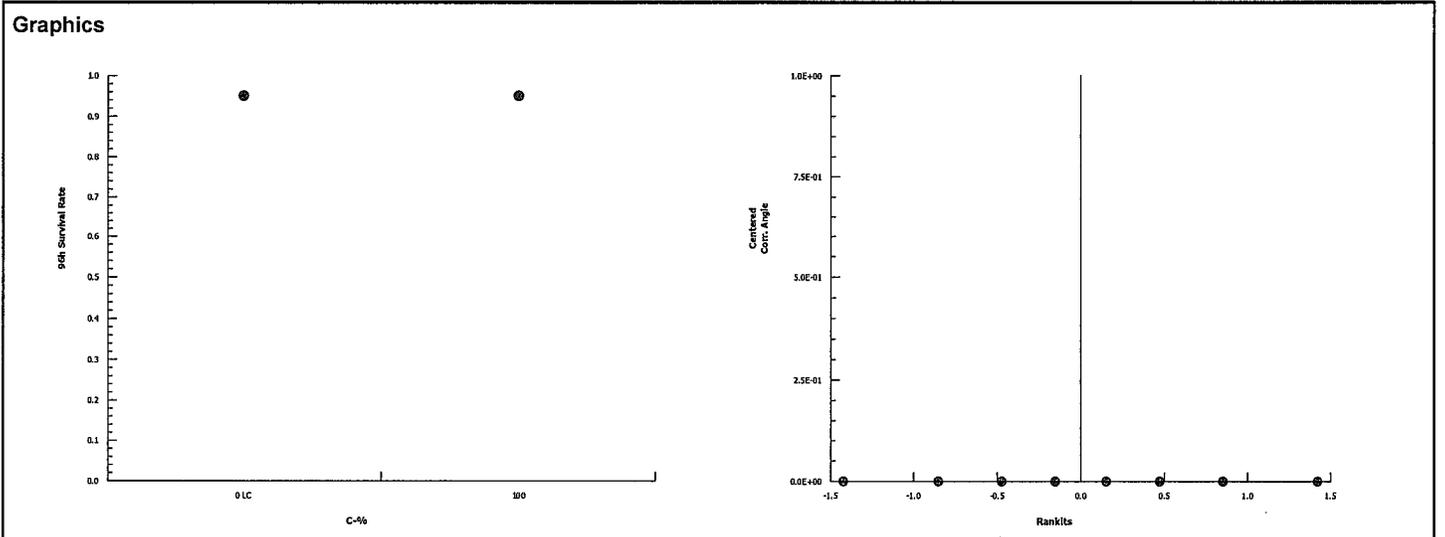
Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0	0	1	65540	<0.0001	Significant Effect
Error	0	0	6			
Total	0		7			

**96h Survival Rate Summary**

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	4	1	1	1	1	1	1	0	0.0%	0.0%
100		4	1	1	1	1	1	1	0	0.0%	0.0%

**Angular (Corrected) Transformed Summary**

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	4	1.345	1.345	1.346	1.345	1.345	1.345	0	0.0%	0.0%
100		4	1.345	1.345	1.346	1.345	1.345	1.345	0	0.0%	0.0%



**CETIS Summary Report**

Report Date: 03 Mar-21 10:22 (p 1 of 1)  
 Test Code: 2102-S179 | 21-2907-8955

**Ceriodaphnia 96-h Acute Survival Test** **Nautilus Environmental (CA)**

<b>Batch ID:</b> 16-9753-0785	<b>Test Type:</b> Survival (96h)	<b>Analyst:</b>
<b>Start Date:</b> 23 Feb-21 13:40	<b>Protocol:</b> EPA/821/R-02-012 (2002)	<b>Diluent:</b> Diluted Mineral Water (8:2)
<b>Ending Date:</b> 27 Feb-21 12:20	<b>Species:</b> Ceriodaphnia dubia	<b>Brine:</b> Not Applicable
<b>Duration:</b> 95h	<b>Source:</b> In-House Culture	<b>Age:</b> <24h

<b>Sample ID:</b> 07-5199-5424	<b>Code:</b> 21-0227	<b>Client:</b> Cardno Hawaii
<b>Sample Date:</b> 20 Feb-21 14:30	<b>Material:</b> Stormwater	<b>Project:</b> ADC Kekaha WQ Monitoring
<b>Receive Date:</b> 23 Feb-21 08:50	<b>Source:</b> Cardno Hawaii	
<b>Sample Age:</b> 71h (3.3 °C)	<b>Station:</b> WW-3	

Comparison Summary							
Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
02-9979-0049	96h Survival Rate	<100	100	NA	18.8%	>1	Wilcoxon Rank Sum Two-Sample Test

Test Acceptability						
Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
02-9979-0049	96h Survival Rate	Control Resp	1	0.9 - NL	Yes	Passes Acceptability Criteria

96h Survival Rate Summary											
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	4	1	1	1	1	1	0	0	0.0%	0.0%
100		4	0.1	0	0.4182	0	0.4	0.1	0.2	200.0%	90.0%

96h Survival Rate Detail					
C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Lab Control	1	1	1	1
100		0	0	0.4	0

# CETIS Analytical Report

Report Date: 03 Mar-21 10:22 (p 1 of 1)  
 Test Code: 2102-S179 | 21-2907-8955

**Ceriodaphnia 96-h Acute Survival Test** Nautilus Environmental (CA)

Analysis ID: 02-9979-0049      Endpoint: 96h Survival Rate      CETIS Version: CETISv1.8.7  
 Analyzed: 03 Mar-21 10:22      Analysis: Nonparametric-Two Sample      Official Results: Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	Test Result
Angular (Corrected)	NA	C > T	NA	NA	18.8%	Fails 96h survival rate

**Wilcoxon Rank Sum Two-Sample Test**

Control	vs	C-%	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		100*	10	NA	0	6	0.0143	Exact	Significant Effect

**ANOVA Table**

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	2.019922	2.019922	1	76.63	0.0001	Significant Effect
Error	0.1581525	0.02635875	6			
Total	2.178074		7			

**Distributional Tests**

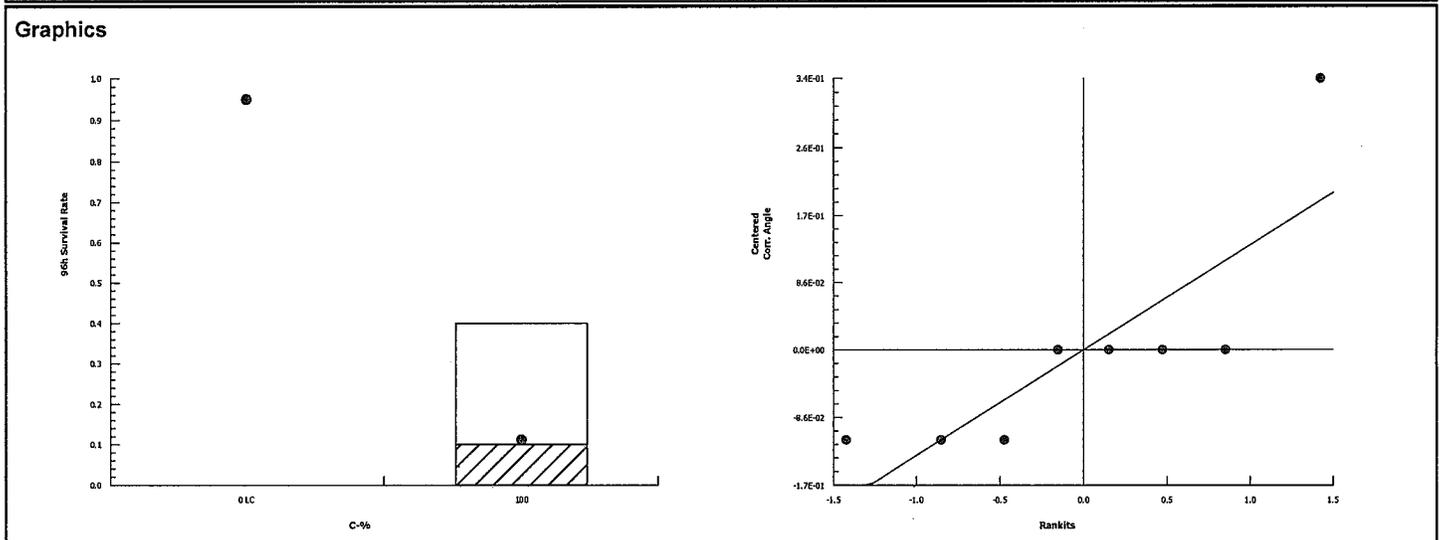
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Mod Levene Equality of Variance	1	13.75	0.3559	Equal Variances
Variances	Levene Equality of Variance	9	13.75	0.0240	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.7065	0.6451	0.0027	Non-normal Distribution

**96h Survival Rate Summary**

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	4	1	1	1	1	1	1	0	0.0%	0.0%
100		4	0.1	0	0.4182	0	0	0.4	0.1	200.0%	90.0%

**Angular (Corrected) Transformed Summary**

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	4	1.345	1.345	1.346	1.345	1.345	1.345	0	0.0%	0.0%
100		4	0.3403	-0.02503	0.7057	0.2255	0.2255	0.6847	0.1148	67.47%	74.7%



96-hour Freshwater Acute Bioassay  
 Static-Renewal Conditions  
 DF-002

Water Quality Measurements  
 & Test Organism Survival

Client: Cardno

Test Species: C. dubia

Sample ID: ADC Kehaha Stormwater Monitoring

Start Date/Time: 2/23/2021 1340

Sample Log-in No.: 21-0226, 21-0227

End Date/Time: 2/27/2021 12-20

Test No.: 2102-5176 a -5179

Tech Initials				
0	24	48	72	96
KL	RT	HH	RT	RT
KL	HH	TN	GH	GH
GH		TN		

Counts:

Readings:

Dilutions made by:

Concentration (%)	Rep	Number of Live Organisms					Conductivity (µmhos/cm)					Temperature (°C)					Dissolved Oxygen (mg/L)					pH (units)				
		0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96
Lab Control	A	5	5	5	5	5	180	189	186	188	204	21.0	20.5	20.3	20.4	19.9	8.2	8.10	8.5	8.8	8.3	8.5	8.10	8.08	8.13	8.25
	B	5	5	5	5	5			196					19.9					9.0					8.14		
	C	5	5	5	5	5																				
	D	5	5	5	5	5																				
WW-2 100%	A	5	5	5	5	5	86	84	52	57	532	20.0	20.3	20.0	20.3	19.9	9.2	8.10	9.5	9.0	8.6	6.28	6.0	6.54	7.10	7.68
	B	5	5	5	5	5			86					19.9					9.5					7.38		
	C	5	5	5	5	5																				
	D	5	5	5	5	5																				
WW-3 100%	A	5	Q13	0	Q13	0	88	90	90	98		19.6	20.2	20.0	20.2	19.9	9.2	8.5	9.1	9.0	8.7	6.01	6.10	6.02	6.5	7.27
	B	5		3		0			96					19.9					8.5					6.69	6.14	
	C	5		3		2																				
	D	5		5		0																				

Initial Counts QC'd by: HH  
 Initiated by: KL

Environmental Chamber: C

Animal Source/Date Received:

Internal / N/A  
2/23/21  
Q13/23/21

Age at Initiation: < 24 hrs

Comments:

i = initial reading in fresh test solution, f = final reading in test chamber prior to renewal

Organisms fed prior to initiation, circle one (y/n)

Feeding Times				
0	24	48	72	96
--	--	1415	--	--
--	--	--	--	--

QC Check: AS 3/2/21

Final Review: AC 3/14/21

**CETIS Summary Report**

Report Date: 03 Mar-21 10:27 (p 1 of 1)  
 Test Code: 2102-S177 | 01-4759-0518

Acute Amphipod Survival Test						Nautilus Environmental (CA)					
Batch ID:	11-5232-2979	Test Type:	Survival (96h)	Analyst:							
Start Date:	24 Feb-21 17:20	Protocol:	EPA/600/R-99/064 (2000)	Diluent:	<sup>a</sup> <del>Coast Filtered Water</del> Dilute Mineral Water						
Ending Date:	28 Feb-21 15:20	Species:	Hyaella azteca	Brine:	Not Applicable						
Duration:	94h	Source:	Aquatic Research Organisms, NH	Age:	13d						
Sample ID:	11-8640-4152	Code:	21-0226	Client:	Cardno Hawaii						
Sample Date:	20 Feb-21 14:00	Material:	Stormwater	Project:	ADC Kekaha WQ Monitoring						
Receive Date:	23 Feb-21 08:50	Source:	Cardno Hawaii								
Sample Age:	4d 3h (2.9 °C)	Station:	WW-2								
Comparison Summary											
Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method				
05-4075-0493	96h Survival Rate	100	>100	NA	38.3%	1	Equal Variance t Two-Sample Test				
Test Acceptability											
Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision					
05-4075-0493	96h Survival Rate	Control Resp	0.675	0.9 - NL	Yes	Below Acceptability Criteria <i>PS</i>					
96h Survival Rate Summary											
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	4	0.675	0.3222	1	0.4	0.9	0.1109	0.2217	32.85%	0.0%
100		4	0.925	0.7727	1	0.8	1	0.04787	0.09574	10.35%	-37.04%
96h Survival Rate Detail											
C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4						
0	Lab Control	0.4	0.6	0.9	0.8						
100		1	0.9	0.8	1						

*@Q18 AC 3/14/21*

**CETIS Analytical Report**

Report Date: 03 Mar-21 10:27 (p 1 of 1)  
 Test Code: 2102-S177 | 01-4759-0518

**Acute Amphipod Survival Test** Nautilus Environmental (CA)

Analysis ID: 05-4075-0493      Endpoint: 96h Survival Rate      CETIS Version: CETISv1.8.7  
 Analyzed: 03 Mar-21 10:27      Analysis: Parametric-Two Sample      Official Results: Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	Test Result
Angular (Corrected)	NA	C > T	NA	NA	38.3%	Passes 96h survival rate

**Equal Variance t Two-Sample Test**

Control	vs C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Lab Control	100	-2.174	1.943	0.280	6	0.9636	CDF	Non-Significant Effect

**ANOVA Table**

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.196325	0.196325	1	4.724	0.0727	Non-Significant Effect
Error	0.2493379	0.04155631	6			
Total	0.4456629		7			

**Distributional Tests**

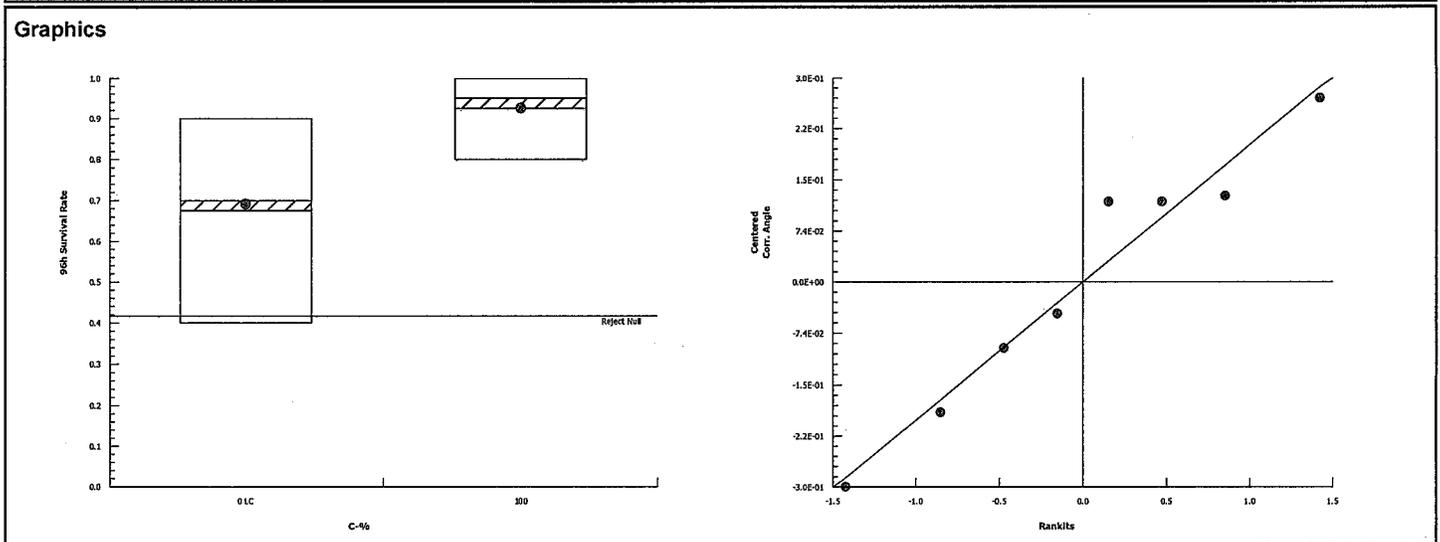
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Variance Ratio F	2.849	47.47	0.4128	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9512	0.6451	0.7229	Normal Distribution

**96h Survival Rate Summary**

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	4	0.675	0.3222	1	0.7	0.4	0.9	0.1109	32.85%	0.0%
100		4	0.925	0.7727	1	0.95	0.8	1	0.04787	10.35%	-37.04%

**Angular (Corrected) Transformed Summary**

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	4	0.9817	0.5871	1.376	0.9966	0.6847	1.249	0.124	25.26%	0.0%
100		4	1.295	1.061	1.529	1.331	1.107	1.412	0.07348	11.35%	-31.91%



**CETIS Summary Report**

Report Date: 03 Mar-21 10:29 (p 1 of 1)  
 Test Code: 2102-S180 | 19-6241-0513

Acute Amphipod Survival Test							Nautilus Environmental (CA)				
Batch ID:	04-6423-4557	Test Type:	Survival (96h)	Analyst:							
Start Date:	24 Feb-21 17:20	Protocol:	EPA/600/R-99/064 (2000)	Diluent:	@ Coast Filtered Water Dilute Mineral water						
Ending Date:	28 Feb-21 15:20	Species:	Hyaella azteca	Brine:	Not Applicable						
Duration:	94h	Source:	Aquatic Research Organisms, NH	Age:	13d						
Sample ID:	05-5743-4708	Code:	21-0227	Client:	Cardno Hawaii						
Sample Date:	20 Feb-21 14:30	Material:	Stormwater	Project:	ADC Kekaha WQ Monitoring						
Receive Date:	23 Feb-21 08:50	Source:	Cardno Hawaii								
Sample Age:	4d 3h (3.3 °C)	Station:	WW-3								
Comparison Summary											
Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method				
17-2662-8913	96h Survival Rate	100	>100	NA	40.4%	1	Equal Variance t Two-Sample Test				
Test Acceptability											
Analysis ID	Endpoint	Attribute		Test Stat	TAC Limits	Overlap	Decision				
17-2662-8913	96h Survival Rate	Control Resp		0.675	0.9 - NL	Yes	Below Acceptability Criteria				
96h Survival Rate Summary											
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	4	0.675	0.3222	1	0.4	0.9	0.1109	0.2217	32.85%	0.0%
100		4	0.875	0.6748	1	0.7	1	0.06292	0.1258	14.38%	-29.63%
96h Survival Rate Detail											
C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4						
0	Lab Control	0.4	0.6	0.9	0.8						
100		0.7	0.9	1	0.9						

@R18 AC 3/14/21

**CETIS Analytical Report**

Report Date: 03 Mar-21 10:29 (p 1 of 1)  
 Test Code: 2102-S180 | 19-6241-0513

<b>Acute Amphipod Survival Test</b>						<b>Nautilus Environmental (CA)</b>	
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Analysis ID: 17-2662-8913	Endpoint: 96h Survival Rate	CETIS Version: CETISv1.8.7
Analyzed: 03 Mar-21 10:29	Analysis: Parametric-Two Sample	Official Results: Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	Test Result
Angular (Corrected)	NA	C > T	NA	NA	40.4%	Passes 96h survival rate

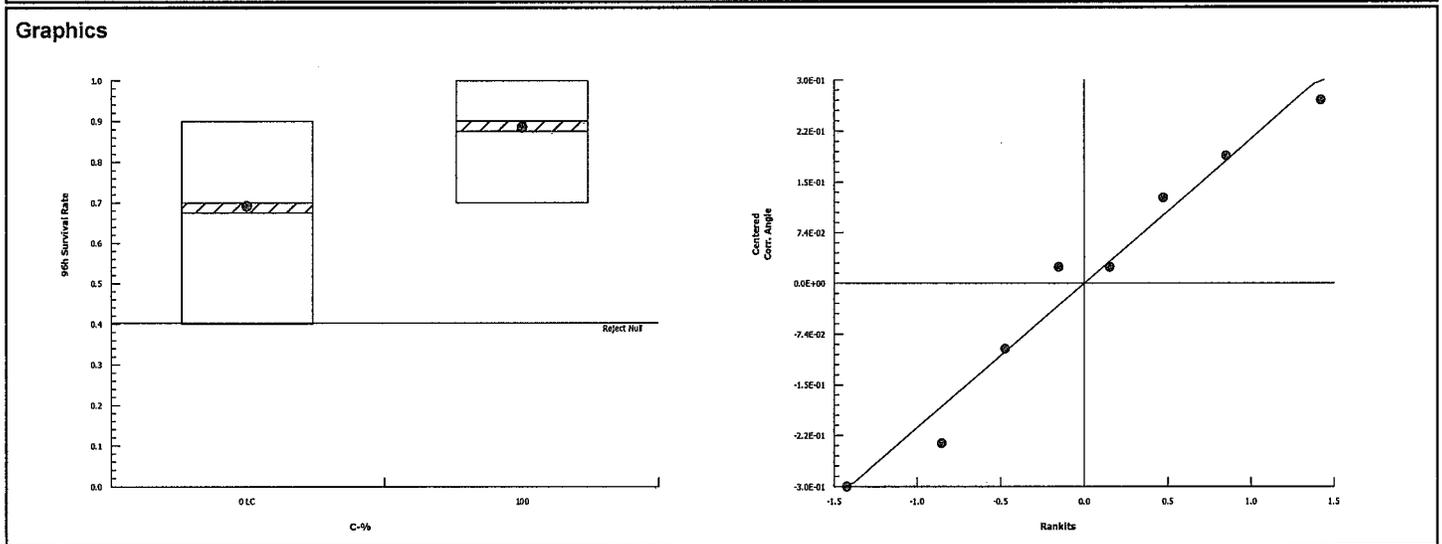
Equal Variance t Two-Sample Test									
Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		100	-1.608	1.943	0.294	6	0.9205	CDF	Non-Significant Effect

ANOVA Table						
Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.1186511	0.1186511	1	2.585	0.1590	Non-Significant Effect
Error	0.2753662	0.04589437	6			
Total	0.3940173		7			

Distributional Tests						
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)	
Variances	Variance Ratio F	2.032	47.47	0.5751	Equal Variances	
Distribution	Shapiro-Wilk W Normality	0.9568	0.6451	0.7787	Normal Distribution	

96h Survival Rate Summary											
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	4	0.675	0.3222	1	0.7	0.4	0.9	0.1109	32.85%	0.0%
100		4	0.875	0.6748	1	0.9	0.7	1	0.06292	14.38%	-29.63%

Angular (Corrected) Transformed Summary											
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	4	0.9817	0.5871	1.376	0.9966	0.6847	1.249	0.124	25.26%	0.0%
100		4	1.225	0.9485	1.502	1.249	0.9912	1.412	0.08699	14.2%	-24.81%



96-hour Freshwater Acute Bioassay  
 Static-Renewal Conditions  
 DF-006

Water Quality Measurements  
 & Test Organism Survival

Client: Cardno

Test Species: H. azteca

Sample ID: ADC Kehaha Stormwater Monitoring

Start Date/Time: 2/22/2021 17:20

Sample Log-in No's.: 21-0226, 21-0227

End Date/Time: 2/27/2021 15:20

Test No's.: 2102-5177 & -5180

Tech Initials				
0	24	48	72	96
PL	TN	TN	DN	#J
HH	TN	GH	GH	KL
CL		TN		

Counts:

Readings:

Dilutions made by:

Sample ID (100%)	Rep	Number of Live Organisms					Conductivity (µmhos/cm)					Temperature (°C)					Dissolved Oxygen (mg/L)					pH (units)				
		0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96
Lab Control	A	10	10	4	4	4	180	193	188	203	199	20.9	20.4	19.3	20.1	20.3	8.0	8.5	8.7	9.0	8.1	8.0	8.0	8.21	8.25	8.15
	B	10	10	7	6	6			198					20.3					8.8					8.16		
	C	10	10	10	8	9																				
	D	10	9	8	8	8											0.14	0.14								
WW-2 100%	A	10	5	3	0	1	509	516	518	540	523	20.8	20.1	19.0	20.0	20.1	9.5	8.6	9.8	9.0	8.0	0.03	6.90	6.29	7.49	7.25
	B	10	↓	0	3	1			531					20.1					8.5		7.9			7.54	7.25	
	C	10	↓	0	0	8																				
	D	10	↓	9	↓	10											0.14	0.14								
WW-3 100%	A	10	5	3	0	1	90	93	89	97	96	20.9	20.5	19.2	20.1	20.2	9.4	8.4	9.5	9.0	7.9	0.01	6.04	6.30	6.81	6.72
	B	10	↓	↓	↓	9			95					20.3					8.6					7.29		
	C	10	↓	↓	↓	10																				
	D	10	↓	↓	↓	9																				
A																										
B																										
C																										
D																										
A																										
B																										
C																										
D																										

Initial Counts QC'd by: TN  
 Initiated by: KL

Environmental Chamber: C

Animal Source/Date Received: ARO 2/24/21 Age at Initiation: 13 days

Animal Acclimation Qualifiers (circle all that apply): Q22 / Q23 / Q24 / (none)

Feeding Times				
0	24	48	72	96
AM:	--	--	6:20	--
PM:	--	--	--	--

Comments: i = initial reading in fresh test solution, f = final reading in test chamber prior to renewal

Organisms fed prior to initiation, circle one (y) (n) (y) Q18 HH 2/24/21

QC Check: ACS 3/2/21

Q18 HH 2/24/21  
Q18 DM 3/2/21

Final Review: AC 3/14/21

## **Appendix B**

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

Client: Cardno Tests Performed: Acute Survival

Project: ADC Kekaha Stormwater Monitoring Test ID No.(s): 2102-S172 to S180

Sample ID:	1) DW-1/ <del>WW-1</del>	2) WW-2	3) WW-3	4)
Log-in No. (21-xxxx):	0225	0226	0227	
Sample Collection Date & Time:	2/20/21 1945 PST	2/20/21 1400 PST	2/20/21 1430 PST	
Sample Receipt Date & Time:	2/23/21 0850	2/23/21 0850	2/23/21 0850	
Number of Containers & Container Type:	2 4L cubi	2 4L cubi	2 4L cubi	
Approx. Total Volume Received (L):	~8L	~8L	~8L	
Check-in Temp (°C)	2.4	2.9	3.3	
Temperature OK? <sup>1</sup>	<input checked="" type="radio"/> Y <input type="radio"/> N	<input checked="" type="radio"/> Y <input type="radio"/> N	<input checked="" type="radio"/> Y <input type="radio"/> N	<input type="radio"/> Y <input type="radio"/> N
DO (mg/L)	9.7	9.5	9.4	
pH (units)	7.46	5.83	5.79	
Conductivity (µS/cm)	2500	513	90	
Salinity (ppt)	1.4	0.2	0.1	
Alkalinity (mg/L) <sup>2</sup>	105	7	13	
Hardness (mg/L) <sup>2,3</sup>	366	50	34	
Total Chlorine (mg/L)	0.05	0.02	(A)	
Technician Initials	HH	HH	HH	

**Freshwater Tests:**

Control/Dilution Water Source: 8:2 Culligan Other: \_\_\_\_\_ Alkalinity: 90 Hardness: 88  
Additional Control?  Y  N = \_\_\_\_\_ Alkalinity: \_\_\_\_\_ Hardness: \_\_\_\_\_

**Marine Tests:**

Control/Dilution Water Source: LAB SW ART SW Other: \_\_\_\_\_ Alkalinity: 175 Salinity: 30 ppt  
Additional Control?  Y  N = Lab SW Alkalinity: 112 Salinity: 30 ppt  
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 (A) not measured due to interference  
(6) ACS 2/28/21; verified DW-1/ww-1 by client

QC Check: ACS 3/2/21

**Sample Descriptions:**

- 1) light brown, clear, no odor, heavy debris
- 2) light orange, slightly opaque, no odor
- 3) dark orange, opaque, no odor, light debris
- 4) light debris

COC Complete?  Y  N

Filtration?  Y  N

Initials: 1) \_\_\_\_\_ 2) \_\_\_\_\_ 3) \_\_\_\_\_ 4) \_\_\_\_\_

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 3/14/21

## **Appendix C**

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

# Enthalpy Analytical - Environmental Toxicology

4340 Vandever Avenue  
 San Diego, CA 92120  
 Phone 858.587.7333  
 infoSD@enthalpy.com

## Chain of Custody

Date 2/22/2021 Page 1 of 1

Sample Collection By:							ANALYSES REQUIRED							Receipt Temperature (°C)	
<b>Report to:</b>  <b>Company</b> <u>Cardno-GS</u> <b>Address</b> <u>737 Bishop St Suite 3050</u> <b>City/State/Zip</b> <u>Honolulu, HI 96734</u> <b>Contact</b> <u>Benjamin Berridge</u> <b>Phone</b> <u>808-476-0067</u> <b>Email</b> <u>benjamin.berridge@cardno-gs.com</u>				<b>Invoice To:</b> Same as Report to <input checked="" type="checkbox"/>  <b>Company</b> _____ <b>Address</b> _____ <b>City/State/Zip</b> _____ <b>Contact</b> _____ <b>Phone</b> _____ <b>Email</b> _____			P. promelas 96-hr Acute Survival	C. dubia 96-hr Acute Survival	H. azteca 96-hr Acute Survival	A. affinis 96-hr Acute Survival	M. beryllina 96-hr Acute Survival	A. bahia 96-hr Acute Survival	Enthalpy Matrix Codes: G = Grab C = Composite FW = Freshwater SW = Seawater Sed = Sediment STRM = Stormwater GW = Groundwater WW = Wastewater O = Other (specify)		
SAMPLE ID	SAMPLE			MATRIX CODE	Container		COMMENTS								
	Date	Time	Type (G or C)	(FW, SW, Sed, STRM, GW, WW, O)	Type	Qty									
1 <b>DW-1/WW-1</b>	2/20/21	17:45 HST	G	STRM	2.5 Gal Plastic	2	Marine Species				X	X	X		
2 <b>WW-2</b>	2/20/21	12:00 HST	G	STRM	2.5 Gal Plastic	2	Freshwater Species	X	X	X					
3 <b>WW-3</b>	2/20/21	12:30 HST	G	STRM	2.5 Gal Plastic	2	Freshwater Species	X	X	X					
4															
5															
6															
7															
8															
9															
10															

PROJECT INFORMATION		SAMPLE RECEIPT		1) RELINQUISHED BY (CLIENT)		2) RECEIVED BY (COURIER)	
Project Name:	ADC Water Quality Monitoring	Total No. of Containers	6	(Signature)	<i>[Signature]</i>	(Time)	14:00
PO No.:		Received Good Condition?	Y	(Printed Name)	Ben Berridge	(Date)	2-22-2021
Shipped Via:	FedEx	Matches Test Schedule?	Y	(Company)		(Company)	
SPECIAL INSTRUCTIONS/COMMENTS: -samples received partially frozen @ temps measured in surrogate cup after thawed.				3) RELINQUISHED BY (COURIER)		4) RECEIVED BY (LABORATORY)	
				(Signature)		(Signature)	<i>[Signature]</i>
				(Time)		(Time)	0850
(Printed Name)		(Printed Name)	Marie Henemann				
(Date)		(Date)	2/23/21				
(Company)		(Company)	EA SD				
		(Log-in #s)	21-022540 -0227				

Additional costs may be required for sample disposal or storage. Payment net 30 unless otherwise contracted.  
 Shaded areas are for lab use only  
 Report turn-around-time varies depending on length of test; please inquire with your project manager.

<http://enthalpy.com/environmental-toxicology-2/>

## **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**

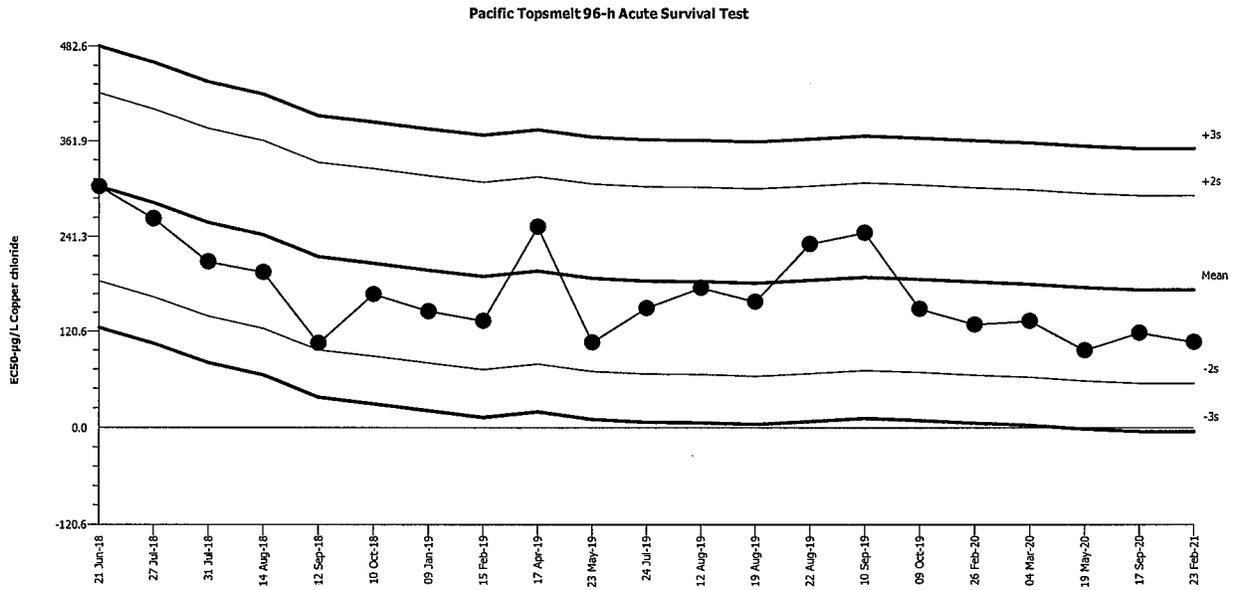
Pacific Topsmelt 96-h Acute Survival Test

Nautilus Environmental (CA)

Test Type: Survival (96h)  
Protocol: EPA/821/R-02-012 (2002)

Organism: Atherinops affinis (Topsmelt)  
Endpoint: 96h Survival Rate

Material: Copper chloride  
Source: Reference Toxicant-REF



Mean: 174.1      Count: 20      -2s Warning Limit: 55.05      -3s Action Limit: -4.446  
Sigma: 59.5      CV: 34.20%      +2s Warning Limit: 293.1      +3s Action Limit: 352.6

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2018	Jun	21	17:15	304.1	130	2.184	(+)		01-0576-9762	09-0246-7639
2		Jul	27	15:45	263.9	89.8	1.509			14-8822-7369	11-4350-5971
3			31	16:30	209.6	35.49	0.5965			19-5107-0005	20-6864-5330
4		Aug	14	16:00	196.4	22.28	0.3745			15-6494-9229	17-8173-7294
5		Sep	12	14:00	105.6	-68.48	-1.151			16-1211-7168	05-2683-6884
6		Oct	10	16:55	168.2	-5.921	-0.09951			03-4460-7421	02-8297-4115
7	2019	Jan	9	16:00	146.4	-27.69	-0.4654			16-8541-8400	01-0716-9806
8		Feb	15	16:10	134	-40.1	-0.674			08-0425-5661	18-0762-3864
9		Apr	17	17:50	253.5	79.45	1.335			05-1475-1452	18-1058-7085
10		May	23	15:30	106.6	-67.47	-1.134			03-2154-6851	19-3512-2662
11		Jul	24	16:25	150.4	-23.69	-0.3982			02-4547-9337	03-4444-2456
12		Aug	12	16:15	176.5	2.373	0.03988			05-6999-0080	19-2452-0933
13			19	19:30	158.7	-15.36	-0.2581			00-1616-6988	16-4823-3084
14			22	16:45	232	57.93	0.9735			14-6253-4066	09-6589-6472
15		Sep	10	11:15	246.2	72.13	1.212			01-3190-7470	00-5901-5932
16		Oct	9	15:40	149.6	-24.5	-0.4118			12-2483-9958	16-7314-6828
17	2020	Feb	26	15:20	129.7	-44.42	-0.7465			04-4275-3329	19-1366-8841
18		Mar	4	17:15	134.1	-40.02	-0.6726			09-0186-0501	09-2347-5750
19		May	19	17:20	96.59	-77.51	-1.303			09-8977-8612	01-6220-7123
20		Sep	17	14:25	118.9	-55.18	-0.9274			07-7701-0607	03-4458-7869
21	2021	Feb	23	16:10	107.2	-66.92	-1.125			15-2183-5128	00-7227-8818

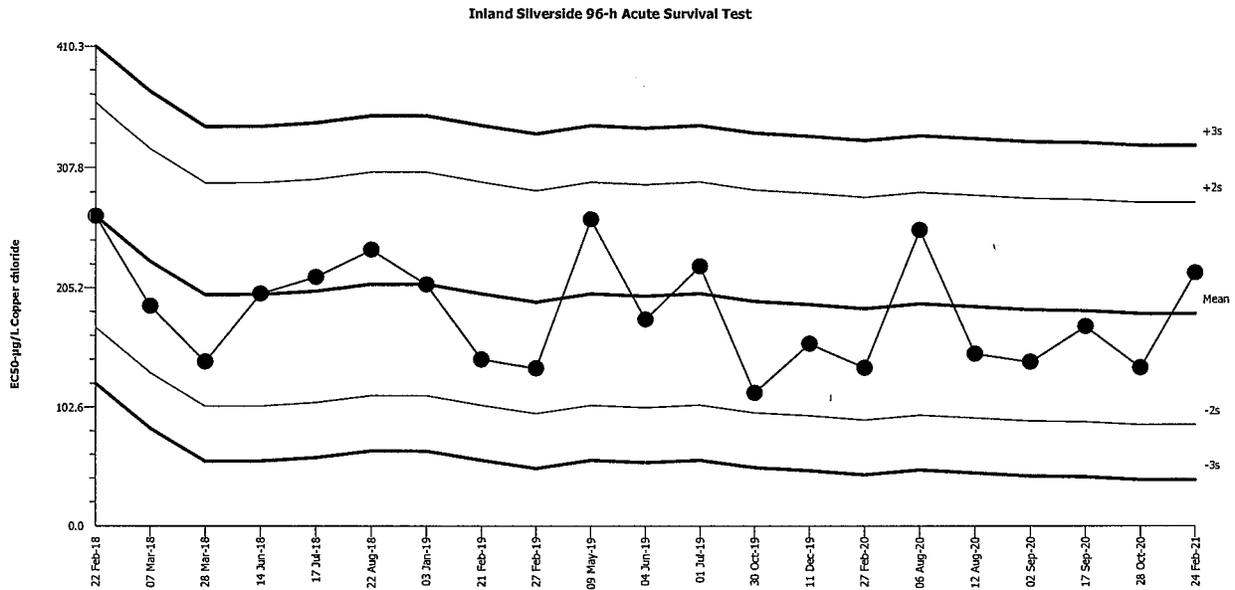
Inland Silverside 96-h Acute Survival Test

Nautilus Environmental (CA)

Test Type: Survival (96h)  
 Protocol: EPA/821/R-02-012 (2002)

Organism: Menidia beryllina (Inland Silverside)  
 Endpoint: 96h Survival Rate

Material: Copper chloride  
 Source: Reference Toxicant-REF



Mean: 183.2      Count: 20      -2s Warning Limit: 87.41      -3s Action Limit: 39.53  
 Sigma: 47.88      CV: 26.10%      +2s Warning Limit: 278.9      +3s Action Limit: 326.8

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2018	Feb	22	17:20	266.7	83.51	1.744			21-2244-9573	15-2512-9013
2		Mar	7	16:25	189.3	6.15	0.1284			06-3891-7579	03-5981-6406
3			28	17:15	141.4	-41.78	-0.8726			18-3798-9831	05-5342-2351
4		Jun	14	14:35	200	16.8	0.3509			01-9952-0614	00-3575-1747
5		Jul	17	14:30	214.4	31.15	0.6507			11-1445-3115	12-3693-5336
6		Aug	22	16:25	237.8	54.64	1.141			08-6172-7555	12-4329-0617
7	2019	Jan	3	16:50	207.9	24.65	0.5149			16-0506-4055	11-1190-1934
8		Feb	21	16:05	143.5	-39.72	-0.8296			10-4228-2556	08-7111-9529
9			27	16:25	135.8	-47.43	-0.9906			14-0947-0420	00-4247-8099
10		May	9	19:10	263.9	80.7	1.685			03-9779-6453	09-3747-7536
11		Jun	4	14:50	177.8	-5.445	-0.1137			00-2136-1210	01-4264-5145
12		Jul	1	15:55	223.6	40.42	0.8441			04-4319-5710	17-4098-1084
13		Oct	30	14:45	114.9	-68.33	-1.427			05-0159-0485	07-6888-5964
14		Dec	11	16:30	156.9	-26.28	-0.5489			11-0566-6524	14-4935-0865
15	2020	Feb	27	17:15	136.4	-46.84	-0.9784			00-2639-4829	10-5059-8408
16		Aug	6	16:00	254.9	71.71	1.498			13-3377-6823	09-5433-0150
17			12	15:20	148.4	-34.84	-0.7277			02-5307-3356	11-5066-6205
18		Sep	2	15:25	141.4	-41.78	-0.8726			09-8373-9144	18-7650-2455
19			17	14:45	172	-11.24	-0.2348			07-8442-4358	02-9347-5784
20		Oct	28	16:35	136.6	-46.6	-0.9732			10-9446-3954	10-4215-8111
21	2021	Feb	24	17:30	218.2	34.99	0.7308			11-4316-4077	02-1492-4727

Mysid 96-h Acute Survival Test

Nautilus Environmental (CA)

Test Type: Survival (96h)

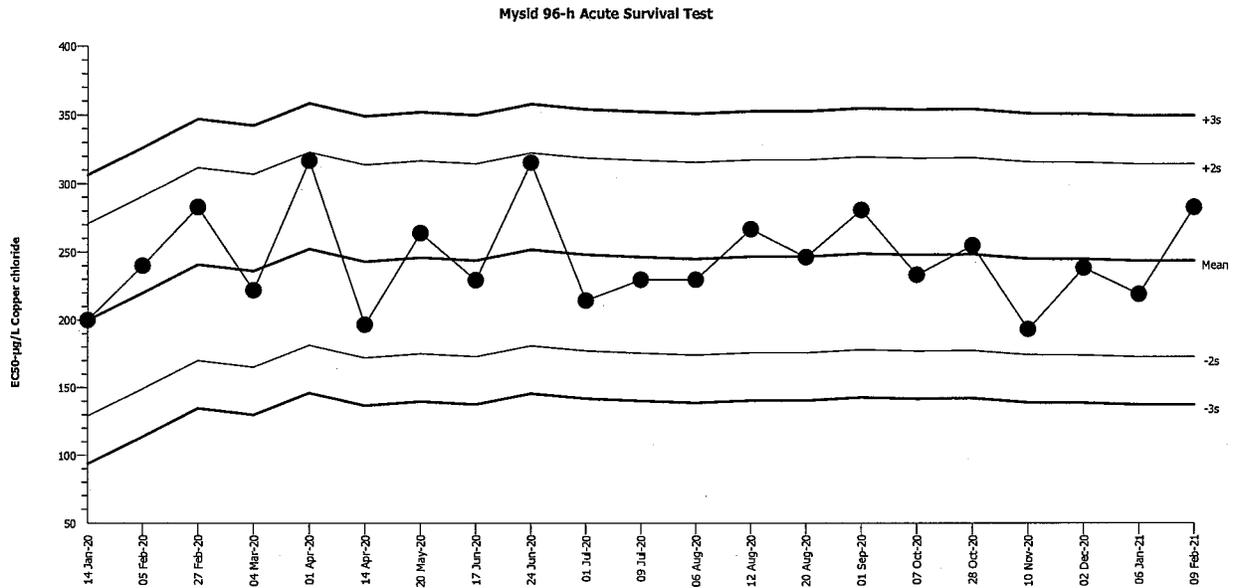
Organism: Americamysis bahia (Opossum Shri

Material: Copper chloride

Protocol: EPA/821/R-02-012 (2002)

Endpoint: 96h Survival Rate

Source: Reference Toxicant-REF



Mean: 243.7      Count: 20      -2s Warning Limit: 172.8      -3s Action Limit: 137.4  
 Sigma: 35.44      CV: 14.50%      +2s Warning Limit: 314.6      +3s Action Limit: 350

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2020	Jan	14	17:10	200	-43.7	-1.233			09-3242-6322	15-8924-0479
2		Feb	5	15:10	239.9	-3.754	-0.1059			06-4700-0567	16-3509-2167
3			27	17:10	282.8	39.14	1.104			16-4090-2608	06-8726-8298
4		Mar	4	16:40	221.9	-21.79	-0.6147			08-2404-4838	16-3243-0975
5		Apr	1	14:40	316.8	73.13	2.063	(+)		01-5241-6442	08-6803-0408
6			14	15:50	196.6	-47.14	-1.33			05-8944-6740	12-4161-6662
7		May	20	15:18	263.9	20.2	0.57			17-7816-4145	16-8137-5941
8		Jun	17	17:00	229.4	-14.3	-0.4036			17-4088-0296	03-9150-0813
9			24	16:50	315.6	71.86	2.028	(+)		18-0749-6976	17-0667-6625
10		Jul	1	15:25	214.4	-29.35	-0.828			18-2892-4402	15-7918-7101
11			9	17:20	229.7	-13.96	-0.3939			03-4895-5452	20-8273-1437
12		Aug	6	16:45	229.7	-13.96	-0.3939			12-3621-4083	04-5216-6972
13			12	15:00	266.7	23.01	0.6492			09-6865-4747	15-4661-4894
14			20	15:10	246.2	2.529	0.07136			11-8933-3936	07-7149-7380
15		Sep	1	17:40	280.9	37.21	1.05			15-1725-8445	02-3217-4494
16		Oct	7	16:50	233.3	-10.39	-0.2933			10-9302-4751	10-8015-5811
17			28	17:30	254.9	11.21	0.3164			05-5138-7579	10-2662-3199
18		Nov	10	16:15	193.2	-50.51	-1.425			18-2802-6809	21-3845-2247
19		Dec	2	16:15	238.4	-5.252	-0.1482			05-6239-9486	13-2389-5949
20	2021	Jan	6	15:40	219.1	-24.6	-0.6942			03-0517-8333	01-7272-9774
21		Feb	9	16:35	282.8	39.14	1.104			18-0066-8687	07-5637-1896

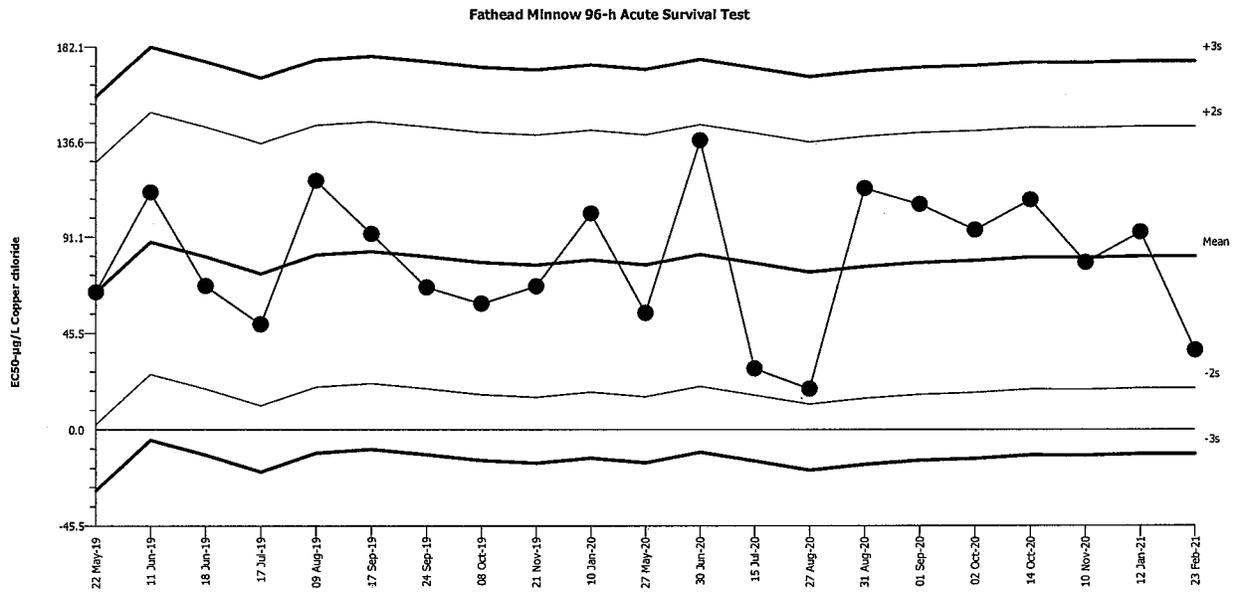
Fathead Minnow 96-h Acute Survival Test

Nautilus Environmental (CA)

Test Type: Survival (96h)  
 Protocol: EPA/821/R-02-012 (2002)

Organism: Pimephales promelas (Fathead Minn  
 Endpoint: 96h Survival Rate

Material: Copper chloride  
 Source: Reference Toxicant-REF



Mean: 81.98      Count: 20      -2s Warning Limit: 19.6      -3s Action Limit: -11.59  
 Sigma: 31.19      CV: 38.00%      +2s Warning Limit: 144.4      +3s Action Limit: 175.6

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2019	May	22	15:55	64.79	-17.19	-0.551			02-1759-3419	15-3388-9003
2		Jun	11	17:20	112.3	30.35	0.9732			16-3477-7401	15-3155-7444
3			18	16:00	67.74	-14.24	-0.4566			19-9791-9157	01-4134-1153
4		Jul	17	16:00	49.72	-32.26	-1.034			03-2107-5289	02-7115-7044
5		Aug	9	16:05	118	36	1.154			14-8182-5304	09-2863-4324
6		Sep	17	16:35	92.53	10.55	0.3383			08-1980-7492	14-4713-0442
7			24	16:50	67.15	-14.83	-0.4754			08-2287-5941	02-7891-5321
8		Oct	8	16:00	59.44	-22.54	-0.7226			04-9867-1678	19-8711-6972
9		Nov	21	16:35	67.63	-14.35	-0.46			04-2957-0529	12-8998-8134
10	2020	Jan	10	17:05	102.3	20.28	0.6503			16-9789-6682	06-6624-2910
11		May	27	16:35	55.05	-26.93	-0.8634			16-5231-1352	08-0130-7710
12		Jun	30	16:25	137.6	55.57	1.782			20-8582-5673	01-3539-9114
13		Jul	15	16:00	28.87	-53.11	-1.703			02-8717-8563	15-0846-2862
14		Aug	27	11:55	19.21	-62.77	-2.012	(-)		21-1958-4827	04-0829-1325
15			31	12:20	114.5	32.52	1.043			04-4264-5870	08-1199-0475
16		Sep	1	18:00	106.8	24.84	0.7964			05-5925-4870	11-8222-6308
17		Oct	2	14:35	94.56	12.58	0.4033			10-6788-7581	10-0094-1709
18			14	15:55	108.9	26.93	0.8634			16-9476-4319	04-8332-8963
19		Nov	10	15:50	79.05	-2.93	-0.09394			10-0925-3953	05-0925-4459
20	2021	Jan	12	16:10	93.54	11.56	0.3706			10-2818-5435	19-4959-1498
21		Feb	23	16:00	37.5	-44.48	-1.426			00-7897-7348	07-5099-8101

Ceriodaphnia 96-h Acute Survival Test

Nautilus Environmental (CA)

Test Type: Survival (96h)

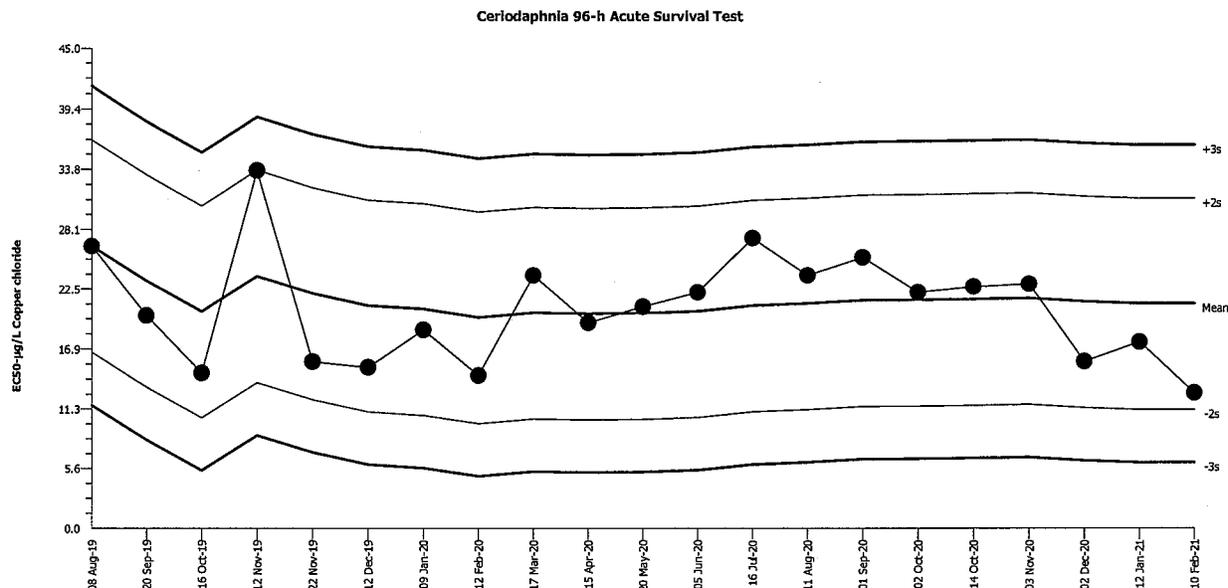
Organism: Ceriodaphnia dubia (Water Flea)

Material: Copper chloride

Protocol: EPA/821/R-02-012 (2002)

Endpoint: 96h Survival Rate

Source: Reference Toxicant-REF



Mean: 21.13      Count: 20      -2s Warning Limit: 11.17      -3s Action Limit: 6.184  
 Sigma: 4.981      CV: 23.60%      +2s Warning Limit: 31.09      +3s Action Limit: 36.07

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2019	Aug	8	14:50	26.54	5.412	1.086			16-9025-1148	02-0239-3729
2		Sep	20	18:20	20	-1.13	-0.2269			02-2453-0660	18-4748-7978
3		Oct	16	16:25	14.64	-6.489	-1.303			20-8293-2680	01-2609-4806
4		Nov	12	15:30	33.64	12.51	2.511	(+)		08-1275-5287	19-9898-2932
5			22	15:30	15.69	-5.438	-1.092			09-3327-8139	04-3248-6422
6		Dec	12	15:55	15.16	-5.973	-1.199			07-4521-5396	10-7315-8492
7	2020	Jan	9	15:20	18.66	-2.469	-0.4958			09-5261-4370	19-5681-9900
8		Feb	12	16:30	14.4	-6.734	-1.352			18-8330-6387	13-9776-1421
9		Mar	17	14:55	23.78	2.654	0.5329			18-8008-6095	05-6082-0450
10		Apr	15	16:10	19.32	-1.811	-0.3636			12-1338-7947	04-4538-1629
11		May	20	13:45	20.84	-0.2905	-0.05832			11-5303-0354	09-0756-9549
12		Jun	5	16:00	22.19	1.061	0.2131			14-1156-1724	03-6374-7457
13		Jul	16	12:45	27.32	6.191	1.243			07-7357-5721	02-9542-5069
14		Aug	11	14:10	23.78	2.654	0.5329			10-2942-2946	03-2270-9930
15		Sep	1	16:40	25.49	4.361	0.8756			08-0309-6057	08-6836-5081
16		Oct	2	13:20	22.19	1.061	0.2131			17-6681-3422	06-7614-6503
17			14	14:35	22.72	1.594	0.32			16-2893-4303	19-0938-0140
18		Nov	3	15:55	22.97	1.844	0.3702			21-1104-6548	14-3781-8992
19		Dec	2	15:10	15.69	-5.438	-1.092			05-6044-5446	17-1353-5910
20	2021	Jan	12	15:20	17.51	-3.618	-0.7264			04-7387-5530	19-1299-4588
21		Feb	10	13:55	12.75	-8.384	-1.683			08-3148-4658	06-6989-2045

Acute Amphipod Survival Test

Nautilus Environmental (CA)

Test Type: Survival (96h)

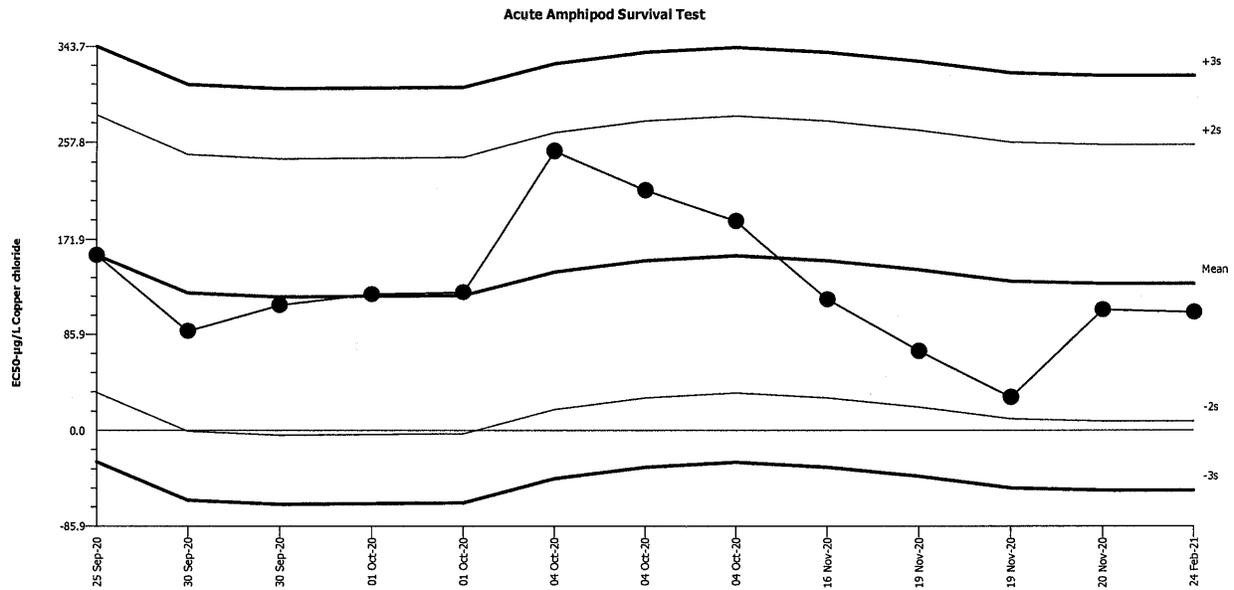
Organism: Hyalella azteca (Freshwater Amphip

Material: Copper chloride

Protocol: EPA/821/R-02-012 (2002)

Endpoint: 96h Survival Rate

Source: Reference Toxicant-REF



Mean: 132.1      Count: 12      -2s Warning Limit: 8.162      -3s Action Limit: -53.8  
 Sigma: 61.96      CV: 46.90%      +2s Warning Limit: 256      +3s Action Limit: 318

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2020	Sep	25	13:40	157.9	25.76	0.4158			16-9081-6764	18-6814-9087
2			30	15:15	88.77	-43.33	-0.6993			07-1981-4343	10-9026-6931
3			30	15:50	112.2	-19.85	-0.3204			11-9201-5159	11-6178-4756
4		Oct	1	14:50	122.2	-9.881	-0.1595			12-4287-6345	20-3445-2394
5			1	15:30	123.9	-8.17	-0.1319			17-5501-7242	20-6772-8270
6			4	14:00	250.3	118.2	1.907			13-1530-9745	13-5170-9478
7			4	14:35	215.4	83.32	1.345			16-7937-3453	09-9493-0420
8			4	15:15	188.3	56.16	0.9063			02-7905-1968	09-8618-9853
9		Nov	16	14:55	117.6	-14.55	-0.2348			05-7327-2673	01-8079-7741
10			19	16:00	70.71	-61.39	-0.9908			00-8858-0066	12-7185-4398
11			19	16:40	29.55	-102.6	-1.655			01-8113-8271	17-2113-7551
12			20	15:10	108.2	-23.9	-0.3858			08-5954-7214	01-8663-5204
13	2021	Feb	24	18:28	105.9	-26.15	-0.4221			17-5815-0110	10-9701-1209