



# ANALYTICAL REPORT

## PREPARED FOR

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## JOB DESCRIPTION

Q4 Acute Biomonitoring

## JOB NUMBER

192-7261-1

# Eurofins Arkansas

## Job Notes

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The test results in this report relate only to the samples as received by the laboratory and will meet all requirements of the methodology, with any exceptions noted. This report shall not be reproduced except in full, without the express written approval of the laboratory. All questions should be directed to the Eurofins Environment Testing South Central, LLC Project Manager.

## Authorization



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Re: Acute Biomonitoring utilizing *Ceriodaphnia dubia*  
Outfall 001 - Spokane Valley, WA  
Client NPDES Permit No. WA0000892  
Control No. 274772-1

This report is the analytical results and supporting information for the samples submitted to Eurofins Arkansas. The following results are applicable only to the sample identified by the control number referenced above. Accurate assessment of the data requires access to the entire document. Each section of the report has been reviewed and approved by the Lab Manager or qualified designee.

Testing procedures and Quality Assurance were in accordance with "Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms" EPA-821-R-02-012, Fifth Edition, October 2002. Test results are summarized below:

Acute *Ceriodaphnia dubia* Survival Test. The permit requirement is NOEC not less than 40%. The following were concluded from the test:

Survival:	NOEC	LOEC	LC50
	100	>100	>100

The sample therefore **PASSED** the *Ceriodaphnia dubia* test.

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## I. Introduction and Summary

Biomonitoring testing of 48-hour non-renewal definitive toxicity tests using *Ceriodaphnia dubia* were performed.

The *Ceriodaphnia dubia* test was conducted from November 29, 2023 at 1625 to December 01, 2023 at 1545.

The tests were performed in accordance with EPA-821-R-02-012. Statistical analyses were performed on the observed data.

The tests were conducted in temperature and light cycle controlled environmental chamber. The test temperature was 25 degrees C +/- 1 degree for the *Ceriodaphnia dubia*.

## II. Control Acceptance Criteria

ORGANISM	CRITERIA	RESULTS	PASS/FAIL
<i>Ceriodaphnia dubia</i>	Control Survival $\geq 90\%$	100	PASS
<i>Ceriodaphnia dubia</i>	Control Dilution CV $\leq 40$	0.00	PASS
<i>Ceriodaphnia dubia</i>	Critical Dilution CV $\leq 40$	0.00	PASS

## III. Outlined Report

### A. Introduction

Permit Number: WA0000892

Test Requirements: 48-hour non-renewal definitive toxicity test using:  
*Ceriodaphnia dubia*

### B. Effluent Samples:

Sampling Point: Outfall 001

Chemical Data:

Analysis	Result
Dissolved oxygen (mg/l)	7.8
pH (standard units)	8.0
Alkalinity (mg/l as CaCO <sub>3</sub> )	170
Hardness (mg/l as CaCO <sub>3</sub> )	200
Conductivity (umhos/cm)	430
Residual Chlorine (mg/l)	<0.05
Ammonia (mg/l)	<0.10

C. Dilution Water Samples: Synthetic Moderately Hard  
Chemical Data:

Analysis	192-7159-A-1
Dissolved oxygen (mg/l)	8.0
pH (standard units)	8.1
Alkalinity (mg/l as CaCO <sub>3</sub> )	59
Hardness (mg/l as CaCO <sub>3</sub> )	90
Conductivity (umhos/cm)	320
Residual Chlorine (mg/l)	<0.05
Ammonia (mg/l)	NA

D. Test Methods

Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms, (Fifth Ed.), EPA-821-R-02-012, 48-hour acute definitive test.

Endpoints:

Death; the criteria employed to establish death are:

No movement

No reaction to gentle prodding

Criteria	<i>Ceriodaphnia dubia</i>
Type and Volume of Test Chamber	30 ml disposable beaker
Volume of Sample	15 ml
Organisms per chamber	5
Replicates per dilution	4
Test Temperature	25 deg. C
Test Initiated	November 29, 2023 at 1625
Test Terminated	December 01, 2023 at 1545
Feeding	None required
Age of Test Organisms	<24 hours

E. Test Organisms

*Ceriodaphnia dubia*

## F. Quality Assurance - Toxicity Tests

Reference Toxicant: Sodium Chloride

Date of test:

*Ceriodaphnia dubia*: October 03, 2023 at 1142 to October 05, 2023 at 1144

Synthetic moderately hard dilution water used

Organism	LC50	Warning Limits
<i>Ceriodaphnia dubia</i>	2.11 g/l	1.53-2.29 g/l

## G. Organism History

*Ceriodaphnia dubia*

Date: November 29, 2023 at 1625

Age: &lt;24 hours

Source: In-house culture

#### IV. Results Summary

*Ceriodaphnia dubia* are exposed in a static non-renewal system to different concentrations of effluent and dilution water. Effluent dilutions for this test were 2.5%, 4.9%, 25%, 40%, 100%. The low-flow concentration was 40%. Test results were based on survival.

##### *Ceriodaphnia dubia*

The *Ceriodaphnia dubia* test was conducted from November 29, 2023 at 1625 to December 01, 2023 at 1545.

Concentration	24 hour % Survival	48 hour % Survival
Control	100	100
2.5%	100	100
4.9%	100	100
25%	100	100
40%	100	100
100%	95.0	95.0



### Appendix (Data)

#### *Ceriodaphnia dubia* Survival Data

Number of organisms per chamber: 5  
Volume of test chamber: 30 ml

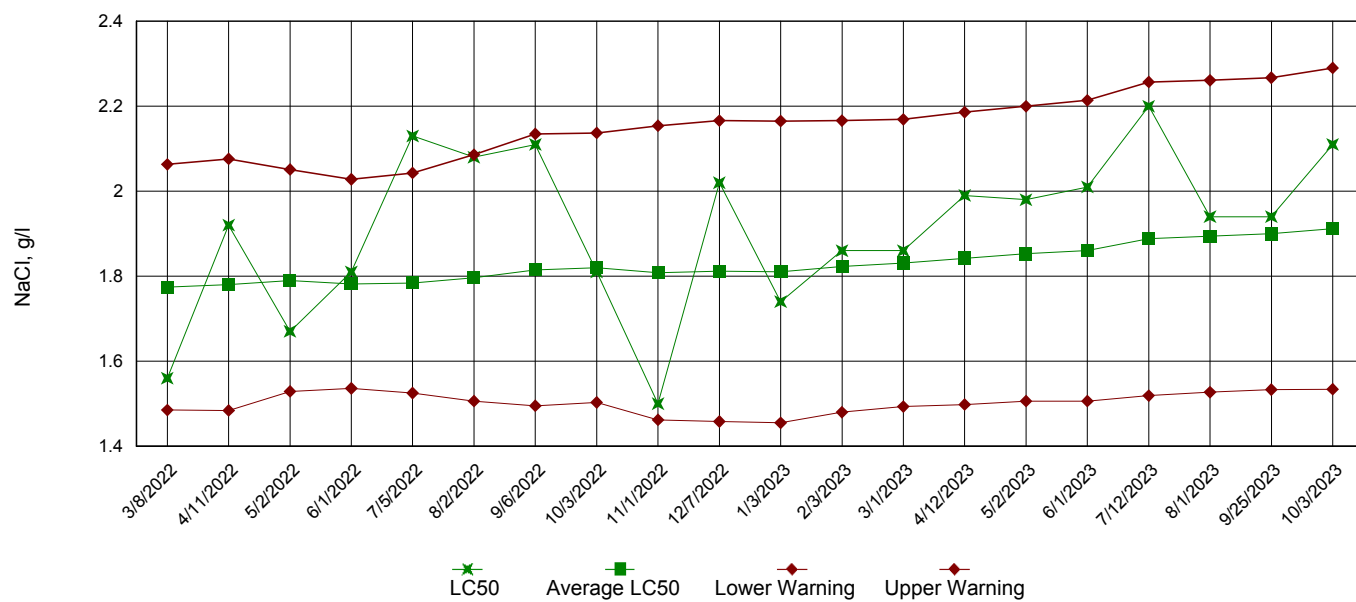
Age of organisms: <24 hours  
Volume of test solution: 15 ml

Effluent Concentration		Number of Survivors		% Survival	CV %
		24 Hours	48 Hours		
Control	rep. A	5	5	100	0.00
	rep. B	5	5		
	rep. C	5	5		
	rep. D	5	5		
2.5%	rep. A	5	5	100	0.00
	rep. B	5	5		
	rep. C	5	5		
	rep. D	5	5		
4.9%	rep. A	5	5	100	0.00
	rep. B	5	5		
	rep. C	5	5		
	rep. D	5	5		
25%	rep. A	5	5	100	0.00
	rep. B	5	5		
	rep. C	5	5		
	rep. D	5	5		
40%	rep. A	5	5	100	0.00
	rep. B	5	5		
	rep. C	5	5		
	rep. D	5	5		
100%	rep. A	4	4	95.0	10.5
	rep. B	5	5		
	rep. C	5	5		
	rep. D	5	5		

CV = Coefficient of variance = standard deviation X 100/mean

Appendix (Reference Toxicant)  
Acute Reference Toxicant, *Ceriodaphnia dubia*

LC50 Survival Data



# Appendix (Water Chemistry)

## Chemical Data for *Ceriodaphnia dubia*

Day 1		Control	2.5%	4.9%	25%	40%	100%
DO, mg/l	Initial	8.0	8.0	8.0	7.8	7.8	7.8
DO, mg/l	Final	7.7	7.9	7.8	8.0	7.9	7.6
pH, su	Initial	8.1	8.0	8.0	8.0	8.0	8.0
pH, su	Final	8.0	8.1	8.1	8.1	8.1	8.5
Alkalinity, mg/l		59	NA	NA	NA	NA	170
Hardness, mg/l		90	NA	NA	NA	NA	200
Conductivity, umho/cm		320	320	320	320	320	430
Residual Chlorine, mg/l		<0.05	NA	NA	NA	NA	<0.05
Day 2		Control	2.5%	4.9%	25%	40%	100%
DO, mg/l	Final	7.5	7.5	7.3	8.1	7.6	7.2
pH, su	Final	8.1	8.1	8.1	8.2	8.2	8.6

# Appendix (Data Sets for DEQ)

## *Ceriodaphnia dubia* Survival Data

Permittee:	Kaiser Aluminum	Critical Dilution:	40%
NPDES No:	WA0000892	Sample Source:	Outfall 001
Contact:	McKynzie Clark	Species Age:	<24 hours
Test Type:	48-hour non-renewal definitive toxicity test	Analysts:	GCX6, V6YL, B6YF, QGL9, WK7B
Dilution Water:	Synthetic Moderately Hard		
Test Initiated:	November 29, 2023 at 1625		
Test Terminated:	December 01, 2023 at 1545		

### PERCENT SURVIVAL

24 hours	Control	2.5%	4.9%	25%	40%	100%
Rep. A	100	100	100	100	100	80.0
Rep. B	100	100	100	100	100	100
Rep. C	100	100	100	100	100	100
Rep. D	100	100	100	100	100	100

48 hours	Control	2.5%	4.9%	25%	40%	100%
Rep. A	100	100	100	100	100	80.0
Rep. B	100	100	100	100	100	100
Rep. C	100	100	100	100	100	100
Rep. D	100	100	100	100	100	100

Dunnett's Procedure or Steel's Many-One Rank Test as appropriate. Is the mean survival at 48 hours significantly different ( $p=0.05$ ) than the control survival for the % effluent corresponding to:

a) Low Flow 40%:	<u>          </u>	Yes	<u>      X      </u>	No
b) 1/2 Low Flow (NA):	<u>          </u>	Yes	<u>          </u>	No

Pass/Fail #TEM3D:           0          

NOEL *Ceriodaphnia dubia* lethality #TOM3D:         100%        

Coefficient of variation for *Ceriodaphnia dubia* survival #TQM3D:           0          

Percent effluent corresponding to LC-50:         >100%

# Appendix (Data Sheets for DEQ)

## *Ceriodaphnia dubia* Chemical Parameters Chart

Permittee:	Kaiser Aluminum	Critical Dilution:	40%
NPDES No:	WA0000892	Sample Source:	Outfall 001
Contact:	McKynzie Clark	Species Age:	<24 hours
Test Type:	48-hour non-renewal definitive toxicity test	Analysts:	GCX6, V6YL, B6YF, QGL9, WK7B
Dilution Water:	Synthetic Moderately Hard		
Test Initiated:	November 29, 2023 at 1625		
Test Terminated:	December 01, 2023 at 1545		

Day 1		Control	2.5%	4.9%	25%	40%	100%
DO, mg/l	Initial	8.0	8.0	8.0	7.8	7.8	7.8
DO, mg/l	Final	7.7	7.9	7.8	8.0	7.9	7.6
pH, su	Initial	8.1	8.0	8.0	8.0	8.0	8.0
pH, su	Final	8.0	8.1	8.1	8.1	8.1	8.5
Alkalinity, mg/l		59	NA	NA	NA	NA	170
Hardness, mg/l		90	NA	NA	NA	NA	200
Conductivity, umho/cm		320	320	320	320	320	430
Residual Chlorine, mg/l		<0.05	NA	NA	NA	NA	<0.05
Day 2		Control	2.5%	4.9%	25%	40%	100%
DO, mg/l	Final	7.5	7.5	7.3	8.1	7.6	7.2
pH, su	Final	8.1	8.1	8.1	8.2	8.2	8.6

# CETIS Summary Report

Report Date: 07 Dec-23 14:19 (p 1 of 1)  
 Test Code/ID: 274772\_CD / 16-4389-8410

## Ceriodaphnia 48-h Acute Survival Test

Eurofins Arkansas

### Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	NOEL	LOEL	TOEL	PMSD	TU
10-3054-8767	48h Survival Rate	Steel Many-One Rank Sum Test	100	>100	---	9.2%	1

### Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	Level	%	95% LCL	95% UCL	TU
19-6402-7655	48h Survival Rate	Linear Interpolation (ICPIN)	LC50	>100	---	---	<1

### Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits		Overlap	Decision
				Lower	Upper		
10-3054-8767	48h Survival Rate	Control Resp	1	0.9	>>	Yes	Passes Criteria
19-6402-7655	48h Survival Rate	Control Resp	1	0.9	>>	Yes	Passes Criteria

### 48h Survival Rate Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
2.5		4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
4.9		4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
25		4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
40		4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
100		4	0.9500	0.7909	1.1090	0.8000	1.0000	0.0500	0.1000	10.53%	5.00%

### 48h Survival Rate Detail

MD5: F2A38373F9C177B450C835AB1BC4199C

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	1.0000	1.0000	1.0000	1.0000
2.5		1.0000	1.0000	1.0000	1.0000
4.9		1.0000	1.0000	1.0000	1.0000
25		1.0000	1.0000	1.0000	1.0000
40		1.0000	1.0000	1.0000	1.0000
100		0.8000	1.0000	1.0000	1.0000

## CETIS Analytical Report

Report Date: 07 Dec-23 14:18 (p 1 of 2)

Test Code/ID: 274772\_CD / 16-4389-8410

## Ceriodaphnia 48-h Acute Survival Test

Eurofins Arkansas

Analysis ID: 10-3054-8767      Endpoint: 48h Survival Rate      CETIS Version: CETIS v2.1.5  
 Analyzed: 07 Dec-23 14:16      Analysis: Nonparametric-Control vs Treatments      Status Level: 1  
 Edit Date: 07 Dec-23 0:00      MD5 Hash: F2A38373F9C177B450C835AB1BC4199C      Editor ID: 004-572-886-9

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	Tox Units	MSDu	PMSD
Angular (Corrected)	C > T	100	>100	---	1	0.09205	9.20%

## Steel Many-One Rank Sum Test

Control	vs	Conc-%	df	Test Stat	Critical	Ties	P-Type	P-Value	Decision(α:5%)
Dilution Water		2.5	6	18	10	1	CDF	0.8333	Non-Significant Effect
		4.9	6	18	10	1	CDF	0.8333	Non-Significant Effect
		25	6	18	10	1	CDF	0.8333	Non-Significant Effect
		40	6	18	10	1	CDF	0.8333	Non-Significant Effect
		100	6	16	10	1	CDF	0.6105	Non-Significant Effect

## Test Acceptability Criteria

## TAC Limits

Attribute	Test Stat	Lower	Upper	Overlap	Decision
Control Resp	1	0.9	>>	Yes	Passes Criteria

## ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.0118142	0.0023628	5	1	0.4457	Non-Significant Effect
Error	0.0425309	0.0023628	18			
Total	0.0543451		23			

## ANOVA Assumptions Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test				Indeterminate
Distribution	Shapiro-Wilk W Normality Test	0.4634	0.884	<1.0E-05	Non-Normal Distribution

## 48h Survival Rate Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	D	4	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.00%	0.00%
2.5		4	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.00%	0.00%
4.9		4	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.00%	0.00%
25		4	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.00%	0.00%
40		4	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.00%	0.00%
100		4	0.9500	0.7909	1.0000	1.0000	0.8000	1.0000	0.0500	10.53%	5.00%

## Angular (Corrected) Transformed Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	D	4	1.3450	1.3450	1.3450	1.3450	1.3450	1.3450	0.0000	0.00%	0.00%
2.5		4	1.3450	1.3450	1.3450	1.3450	1.3450	1.3450	0.0000	0.00%	0.00%
4.9		4	1.3450	1.3450	1.3450	1.3450	1.3450	1.3450	0.0000	0.00%	0.00%
25		4	1.3450	1.3450	1.3450	1.3450	1.3450	1.3450	0.0000	0.00%	0.00%
40		4	1.3450	1.3450	1.3450	1.3450	1.3450	1.3450	0.0000	0.00%	0.00%
100		4	1.2860	1.0960	1.4750	1.3450	1.1070	1.3450	0.0595	9.26%	4.43%

## 48h Survival Rate Detail

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	1.0000	1.0000	1.0000	1.0000
2.5		1.0000	1.0000	1.0000	1.0000
4.9		1.0000	1.0000	1.0000	1.0000
25		1.0000	1.0000	1.0000	1.0000
40		1.0000	1.0000	1.0000	1.0000
100		0.8000	1.0000	1.0000	1.0000

# CETIS Analytical Report

Report Date: 07 Dec-23 14:18 (p 2 of 2)  
Test Code/ID: 274772\_CD / 16-4389-8410

## Ceriodaphnia 48-h Acute Survival Test

Eurofins Arkansas

Analysis ID: 10-3054-8767 Endpoint: 48h Survival Rate CETIS Version: CETIS v2.1.5  
Analyzed: 07 Dec-23 14:16 Analysis: Nonparametric-Control vs Treatments Status Level: 1  
Edit Date: 07 Dec-23 0:00 MD5 Hash: F2A38373F9C177B450C835AB1BC4199C Editor ID: 004-572-886-9

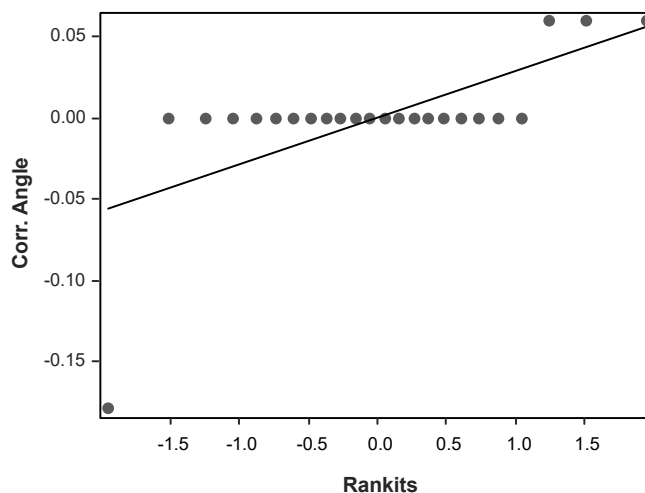
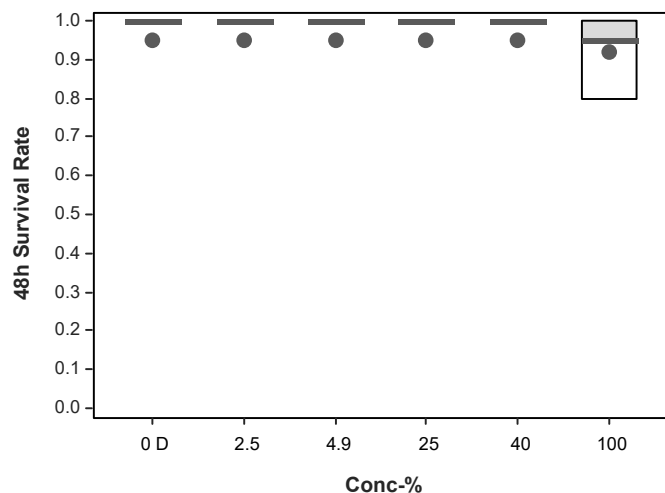
### Angular (Corrected) Transformed Detail

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	1.3450	1.3450	1.3450	1.3450
2.5		1.3450	1.3450	1.3450	1.3450
4.9		1.3450	1.3450	1.3450	1.3450
25		1.3450	1.3450	1.3450	1.3450
40		1.3450	1.3450	1.3450	1.3450
100		1.1070	1.3450	1.3450	1.3450

### 48h Survival Rate Binomials

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	5/5	5/5	5/5	5/5
2.5		5/5	5/5	5/5	5/5
4.9		5/5	5/5	5/5	5/5
25		5/5	5/5	5/5	5/5
40		5/5	5/5	5/5	5/5
100		4/5	5/5	5/5	5/5

### Graphics





## CETIS Analytical Report

Report Date: 07 Dec-23 14:18 (p 1 of 2)  
 Test Code/ID: 274772\_CD / 16-4389-8410

## Ceriodaphnia 48-h Acute Survival Test

Eurofins Arkansas

Analysis ID: 19-6402-7655      Endpoint: 48h Survival Rate      CETIS Version: CETIS v2.1.5  
 Analyzed: 07 Dec-23 14:17      Analysis: Linear Interpolation (ICPIN)      Status Level: 1  
 Edit Date: 07 Dec-23 0:00      MD5 Hash: F2A38373F9C177B450C835AB1BC4199C      Editor ID: 004-572-886-9

## Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1929330	1000	Yes	Two-Point Interpolation

## Test Acceptability Criteria

Attribute	Test Stat	TAC Limits		Overlap	Decision
		Lower	Upper		
Control Resp	1	0.9	>>	Yes	Passes Criteria

## Point Estimates

Level	%	95% LCL	95% UCL	Tox Units	95% LCL	95% UCL
LC50	>100	---	---	<1	---	---

## 48h Survival Rate Summary

Conc-%	Code	Count	Calculated Variate(A/B)							Isotonic Variate	
			Mean	Median	Min	Max	CV%	%Effect	ΣA/ΣB	Mean	%Effect
0	D	4	1.0000	1.0000	1.0000	1.0000	0.00%	0.00%	20/20	1.0000	0.00%
2.5		4	1.0000	1.0000	1.0000	1.0000	0.00%	0.00%	20/20	1.0000	0.00%
4.9		4	1.0000	1.0000	1.0000	1.0000	0.00%	0.00%	20/20	1.0000	0.00%
25		4	1.0000	1.0000	1.0000	1.0000	0.00%	0.00%	20/20	1.0000	0.00%
40		4	1.0000	1.0000	1.0000	1.0000	0.00%	0.00%	20/20	1.0000	0.00%
100		4	0.9500	1.0000	0.8000	1.0000	10.53%	5.00%	19/20	0.9500	5.00%

## 48h Survival Rate Detail

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	1.0000	1.0000	1.0000	1.0000
2.5		1.0000	1.0000	1.0000	1.0000
4.9		1.0000	1.0000	1.0000	1.0000
25		1.0000	1.0000	1.0000	1.0000
40		1.0000	1.0000	1.0000	1.0000
100		0.8000	1.0000	1.0000	1.0000

## 48h Survival Rate Binomials

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	5/5	5/5	5/5	5/5
2.5		5/5	5/5	5/5	5/5
4.9		5/5	5/5	5/5	5/5
25		5/5	5/5	5/5	5/5
40		5/5	5/5	5/5	5/5
100		4/5	5/5	5/5	5/5

# CETIS Analytical Report

Report Date: 07 Dec-23 14:18 (p 2 of 2)  
Test Code/ID: 274772\_CD / 16-4389-8410

## Ceriodaphnia 48-h Acute Survival Test

Eurofins Arkansas

Analysis ID:	19-6402-7655	Endpoint:	48h Survival Rate	CETIS Version:	CETIS v2.1.5
Analyzed:	07 Dec-23 14:17	Analysis:	Linear Interpolation (ICPIN)	Status Level:	1
Edit Date:	07 Dec-23 0:00	MD5 Hash:	F2A38373F9C177B450C835AB1BC4199C	Editor ID:	004-572-886-9

### Graphics

